Impact of partial replacement of saturated with monounsaturated fatty acids in dairy foods on markers of cardiovascular risk

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A thesis is part fulfilment of the requirement for the degree of

Doctor of Philosophy

School of Chemistry, Food and Pharmacy

School of Agriculture, Policy and Development

September 2017

DECLARATION

I confirm this is my own work and the use of all material from other sources has been properly and fully acknowledged.

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September 2017

ACKNOWLEDGEMENTS

The journey towards completing my doctorate has certainly been a learning process, filled with excitement, challenges and new perspectives. For giving me the opportunity to undertake this project and their invaluable support throughout these past 4 years, I would first and foremost wish to thank my supervisors, Professor Julie Lovegrove, Professor Ian Givens and Dr Kim Jackson. I appreciate all the encouragement, productive discussions, academic support and for allowing me to take many opportunities outside the sphere of the PhD – which allowed me to develop other skills. To Dr Oonagh Markey, who has been on this journey for these past few years – I am grateful for all your continuous support and encouragement, especially during tougher times. To everyone who formed the RESET team and whose important contributions helped shape and bring the project to its successful end: Drs Sue Todd, Kirsty Kliem, Colette Fagan, Alistair Grandison and Mr Dave Humphries. The large and multifaceted nature of human clinical trials required a lot of woman-power and RESET had a great army of students and assistants over the course of the project. From printing recruitment flyers, data entering, lab & pilot plant assistance to making hundreds of grilled-cheese and butter toasties in the early mornings, thank you to *Trisha, Victoria, Caroline, Ash, Laury, Kate, Jane, Honglin, Heshan, Elly and Alba*. My gratitude to everyone in HSNU who may not have been directly linked to RESET but provided help during the human trial.

A massive thank you for being there to my friends, both in Reading and scattered among a few other cities and countries – my sincere thanks for the laughs, the more serious discussions, the sometimes-pointless ones, the much-needed caffeine or wine breaks and ultimately, the moral support. To the aptly named '*Hungry thoughts*' (Agi, Angelika, Thanasis and Virag), Aga, Yianna, Valeria, Giulia, Natasa, Ioanna, Gessica, Katerina, my thanks for being there.

Lastly, but certainly not least, I would like to thank my parents – who perhaps didn't always understand what I've been doing these past 4 years but never stopped encouraging me to keep going.

LIST OF COMMONLY USED ABBREVIATIONS

AIx, augmentation index

ApoB, apolipoprotein B

ApoE, apolipoprotein E

AUC, area under the curve

BMI, body mass index

BP, blood pressure

CHD, coronary heart disease

cIMT, carotid intima media thickness

CVD, cardiovascular diseases

CMax, maximum concentration

CRP, C-reactive protein

DASH, dietary approaches to stop hypertension

DIVAS, dietary intervention and vascular study

DBP, diastolic blood pressure

DH, Department of Health

EDTA, ethylenediaminetetracetic acid

ELISA, enzyme linked immune-assay

FA, fatty acids

FMD, flow mediated dilatation

HDL-C, high density lipoprotein cholesterol

HPLC, high-performance liquid chromatograph

HOMA-IR, homeostatic model assessment of insulin resistance

iAUC, incremental area under the curve

iCAM-1, intercellular adhesion molecule 1

IL, interleukin

LDL-C, low density lipoprotein cholesterol

LPS, lipopolysaccharide

MUFA, mono-unsaturated fatty acids

NDNS, National Diet and Nutrition Survey

NEFA, non-esterified fatty acids

NO, nitric oxide

PCR, polymerase chain reaction

PL-FA, phospholipid fatty acids

PLS-DA, partial least squares discriminant analysis

PP, pulse pressure

PUFA, poly-unsaturated fatty acids

PWA, pulse wave analysis

PWV, pulse wave velocity

RCT, randomized controlled trial

RESET, replacement of saturated fat in dairy on total cholesterol

rQUICKI, revised quantitative insulin sensitivity check index

RR, relative risk

rTFA, *ruminant* trans fatty acids

SBP, systolic blood pressure

SATgene, Study Into Genetic Influence on Cholesterol Response to Dietary Fat

SEM, standard error of the mean

SFA, saturated fatty acids

TAG, triacylglycerol

TE, total energy

TC, total cholesterol

TFA, *trans* fatty acids

TNF-α, tumor necrosis factor alpha

TTMax, time to reach maximum concentration

TRL, triacylglyceride-rich lipoproteins

UHT, ultra-high temperature

VCAM-1, vascular cell adhesion molecule 1

WBC, whole blood culture

WHO, World Health Organization

Δ, change from baseline values

ABSTRACT

Cardiovascular diseases (CVD) remain one of the leading causes of morbidity and mortality worldwide. Current dietary guidelines advocate for a restriction of high intakes of saturated fatty acids (SFA), an important risk factor for the development of CVD due to their association with increased low-density lipoprotein cholesterol (LDL-C). Dairy foods are major contributors of total SFA intake. The literature review explored aspects of dairy consumption on CVD risk, highlighting current evidence which suggests a beneficial effect of certain dairy products. A reduction of dietary SFA is also dependent on a suitable replacement macronutrient. Supplementation of the dairy cow diet with plant oils/seeds provides an alternative strategy to partially replacing SFA in milk fat with monounsaturated fatty acids (MUFA). Few interventions studies have extensively investigated the impact of fatty acid (FA) modified dairy products on traditional and novel CVD risk markers. The RESET intervention study aimed to address this knowledge gap in both a chronic and acute-within-chronic setting in adults at moderate CVD risk. A food-exchange model (38% total energy (%TE) from fat) was implemented and adherence assessed from 4 day weighed food diaries and phospholipid FA (PL-FA) analysis. Consumption of the FA-modified diet for 12 weeks (dietary target: 16%TE SFA; 14%TE MUFA) led to a significant attenuation in fasted LDL-C concentrations from baseline values and compared to matched control products (19%TE SFA; 11%TE MUFA). At the beginning and end of each dietary intervention, the postprandial investigation was performed using sequential test meals representative of the assigned dairy diet. Following the FA-modified diet and test meals, a decrease in the postprandial iAUC summary response for apolipoprotein B (apoB) was observed, suggestive of an effect on triacylglyceride-rich lipoproteins (TRL). Changes in postprandial summary responses of plasma total lipid FA reflected the intake of the test meals. Additionally, the FA-modified diet appeared to modestly impact on both fasted concentrations and postprandial responses of nitrite, which may be indicative of an effect on endothelial function. Little to no impact was observed for other outcome measures. The impact of *APOE* genotype, retrospectively assessed, fasted and postprandial outcome measures following the two dairy diets, was explored. Significant pre-intervention fasted baseline differences in high-density lipoprotein cholesterol (HDL-C), small and medium HDL particle distribution and non-esterified fatty acids (NEFA) between *E4* carriers and *E3/E3*, were observed. There was no interaction between genotype and treatment on any of the outcome measures. In summary, consumption of FA-modified dairy products appear to beneficially impact on certain markers of CVD risk,

compared to matched control products. However, further research should address the impact of other types of FA-modified dairy products in both healthy and at risk populations.

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Chapter I: Literature review: Dairy and cardiovascular risk: friend or foe?

The aim of this chapter is to provide a review of the current evidence on the association between dairy intake and CVD risk and present evidence of the impact of fat modified dairy products on risk factors of cardiovascular disease.

Elements from this chapter have been previously published in the following invited review (**Appendix I**) and may appear word for word in the present chapter:

Markey O, Vasilopoulou D, Givens DI & Lovegrove JA (2014) Dairy and cardiovascular risk: friend or foe? *Nutrition Bulletin* **39**; 161-171.

DV and OM are co-authors of the published review, which was approved by all authors.

DV expanded on elements of the review presented in this thesis, by including updated data and expanding on methodologies relevant to the intervention trial.

1.1 Introduction to cardiovascular disease risk

Cardiovascular diseases (CVD) are ranked as the leading causes of death in the UK and worldwide, with a number of modifiable and non-modifiable risk factors (1). Considered an umbrella term for a group of diseases related to perturbation of blood vessels and heart function, conditions include coronary heart disease (CHD), stroke, peripheral artery disease, heart attack and pulmonary embolisms (1). In the UK, CVD accounts for 26% of all deaths (2). Annual mortality rates have declined in the UK between 1980 and 2015, mainly due to lifestyle and dietary changes and better access to treatment (2). Mortality rates within Europe vary substantially between countries, with higher figures observed in Eastern Europe, attributed predominantly to an increased total energy and fat consumption and a higher smoking prevalence (1). The global prevalence of CVD is anticipated to increase and is expected to be responsible for over 23 million deaths by 2030, in part due to demographic changes with higher obesity rates and an ageing population (3). The financial burden of CVD within the European Union (EU) reached an annual cost of €210 billion in 2015, in both direct and indirect costs, with the cost per person in the UK exceeding the EU average (4, 5).

The development and progression of CVD is associated with a number of modifiable and non-modifiable risk factors. Modifiable risk factors include physical inactivity, high blood pressure, dyslipidaemia (i.e. elevated total and low-density lipoprotein-cholesterol (LDL-C) and triacylglycerols (TAG)) and being overweight/obese. Hypertension represents the greatest risk factor associated with the development of CHD and stroke in particular (6). Dietary risks also account for the greatest loss of global disability-adjusted life years (DALY) for disease risk factors, according to more recent data (7). CVD is one of the main contributors to lost DALY and an atherogenic diet represents a leading risk factor for CVD (7, 8). Therefore, on a population level, it remains important to develop effective dietary strategies aimed at reducing risk factors associated with CVD development and progression.

It is well established that a high intake of total dietary saturated fatty acids (SFA) and *trans* fatty acids (TFA) leads to a higher risk of CVD, an effect linked to an increased plasma concentration of fasting LDL-C (9). Current dietary guidelines recommend aiming for a population average intake of <10% of total energy intake from SFA and 2% of total enegery intake total TFA (10). At present, this target is exceeded by the UK adult population (men and women), with the most recent UK National Diet and Nutrition Survey (NDNS) reporting a mean population SFA intake of >12% of total energy intake (10). Intakes of total TFA in the UK do not exceed dietary recommendations and are estimated at 1.2-1.3% on average (10). Data from the current rolling UK NDNS on SFA intake, which show consistently higher intakes across all age groups, are summarised in **Table 1.1**.

Table 1.1 Average intake of saturated fatty acids in absolute terms and as percentages of food and total energy, by age and gender (NDNS 2012/13 – 2012/14)

	Boys/Men			Girls/Women		
	11-18 years	19-64 years	≥65 years	11-18 years	19-64 years	≥65 years
SFA (g/day)	27.1 ± 10.5	28.4 ± 11.2	25.6 ± 9.6	23.0 ± 8.8	22.1 ± 9.6	22.1 ± 8.3
% food energy	12.5 ± 2.7	12.7 ± 2.9	13.2 ± 3.2	12.7 ± 2.6	12.7 ± 3.6	13.7 ± 3.4
% total energy	12.5 ± 2.7	12.0 ± 3.0	13.2± 3.2	12.6 ± 2.6	12.3 ± 3.5	13.3 ± 3.2

Values are mean ± SD. NDNS, National Diet and Nutrition Survey. SFA, saturated fatty acids.

Adapted from Bates et al. (10)

1.2 The process of atherosclerosis

Atherosclerosis, which initiates in the sub-endothelial arterial space (intima) at regions of disturbed blood flow, is triggered by interplays of both endothelial dysfunction and sub-endothelial lipid retention (11). Vascular endothelial cells comprise a dynamic lining of the vascular system, responding to both mechanical and humoral stimuli to maintain vascular homeostasis (11). The endothelium is the major regulator of vascular wall homeostasis, whereby a balanced vascular tone and low levels of oxidative stress is maintained by the release of mediators, such as nitric oxide (NO), prostacyclin (PGI2) and endothelin (ET-1) and control of local angiotensin-II activity (11). Vascular permeability to plasma constituents, platelet and leukocyte adhesion and aggregation is also regulated by the endothelium (11, 12). Therefore, endothelial dysfunction is characterised by progressive dysregulation of vascular wall homeostasis. Increases in the expression of adhesion molecules, synthesis of pro-inflammatory and pro-thrombotic factors, oxidative stress and an abnormal modulation of vascular tone are all factors which progressively lead to impaired endothelium-dependent vasodilation (11).

Lipoproteins, responsible for the transport of fat within the bloodstream, are divided into five major classes based on their relative density: chylomicrons, very low-density lipoproteins (VLDLs), intermediate-density lipoprotein (IDLs), LDLs and high-density lipoproteins (HDLs) (13). Apolipoproteins (apo), proteins bound to the surface of lipoproteins, function as regulators of lipoprotein metabolism (13). They comprise a large class of proteins (grouped by function into A, B, C and E). The present thesis will present data on two classes, relevant to the dietary intervention study: a) apoB, which is recognised by cell-surface receptors that mediate endocytosis (13). Hepatic derived apoB-100 on VLDL, IDL and LDL functions as a ligand for LDL receptors (13). ApoB-48, synthesized by the small intestine, is present on chylomicrons and their remnants and is essential for the intestinal absorption of dietary fats (14). As LDL particles are derived from the lipolysis of

TAGs in VLDLs, the rate of VLDL production and lipolysis of VLDL TAGs influences circulating levels of LDL-C (15). Most circulating LDL particles are taken up by the liver, via LDL receptors (15). In the liver, LDL receptor expression is regulated by hepatocyte cholesterol concentrations (15). However, low LDL receptor activity reduces the rate of LDL clearance, which results in prolonged LDL residence in the circulation (15). Consequently, the retention of circulating LDL particles and other apoB-containing lipoproteins (such as lipoprotein(a)) and remnants undergo modifications, including aggregation, oxidation and lipolysis in arterial vessel walls (15). Moreover, the progression of atherosclerosis is also influenced by the fed state and particularly following a fat-rich meal, the clearance of chylomicrons and their remnants.

Over time, the sub-endothelial retention of apoB-containing lipoproteins, VLDL-C and chylomicron remnants in arterial regions where laminar flow is disturbed by branch points or arterial bends leads, leads to endothelial dysfunction, pathogenic risk factors and ultimately atherosclerosis (11). Retained lipoproteins within the arterial wall likely trigger low-grade inflammatory responses, which will be discussed further below. Additionally, b) apoE (apoE2, -E3 and -E4) plays a critical role in the clearance of VLDL and chylomicron remnants from the circulation (13, 16). ApoE is primarily recognised as a regulator of lipid levels, playing a role in the homeostatic control of both circulating and tissue lipid content in part by binding to high affinity cell-surface lipoprotein receptors (17). The apoE isoforms differ in their ability to interact with apoE-containing lipoproteins and lipid complexes to the LDL receptor, the LDL-receptor related protein, the VLDL receptor and the apoE-receptor-2 (17). Additionally, apoE also binds to cell surface heparan sulfate proteoglycans (HSPGs) in accordance to differences in the binding affinity of apoE isoforms. The interaction with HSPGs attracts and sequesters apo-E containing lipoproteins at cell surfaces, thereby facilitating their interaction with the LDL receptor related protein and potentially other receptors (16). As atherosclerosis is recognised as a major cause of CVD, low-grade

inflammation plays a central role in the development of atherosclerosis and will be discussed further below.

1.3 Dietary fatty acids and CVD risk

Recent meta-analyses of prospective studies (18-20) have questioned whether high SFA intake is always a risk factor for CVD. It is also important to recognise that although there is strong consistent evidence linking high dietary SFA consumption to an increased risk of CVD events, this relationship is complex and influenced by multiple contributing factors (21). Among these, but not limited to, are structural variations such as chain length, which impact absorption and transport, the inter-individual variability to dietary fat response and the overall dietary pattern and food matrix in which SFAs are consumed (22). Additionally, in the case of dietary SFA, where the aim is to reduce total intake, it is imperative to consider the optimal food or nutrient replacement which would lead to a reduction in CVD risk (23, 24). The most significant reduction in CHD risk has been observed when dietary SFA are replaced with *cis*-polyunsaturated fatty acids (*cis*-PUFA), however little effect was seen on risk of stroke (24). Collectively, the evidence from randomized controlled trials (RCT) showed that a replacement of 10% and 5% of energy from SFA by cis-PUFA led to a reduction of CVD event by 27% and 10% respectively (24, 25). Two classes of PUFA, n-3 and n-6, have been widely studied for their impact on CVD risk. In the context of SFA replacement, mixed PUFA (n-3 + n-6) reduces CVD risk by improving the lipid profile (26). However, studies have also shown that high intakes of n-6 PUFA alone may increase oxidation susceptibility of LDL-C (27). This highlights the importance of an adequate n-6/n-3 ratio in the diet in contrast to individual nutrients. Replacement of SFA with n-6 PUFA reduces the risk of CVD events and mortality, as observed by a meta-analysis of prospective cohort studies, where a 2% energy replacement from SFA with n-6 PUFA was associated with an 11% reduced risk of CVD mortality and a 7% reduced risk of total mortality (28). However, the food source of SFA replacement also needs to be considered. In particular, secondary analysis of the Sydney Diet

Heart Study (1966-1973) which investigated the replacement of SFA with linoleic acid explained the original divergent results. The study recruited participants who had suffered an acute coronary event and were randomly allocated to either a high n-6 PUFA, low SFA diet (PUFA 15% and SFA <10% total calories) or a control group. The participants in the high n-6 PUFA diet had higher rates of all-cause, CHD and CVD death following the 3 year intervention (29). These observations were explained by the TFA present in the primary source of linoleic acid (stick margarines). (29). Fewer large scale studies have extensively reviewed the replacement of SFA with n-3 PUFA, as these are found in smaller amounts in dietary sources and usually represent a small proportion of overall dietary fat intake. However, analysis of the Nurses' Health Study and Health Professionals Follow-up Study observed a 5% reduction in all-cause mortality, but not CVD mortality, following a replacement of 0.3% of energy from SFA with n-3 PUFA (28). Intake of n-3 PUFA has been observed to decrease TAG and VLDL-C concentrations (by controlling TAG uptake and synthesis in the liver), apoB production and may moderately elevate HDL-C concentrations (30). Additionally, n-3 PUFA may also confer beneficial effects on blood pressure, by promoting NO production and consequently vasodilation and decreasing the expression of pro-inflammatory markers, such as adhesion molecules (30). As previously mentioned, the beneficial impact of n-6 PUFA largely depends on the n-6/n-3 ratio, as excess n-6 PUFA intake have been associated with pro-inflammatory and oxidative effects, thereby adversely impacting on CVD risk by affecting vascular function (27).

In the case of *cis*-monounsaturated fatty acids (*cis*-MUFA), recent reviews report that there is still insufficient data to draw definite conclusions (23, 24). A meta-analysis of long term studies (>6 months) which compared high *cis*-MUFA diets (>12% total calories) to lower *cis*-MUFA diets (<12% total calories), concluded that high *cis*-MUFA diets were associated with lower systolic and diastolic blood pressure and lower fat mass (31). Additionally, a recent RCT which compared diets high in *cis*-PUFA with diets high in *cis*-

MUFA observed that the latter led to a reduction in abdominal obesity (mainly in men) and concomitant reductions in diastolic blood pressure and serum TAGs (32). When considering replacing SFA with *cis*-MUFA, a meta-analysis of RCTs concluded that specific risk biomarkers of CVD were reduced (total cholesterol, LDL-C and TAGs) (33). However, the effect on CVD, CVD mortality and all-cause mortality following a replacement of SFA with *cis*-MUFA shows mixed results (23). An explanation for these findings may be that dietary sources of fatty acids vary among different studies, making it difficult to draw definite conclusions (23). The observed beneficial impact of MUFA on CVD risk may be in part explained by an improvement in insulin sensitivity (30), however this mechanisms has not been fully explored in dietary replacement studies. Lastly, there appears to be no clear beneficial evidence of substituting carbohydrates (CHO) for SFA in the prevention of CVD (23, 34, 35). This may be explained by the fact that only CHO from fibre rich wholegrains appear to confer a modest beneficial impact, while sources of refined CHO do not (23). Furthermore, the Cochrane meta-analysis of Hooper *et al.* (35), identified that a reduction of dietary SFA intake with a concomitant replacement by unsaturated fatty acids, and/or reduction of total fat, lowered the risk of CVD events by 14% (relative risk (RR) 0.86; 95% confidence interval (CI) 0.77-0.96) but had no effect on CVD or total mortality (23).

The evidence to date suggests that a replacement of SFA with *cis*-PUFA (with an appropriate n-6/n-3 ratio) and *cis*-MUFA may lead to a beneficial impact on CVD risk. Evidence suggests that the beneficial impact on CVD risk of these unsaturated FA, compared to SFA, may be explained through reductions of TC, LDL-C, apoB, TAG and pro-inflammatory markers with a parallel increase in HDL-C and production of NO, among other factors. However, the evidence is not conclusive as observed beneficial impact on risk markers following SFA replacement with UFAs are dependent on a number of factors, including the replacement foods within the diet rather than just single nutrients. These factors raise important questions when considering milk and dairy products (including butter), which

represent up to 35% of the average total dietary SFA intake in the UK (10). A reduction or substitution with low-fat dairy counterparts has been a strategy traditionally implemented to reduce CVD risk at a population level; however, the bulk of the evidence from both prospective cohort studies and RCTs does not support an association between dairy food intake and an increased risk of CVD, with the exception of butter (36). The case of butter intake and CVD risk however still remains a matter of debate, as a recent systematic review and meta-analysis of prospective observational studies observed a small positive association between butter consumption and all-cause mortality (n = 9 country-specific cohorts as 14 g per day: RR = 1.01, 95% CI = 1.00, 1.03, *P* = 0.045) but no association with incident CVD (n = 4; RR = 1.00, 95% CI = 0.98, 1.02, *P* = 0.704) or CVD subtypes (for CHD, n = 3; RR = 0.99, 95% CI = 0.96, 1.03; *P* = 0.537; for stroke n = 3; RR = 1.01, 95% CI = 0.98, 1.03; *P* = 0.737) (37). However, as the authors also highlight, substitution of butter for healthier fats, such as olive oil, would lead to a greater reduction in CVD risk.

1.4 The consumption of milk and dairy products in the UK

1.4.1 Changing trends in UK dairy consumption

Dairy product consumption in the UK has changed markedly over recent years, a trend which may be in part attributed to negative connotations surrounding dairy products and SFA content, especially whole fat versions, and a parallel increase in the availability of alternative products. According to the *Family Food Survey* published by the Department of Environment, Food and Rural Affairs (DEFRA (38)), average consumption of milk is about 1.5 litres per person per week, the majority of which is consumed as semi-skimmed milk (70%), followed by whole milk (20%) and skimmed milk (10%). Since 2003, there has been an almost 14% decline in consumption of total milk, with whole milk consumption declining by an average of 50% (38). Cheese and

cream consumption trends have remained fairly constant in recent years while consumption of yogurt and fromage frais has steadily increased compared with intakes in 1970, reaching 200 ml per person per week in 2013 (38). In contrast, butter consumption has markedly decreased since the 1970s by approximately 70% and is now estimated at 42 g per person per week (38). The average contribution of daily total SFA and TFA intake from milk and specific dairy products for both children and adults is presented in **Table 1.2**.

Table 1.2 Percentage contribution of dairy products to average daily saturated and trans fatty acid intake, by age (NDNS 2012/13 – 2012/14).

	total SFA contribution (%)					total TFA contribution (%)				
	1.5-3 yrs	4-10 yrs	11-18 yrs	19-64 yrs	≥65 yrs	1.5-3 yrs	4-10 yrs	11-18 yrs	19-64 yrs	≥65 yrs
Milk and milk products	48	31	22	22	24	55	42	32	31	32
of which:										
Whole milk (3.8% fat)	21	8	3	2	2	19	7	3	2	2
Semi skimmed milk (1.8% fat)	5	5	4	4	6	9	10	7	7	9
Other milk and cream	4	2	2	2	2	2	3	2	2	2
Cheese	9	8	8	10	9	15	14	14	16	15
Cheddar cheese	6	5	6	7	6	9	9	10	11	10
Other cheese	3	3	2	3	3	6	5	4	5	4
Yoghurt, fromage frais and other dairy desserts	6	3	2	2	2	7	5	3	2	2
Ice cream	3	4	3	2	2	3	3	3	1	2
Fat spreads	7	9	8	10	14	6	7	7	9	12
of which:										
Butter	3	4	4	6	8	5	6	5	7	10

Values are average percentages. Abbreviations: NDNS, National Diet and Nutrition Survey; SFA, saturated fatty acids; TFA, *trans* fatty acids; yrs, years.

Adapted from Bates et al. (10)

1.4.2 Potential benefits of milk and dairy consumption

Ruminant derived milk and dairy products are regarded as complex foods, containing a number of different components. It remains a matter of debate as to whether nutrients within dairy foods act independently or synergistically in relation to chronic disease risk, leading to the suggestion that the association of dairy SFA with CVD may be dependent on other nutrients/macronutrients present within the dairy matrix (39). Despite the heterogeneity of dairy products in terms of nutrient content and physical structures, the components that have been observed to have an impact on health are the numerous classes of fatty acids, proteins (casein and whey), minerals (magnesium, phosphate and calcium) and the components of the milk fat globule membrane (MFGM) (21). Table **1.3** provides an overview of the average nutritional composition of three dairy products, relevant to the present thesis. Additionally, the bacteria present in fermented dairy products, such as yogurt and cheese, may produce bioactive peptides and short-chain fatty acids (SCFAs) and may lead to improvements in insulin sensitivity and a reduction in inflammation through microbiome and vitamin K related pathways (21). Moreover, mineral bioavailability is enhanced by the lack of inhibitors present in milk, including phytates and oxalates, and by the presence of lactose and certain amino acids that may promote mineral absorption (40). Furthermore, lipids present in dairy products mediate the delivery of essential fat-soluble vitamins (such as vitamins A and D), iodine and specific fatty acid classes, such as conjugated linoleic acids (CLA), may also have cardio-protective properties, although further studies are needed to confirm these associations (41). Therefore, although consumption of dairy products significantly contributes to total SFA intake at a population level, it is important to recognise that other dairy components also play a role in human health and development.

Table 1.3 Average energy content and nutritional composition of milk (by percentage fat content), Cheddar cheese and butter (per 100g).

	Milk (3.5% fat)	Milk (1.7% fat)	Milk (0.3% fat)	Cheddar Cheese	Butter
Energy (MJ)	0.3	0.2	0.1	1.7	3
Total fat (g)	3.5	1.7	0.3	35	81
SFA (g)	2.3	1.1	0.1	22	51
MUFA (g)	1.1	0.4	0.1	9	21
PUFA (g)	0.1	Trace	Trace	0.9	3
trans (g)	0.1	0.1	Trace	1.4	3.3
Total protein (g)	3.3	3.6	3.6	25	1
Lactose (g)	4.7	4.8	4.9	0.3	Trace
Calcium (mg)	116	120	120	659	15
Phosphorus (mg)	93	80	85	510	24
Potassium (mg)	160	161	167	98	25
Vitamin A (as μg retinol equivalent)	31	20	1	20	10

All nutrient values are expressed per 100 g of product and represent average values.

Adapted from Roe et al (42)

1.5 The impact of dairy product consumption on cardiovascular disease

1.5.1 Evidence from prospective studies

The relationship between milk and dairy product intake with different CVD events has been investigated in a number of studies. However, a large body of the evidence points towards either no relationship or an inverse association between consumption of dairy products and CVD risk.

A meta-analysis of prospective cohort studies, which examined the associations between milk and dairy products with health and overall mortality, concluded that the available data gathered from fifteen studies indicated a potentially beneficial effect of milk and dairy product consumption on CVD risk (43). High milk and dairy product intake resulted in a relative risk (RR) of stroke and ischaemic heart disease (IHD) of 0.79 (95% CI 0.75, 0.82) and 0.84 (95% CI 0.76, 0.93) respectively, relative to low consumption of milk and dairy products (43). In a second more extensive meta-analysis, the authors investigated the relationship between different types of dairy products with the incidence of vascular disease (44). A reduction in RR was observed in subjects with the highest dairy product consumption, relative to subjects with lowest dairy intakes: 0.87 (95% CI 0.77, 0.98) for all-cause deaths, 0.79 for IHD (95% CI 0.68, 0.91) and 0.85 for stroke (95% CI 0.68, 0.91) (44). Furthermore, a dose response meta-analysis of seventeen prospective studies resulted in a marginally significant inverse association between milk intake and CVD events, although no significant association was reported with risk of stroke or CHD (45). In support of these observations, a meta-analysis conducted by Qin et al. also reported an inverse association between dairy product consumption and overall risk of CVD (nine studies; RR 0.88; 95% CI 0.81, 0.96) and stroke (twelve studies; RR 0.87, 95% CI 0.77, 0.99) (46). For specific dairy product, only cheese was inversely associated with risk of stroke (RR: 0.91, 95% CI 0.84, 0.98). However,

this meta-analysis also reported no association between dairy product consumption, analysed as low or high fat dairy, with CHD risk (46). Although it may be suggested that the inverse association observed for risk of stroke but not CHD for total dairy and cheese in particular may be in part due to the hypotensive effects of specific nutrients within the dairy matrix, the meta-analysis was limited due to a lack of dose-response analysis and the broad categorisation of the dairy products and outcome variables of the studies included (21). A further meta-analysis investigated the association of dairy fat consumption with risk of CVD, CHD and stroke, using data from three large US cohorts (47). Dairy fat intake resulted in no significant association with risk of CVD, CHD and stroke (for a 5% increase in energy from dairy fat, RR 1.02; 95% CI 0.98, 1.05; RR 1.03; 95% CI 0.98, 1.09; RR 0.99; 95% CI 0.93, 1.05 respectively). On the other hand, a 5% energy substitution of dairy fat with an equivalent energy intake of *n-6* PUFA or vegetable fat led to a 24% (RR 0.76; 95% CI 0.71, 0.81) and 10% (RR 0.90; 95% CI 0.87, 0.93) lower risk of CVD, respectively (47). In contrast, exchanging dairy fat with a 5% energy intake from animal fat was associated with a 6% increase in CVD risk (RR 1.06; 95% CI 1.02, 1.09). It is important to note that the origin of animal fat which was used as an energy replacement was not specified. Additionally, the food in which the dairy fat is contained is not considered, thereby any assessment of the dairy food matrix cannot be determined. Nevertheless, the study adds further evidence to confirm that dairy fat does not appear to be associated with an increased risk of stroke, CHD and CVD. Furthermore, it also highlights the importance of considering which macronutrient replacing SFA (or a high SFA food group) would lead to a substantial reduction in CVD risk. The most recent dose-response meta-analysis included 29 cohort studies and found no associations for total dairy (200 g/day) and milk (12 cohorts; 244 g/day) with all-cause mortality (RR 0.99; 95% CI 0.96, 1.03; RR 1.00, 95% CI, 0.93, 1.07 respectively), CHD (12 cohorts, RR 0.99, 95% CI, 0.96, 1.02; RR 1.01; 95% CI, 0.96, 1.06 respectively) and CVD risk (RR 0.97, 95% CI 0.91, 1.02; RR 1.01; 0.93, 1.10 respectively) (48). However, marginal inverse association

were observed between total fermented dairy (defined as cheese, yoghurt or sour milk; per 20 g/day) with mortality (RR 0.98; 95% CI 0.97, 0.99) and CVD risk (RR 0.98; 95% CI 0.97, 0.99) (48). More detailed analyses of individual fermented dairy products (cheese and yoghurt) led to a 2% lower risk of CVD for cheese (RR 0.98, 95% CI, 0.95, 1.00 per 10 g/day) but not yoghurt (48). Although the authors indicate that there was a high degree of heterogeneity between the included studies of the meta-analysis, their observations add evidence to further examine the impact of specific dairy products on risk markers of CVD in well-designed RCTs.

1.5.2 Cardiometabolic risk factors

Dietary guidelines which focus on reducing CVD risk have traditionally aimed to limit the intake of foods and nutrients which raise fasting LDL-C concentrations, an important CVD risk factor (9). The Friedewald equation, first developed in 1972 (49), provides an adequate estimate of fasting LDL-C without the use of laborious and expensive ultracentrifugation techniques and is routinely used in population studies and RCTs. Additionally, it was demonstrated that the concentration of VLDL-C could be estimated by a ratio of TAG/2.2 (in mmol/l) (49) (REF), resulting in the following equation of LDL-C:

LDL-C (mmol/L) = total cholesterol – HDL-C – (TAG/2.2)

It is worth noting that the equation assumes a constant ratio of TAG to cholesterol in VLDL particle, which may not always be the case for individuals with certain conditions such as dyslipidaemia and insulin resistance (50). Additionally, Friedewald's equation for serum LDL-C represents the cholesterol present in all LDL particles, regardless of density and particle size. Measurement of LDL particle (LDL-P) number and distribution has been suggested as a more sensitive indicator than LDL-C when assessing CVD risk (50). It has been proposed that sdLDL-P are particularly atherogenic due to a number of reasons, including an increased susceptibility to oxidation, high sub-endothelial permeability,

decreased LDL receptor affinity and an increased interaction with food matrix components (51, 52). However, estimation of LDL-C using Friedewald's equation remains an important biomarker and is more cost-effective and quick to assess in intervention trials than LDL-P size and distribution by nuclear magnetic resonance (NMR) or density gradient ultracentrifugation (53).

As the it has been estimated that every 1% reduction in LDL-C translates to a 1-2% reduction in population CVD risk (9), this risk marker remains one of the most widely used to assess the impact of dietary fat replacement in intervention studies. Evidence from animal, human and *in vitro* studies suggest that SFA intake leads to inhibition of LDL-C receptor activity, enhancement of apoB production and therefore an elevation in TC, LDL-C and LDL-C:HDL-C ratio which are all risk markers for CVD. On the other hand, MUFA intake may lead to a beneficial outcome in CVD risk via a number of suggested mechanisms. These include an alteration of the lipid/lipoprotein profile, for example inactivation of sterol regulatory element binding protein (SREBP), a transcriptor factor which regulates cholesterol synthesis, and increasing the expression of hepatic LDL receptor via stimulation of acyl-CoA cholesterol acyltransferase in the liver (54). Additionally, it has also been suggested that MUFA lead to a greater diet-induced thermogenesis and greater fat oxidation rates in comparison to SFA intake (55). Evidence suggests that total PUFA consumption leads to beneficial effect on TC:HDL-C, mainly by modulating a reduction in LDL-C concentrations and the apoB pool size (26). Potential mechanisms from animal studies include a 2 fold increased LDL fractional catabolic rate when compared to SFA intake (56)

In the case of dairy foods, low fat dairy products have been advocated as the preferred choice for consumers to limit high consumption of whole fat and total dairy products. At the same time, individual SFAs present in foods differentially affect blood lipids: elevated serum concentration of LDL-C are associated with intakes of lauric (12:0), myristic

(14:0) and palmitic (16:0) acids, whereas limited effects are observed with intake of stearic acid (18:0), which is poorly absorbed (9). A number of studies have shown that SFA intake from dairy foods may lead to increases in larger, rather than smaller, LDL particle size (57-59), although the significance of this pattern and its relationship with CVD risk remains to be determined.

A small number of RCTs have compared low- and high-fat dairy foods on LDL-C concentrations. Consumption of a high-fat dairy and Dietary Approaches to Stop Hypertension (DASH)-type diet did not lead to significant increases in LDL-C concentrations compared with the same diet with low-fat dairy in both men and women in a crossover RCT (60). On the other hand, the food matrix of dairy products may exert an effect on the change observed in LDL-C rather than dairy fat per se. A meta-analysis conducted by Goede *et al.* compared the effect of cheese and butter consumption on blood lipids in five RCTs. Serum LDL-C concentrations were significantly lower following intake of cheese, compared to butter consumption as observed from pooled data of four of the five RCTs (ΔLDL-C: 0.22 mmol/L; 95% CI 0.29, 0.14 mmol/L) (61). Ultimately, although it appears that certain types of dairy products such as milk, cheese or yoghurt appear to have a relatively neutral effect on LDL-C, more robust evidence is needed to assess differences not only in specific dairy foods but also dairy fat content on LDL particle size and number.

SFA intake also increases HDL-C concentrations, leading to the suggestion that some SFAs have a neutral effect on the total cholesterol:HDL-C ratio and consequently on CVD risk (9, 62). Despite extensive evidence showing that low HDL-C concentrations are associated with a greater risk of CVD (63), there is still disparity on this association as serum HDL-C concentrations alone may not reflect the functionality and capacity of HDL to reduce risk (63). Drouin-Chartier *et al.* reviewed the limited studies which have assessed the effect of dairy intake on circulating HDL concentrations, concluding that the evidence shows a

neutral effect although recognising that a knowledge gap remains as studies which assess the impact of HDL functionality are still needed (64).

Hypertension

Hypertension is also a key risk factor in the progression of CVD development, affecting up to 30% of adults in the UK (4). Defined as systolic blood pressure (SBP) ≥140 mm Hg and/or diastolic blood pressure (DBP) ≥90 mm Hg, it is influenced by a number of variables such as gene polymorphisms, diet, environmental determinants and interactions between these factors (65). It is recognized as a major risk factor for stroke, CHD and heart failure, among other conditions (65). The most common method for the measurement of blood pressure (BP) to assess heamodynamic stability, is with the use of a sphygmomanometer (66). However, this measurement has potential limitations in reproducibility partly due to the 'white-coat effect' (67). A further, more reliable assessment is measurement of ambulatory BP over the course of 24h, considered a predictive measurement for CVD events, mortality and morbidity associated with hypertension (67). As previously mentioned, milk and dairy products are a source of micronutrients (such as calcium, potassium, iodine) and protein (casein, whey and specific bioactive peptides), which may be associated with beneficial hypotensive effects in an independent or synergistic manner (68). A number of mechanisms by which dairy components may reduce BP have been proposed (69). The bioactive peptides present in whey and casein proteins have been observed to play a role in controlling BP by inhibiting the action of angiotensin-I-converting enzyme, resulting in vasodilation (70), by modulating the release of endothelin-1 by endothelial cells (71) and acting as opioid receptor ligands increasing nitric oxide products, which mediates arterial tone (68). Several studies have looked at this relationship, with the DASH study considered as one of the first which observed a beneficial association between dairy consumption and a reduction in BP (72). However, conclusions cannot be easily drawn from this study alone due to differences in

macronutrient composition of the diets in the original DASH trial (73). In a RCT which compared a DASH diet with high-fat dairy to one with low-fat dairy, Chiu et al. reported no significant effect on BP (60). Furthermore, a meta-analysis of 7 RCTs also concluded that there was no significant BP lowering effect of total dairy intake on both systolic and diastolic BP (74). In contrast, a meta-analysis of 7 cohort studies which assessed the dose-response association between milk intake and risk of hypertension, concluded that each increment of 200 g/d of milk consumed was associated with a significant 4% reduction in the risk of hypertension (RR 0.96; CI 0.94, 0.98) (75). An important consideration is the potential impact of a threshold dependency mechanism, whereby benefit is conferred in those at low nutrient status (such as calcium), whereas in individuals with adequate baseline status, little effect is observed (76). Furthermore, as outlined in Drouin-Chartier *et al.*, short-term RCTs suggest no significant effect on BP irrespective of type and fat content (with the exclusion of butter) while evidence from prospective cohort studies on total dairy, low-fat dairy and milk intake suggest an association towards a reduction in risk of hypertension (77). A suggested explanation for this discrepancy is that long term dairy intake may attenuate the increase in BP seen as a result of aging and weight gain, thereby reducing the risk of hypertension (77). This, and other suggested mechanisms, need to be further investigated.

Vascular function and elasticity

Dietary patterns may impact the elasticity and function of blood vessels (78). The endothelium, defined as the inner layer of cells of the vascular wall, is a key regulator in vascular homeostasis (79). As previously mentioned, NO is the main endothelium-derived vasodilator, produced from its precursor L-arginine via the enzymatic action of endothelial NO synthase (eNOS) (80). Disturbances in endothelial function, such as reduced vasodilation and activation of inflammatory pathways, progressively leads to the development of atherosclerosis (79, 81). Assessment of vascular dysfunction, which includes both endothelial

dysfunction and arterial stiffness, can be assessed in a number of ways (79). Flow-mediated dilatation (FMD), which measures the vasodilatory response of the brachial artery to an increase in blood flow-associated shear stress and carotid intima-media thickness, can be used to non-invasively assess the endothelial function and arterial structural changes, respectively (82). Is it considered as the 'gold standard' method to assess endothelial function, with some studies regarding FMD response a superior predictive assessment to traditional risk factors, including elevated cholesterol concentrations, in individuals with established CVD (83). Of note however is the extensive training required to properly conduct this measurement and assess response, which may be viewed as a limitation. A surrogate measurement, which is not operator dependent like FMD, is the assessment of the carotid intima thickness (cIMT), defined as the distance between the media-adventitia interface to the intima-lumen interface on the right and left common carotid artery (CCA) (84). It is considered an early marker of atherosclerosis (85). However, due to methodological variations and a limited number of short term intervention studies, it remains unclear whether cIMT progression can be considered a predictor of future vascular events (84, 85). Furthermore, change in the IMT of the carotid artery progresses over time and few studies have observed differences as a response to diet alone. The intake of total dietary SFA and TFA have been observed to be positively associated with cIMT. In particular, it has been shown that a 10 g/day increase in total SFA intake and a 1 g/day TFA intake resulted in a 0.03 mm greater cIMT value following multivariate adjustments (85, 86). To date few studies have investigated the effect of dairy product consumption in relation to changes in cIMT. A cross-sectional study examined high (> 100 g/day) and low (< 100 g/day) yoghurt consumption in elderly women (> 70 years) (87). It was observed that high consumption correlated with a 0.023 mm lower cIMT, compared to low consumption following baseline, dietary and lifetime adjustments ($p < 0.003$; (87)). However, the same study concluded that total intake of dairy products, milk and cheese was not associated with change in cIMT (87).

Cardiovascular events and all-cause mortality are also independently predicted by carotid-femoral pulse wave velocity (PWV), regarded as the gold standard measurement to assess arterial distensibility and stiffness by applanation tonometry (88, 89). The use of this methods is relatively simple and non-invasive, and its principle is that the higher velocity of pulse waves measured between two sites translates to stiffer arteries (90). Additionally, PWV may also be used as a measure of endothelial function (91), due to the fact that arterial compliance is in part determined by endothelium-dependent vasodilation (92). Moreover, the augmentation index (AIx), an indirect measure of arterial stiffness, represents the ratio of the magnitude of the reflected pulse wave to the initial wave (93). Evidence from the *Caerphilly Prospective Study* highlighted that, with the exception of butter intake, dairy product consumption does not impact negatively on PWV (94). Furthermore, the AIx was 1.8% lower in men with the highest quartiles of dairy product intake (94). Similarly, findings from a cross-sectional study illustrated that dairy food intake was inversely correlated with PWV (95). However, cross-sectional studies present predictive limitations, whereby exposure and outcome are assessed simultaneously thereby negating a temporal relationship between the two (96).

Inflammation

A low-grade chronic inflammation and oxidative stress is recognised as a major factor contributing to the initial stages of atherosclerosis and disease progression (97, 98). The recruitment of leucocytes into the sub-endothelial arterial wall initiates a cascade of reactions, primarily mediated by inflammatory mediators (98). Specifically, the increased presence of chemokines and cytokines progressively leads towards atherosclerosis via four stages: a) increased development of chemokine production followed by expression of adhesion molecules (VCAM-1, ICAM-1) on the endothelium which stimulates further leucocyte recruitment; b) as a consequence, monocyte entry and differentiation into macrophages,

promoting ‘foam cell’ formation beneath a progressively dysfunctional endothelial lining; c) initiation of smooth muscle cell proliferation and 4) plaque instability and eventual rupture (12, 98). Of interest to the present thesis are inflammatory cytokines, which lead to the generation of endothelial adhesion molecules (such as P selectin and VCAM-1), which in turn may enter the circulation in soluble form (99). Additionally, from the initial stages of a pro-inflammatory state, cytokines such as IL-6, IL-8 and TNFα may mediate the increased production of acute-phase reactants, such as C-reactive protein (99). Conversely, a healthy endothelium is characterized by a balanced concentration of vasoactive mediators, such as the potent vasodilator NO which, in response to disturbed blood flow within arteries and increased concentration of adhesion molecules, results in a suppressed of NO synthesis (97). It is well known that inflammatory processes are affected by dietary fatty acids, impacting on membrane and lipid raft composition and function (98). In the case of dietary SFA, there is some evidence for a pro-inflammatory effect through increases of fibrinogen and TNF-alpha, although most of the evidence is derived primarily from cross-sectional, animal and *in vitro* studies, with limited RCTs comparing SFA replacement and inflammation (98). Several studies have investigated the effect of n-3 PUFA on inflammation. Overall, it appears that the anti-inflammatory properties of n-3 PUFA may in part be explained by their conversion to biologically active signaling molecules, such as eicosanoids which in turn may lead to suppression of inflammation (100). The evidence for cis-MUFA is less clear, although proposed mechanisms for a favourable effect on inflammation include a beneficial modulation on adhesion molecule production (100).

Evidence from a review on dairy consumption and inflammatory biomarkers did not conclude an adverse association in both overweight and obese individuals, although the authors highlighted that no definite conclusions could be drawn due to the heterogeneity of the studies (101). In a meta-analysis of six RCTs by Benatar *et al.*, high dairy consumption was shown not to significantly impact on plasma CRP concentrations, compared with low

dairy consumption (74). Although there is some evidence supporting a beneficial association between dairy consumption and inflammation, the mechanisms involved to support a beneficial association between dairy intake and inflammation remain unclear and the limited studies available are either underpowered or compare different types of dairy products, making it difficult to draw definite conclusions (101).

In order to monitor changes in inflammatory biomarkers following a dietary intervention, a few points need to be considered. Firstly, it is worth noting that in most cases a number of inflammatory biomarkers are measured in RCTs as there is no consensus as to which in particular realistically represent change in low-grade inflammation (102) in an acute or chronic setting (98, 103). One of the main concerns when interpreting observed data from intervention studies is that inflammatory biomarkers by themselves do not represent metabolic low-grade inflammation (98). Furthermore, a number of modifying factors (age, gender, genetics among others) affect the concentration of inflammatory biomarkers at any given time, meaning that in both healthy and at risk individuals there will be a wide variability in measurements (98). At the same time, measuring changes in the concentration of inflammatory biomarkers in response to a dietary challenge still provides useful information in relation to the study in question.

Insulin resistance

The increasing prevalence of type 2 diabetes (T2D) has also been a focus of tailoring dietary recommendations for at risk populations. Within the human body, insulin has a wide range of biological functions (104). It functions not only as a key regulator of metabolic energy storage and disposal in tissues, but also as an integral factor in the haemodynamic activities associated with increased blood flow and cardiac output, probably through increased NO production. In contrast, insulin resistance plays a key role in mediating cardio-metabolic disorders (105). Measurement of both glucose, insulin and non-esterified fatty acid (NEFA)

concentrations allows indices of insulin resistance, such as the homeostatic model assessment of insulin resistance (HOMA-IR) and the revised quantitative insulin sensitivity check index (rQUICKI) to be derived (106). Epidemiological evidence on dairy consumption and insulin resistance appears to indicate a potentially favourable effect (36). However, there is a need for suitably powered intervention studies to assess the impact of both dairy fat and specific dairy products on insulin sensitivity (64).

Postprandial lipaemia

An aspect which has not been extensively investigated in the context of dairy product intake, is the impact on the postprandial (fed) state, where raised concentrations of TAG are considered an independent CVD risk factor (107). Standardized methodological protocols, using one or two test meal challenges, are used to investigate the impact on postprandial lipid, inflammatory and vascular biomarkers following the consumption of FA from different sources (108).

It is well established that the amount and type of dietary fat influences the postprandial lipaemic response (108). Following meal consumption, the duration and magnitude of postprandial TAG response is in turn influenced by metabolic factors including: the rate of TAG secretion from both the intestine and liver; enzymatic activity related to the processing of TAG-rich lipoproteins (TRLs; chiefly lipoprotein lipase and hepatic lipase); and the TRL remnant clearance rate by receptor mediated processes (107). Additionally, as previously mentioned for low-grade inflammation, inter-individual variability affects postprandial TAG response to dietary intervention. As detailed in Jackson et al (107) several mechanisms have been proposed to explain the effect of TRLs on the vascular wall and how these related to CVD risk. Of interest is the impact of a delayed clearance of TRLs from the circulation on HDL and LDL metabolism, inflammation and vascular function. In particular, the delayed clearance of TRLs from the circulation leads to a remodelling of the lipid contents of LDL

and HDL, turning them into substrates for lipase action and leading to the formation of sdLDL and sdHDL particles, a process in neutral lipid exchange (107). The latter are quickly removed from blood circulation, resulting in decreased HDL-C concentrations (107). This results in sdLDL, which have a lower binding affinity to the LDL-C receptor and remain present within the circulation for an extended period of time, increasing their infiltration rate into the arterial wall (107). It has been observed that elevated serum TAG (> 1.5 mmol/l) is associated with the remodelling of LDL into sdLDL while in contrast, a reduction in concentration (< 1.5 mmol/l) redistributes LDL subclasses into larger particles (53).

Taken together, the current existing evidence suggests that milk and certain dairy products have a neutral impact on lipid-related cardiometabolic risk factors, low-grade systemic inflammation and vascular function. There is consistent evidence that milk intake in particular appears to be associated with a lower risk of hypertension, although this may also be due to other factors such as dietary patterns. It therefore remains challenging to determine whether intake of specific dairy products may lead to a beneficial impact on outcome measures associated with CVD risk factors. Further long-term, well designed studies are needed to fully explore both dairy fat content and product type in relation to defined primary CVD risk outcomes and to determine the mechanisms involved.

Genetic factors and CVD risk

As has been mentioned, responsiveness to dietary fat replacement measured via defined risk markers of CVD presents a degree of inter- and intra-individual heterogeneity. It is therefore also of interest to examine non-modifiable risk factors, such as gene polymorphisms on the response to specific dietary interventions. A number of gene variants are associated with increased CVD risk and have been previously described (109, 110). Of interest to the present thesis is the *APOE* genotype, as it is a well-recognised regulator of lipoprotein metabolism and accounts for up to 7% variation in total and LDL cholesterol

(111). The polymorphic protein, which result in 6 *APOE* genotypes derived from three common alleles (ε2, ε3, ε4) (112). The ε4 allele is of interest as individuals carrying this allele present increased concentrations of total cholesterol, LDL-C, TAG and reduced HDL-C concentrations compared to the ε3 allele (111-115). Furthermore, as reported in a meta-analysis, these increased biomarkers of CVD risk translated into a moderate association between ε4 and CAD mortality (OR 1.06, 95% CI 0.99 – 1.12) (112). Results from dietary interventions suggest that *E4* carriers appear more sensitive to changes in total fat, SFA, dietary cholesterol and long chain n-3 PUFA intake compared to E3 carriers (116-119). However, RCTs which have investigated dietary FA manipulation and *APOE* genotype have primarily focused on changes in fasted lipid concentrations with a small number of RCTs investigating the postprandial state(116, 119, 120). Thus, there is a need for future studies to examine the impact of *APOE* genotype following FA manipulation in the postprandial state and with both traditional and novel biomarkers of CVD risk.

1.6 Altering the bovine diet to manipulate the fatty acid profile of milk

Low-fat dairy consumption has been promoted as a healthier consumer choice, aimed at reducing total SFA intake from all food sources. Altering the fatty acid profile of milk offers an alternative strategy to lowering SFA intake from dairy products, while at the same time preserving the beneficial contributions to the human diet. Supplementation of the bovine feed is a strategy that has been widely explored, through inclusion of oilseeds or plant oils in forages, leading to partial replacement of SFA with *cis*-MUFA and, to a lesser extent, *cis*-PUFA (121). Considering that the rumen is the primary site for microbial fermentation and fat metabolism, microbes transform dietary FAs via two main processes, lipolysis and biohydrogenation (122). In the case of biohydrogenation, unsaturated FA are isomerised and hydrogenated, with bacterial enzymes as catalysts, to produce a number of conjugated linoleic

acid (CLA) isomers and *trans*-MUFA, which collectively denote ruminant TFA (rTFA) (123). The fat in milk and dairy products of conventional dairy products typically contain 2-6% TFA (124, 125). Additionally, the predominant CLA isomers in milk and dairy products are *cis*-9, *trans*-11, followed by *trans*-10, *cis*-12-18:2 (126).

There is strong evidence from both observational and controlled feeding studies that substitution of SFA or UFA for TFA detrimentally impact on CVD risk markers. The adverse effects of TFA are well established as their consumption raises LDL-C while reduce HDL-C and thereby raise the TC:HDL-C ratio (100). Additionally, TFA intake detrimentally affect endothelial function, promote low-grade inflammation and reduce the particle size of LDL-C and the concentrations of lipoprotein(a) (100, 127). However, the majority of studies have focused on the impact of iTFA (18:1*t*9, trans elaidic, being the most abundant in the diet) as these were commonly consumed in partially hydrogenated vegetable oils. However, the association between TFA and CVD remains inconclusive, with some studies showing a cardio-protective association of rTFA (128, 129) while others an overall detrimental effect regardless of TFA source (130). Considering that the latter was published in 2001 a more recent a systematic review and meta-analysis was undertaken by Bendsen *et al.* (2011) in an attempt to resolve conflicting reports (131). They reported that the RR for high vs. low quintiles of total TFA intake (10 g to approximately 2.8 g/day) was 1.22 (95% CI: 1.08, 1.38) for CHD events and 1.24 (95% CI: 1.07, 1.43) for fatal CHD. rTFA intake (0.5-1.9 g/day) was not significantly associated with CHD risk (RR 0.92; 95% CI 0.76, 1.11) although neither was iTFA. There was however, a trend towards a positive association (RR 1.21; 95% CI 0.97, 1.50, $P = 0.09$). The authors concluded that while iTFA is be positively related to CHD, rTFA is not, although the limited number of studies available prevented any firm conclusions concerning whether the source of TFA is important (131). Gebauer *et al* (132) compared the effects of the two predominant stereoisomers of rTFA (vaccenic acid, VA; 18:1*t*11) and iTFA (elaidic acid, 18:1*t*9) respectively, in addition to the most predominant CLA, c9, t11. The

diets of this RCT were designed to replace stearic acid with 0.1% of mixed isomers of TFA (control), ~3% VA, ~3% elaidic acid (iTFA diet) or 1% *c*9, *t*11 of total energy respectively consumed in a cross-over design over 24 days by a total of 106 healthy adults (132). The authors observed higher concentrations of TC, LDL-C, TAG, lipoprotein(a) and apoB following the VA diet and compared to the iTFA diet. Interestingly, HDL-C and apoAI concentrations were higher following the VA diet. However, both the VA and iTFA diets led to increases in TC, LDL-C, TC:HDL-C and apoB (2 – 6% change, $P < 0.05$) compared to the control diet. On the other hand, the CLA diet lowered fasted TAG ($P < 0.01$) and did not impact on the other lipoprotein risk markers (132). It is worth noting that VA may be the predominant rTFA, but is also present in partially hydrogenated vegetable oils, ranging from 15% to 24% of total trans-18:1 isomers (132, 133). Further research is therefore needed to draw firm conclusion on the effect of VA on lipoprotein risk markers, by investigating and comparing their effect from different food sources.

The current dietary intake of TFA in the UK (0.5-0.6% of food energy in adults; DH 2016) is still below the recommended population maximum (2% of food energy intake (DH 1991), with milk and milk products contributing to around 25% of this intake (DH 2016). Consequently, rTFA intake is not seen as a major cause of concern to CVD health at a population level (127). However, it has yet to be determined whether increasing rTFA intake, through manipulation of the fatty acid profile of milk and dairy products to reduce SFA content, impacts on CVD health (134).

1.6.1 Impact of fatty acid modified dairy products on cardiovascular disease risk factors

A limited number of studies have investigated the impact of modified feed-reduced SFA milk and milk products in healthy and hypercholesterolaemic populations when compared to commercially available whole milk dairy products (134). However, the studies performed to date have relied on changes in circulating blood lipid levels as predictors of

CVD risk and on butter as the main test food (134). Some selected data illustrate that, in comparison to conventional milk, cheese, butter and ice cream (70 g/100 g SFA, 28 g/100g *cis*-MUFA), TC and LDL-C were significantly lowered (-0.28 mmol/L, $P < 0.001$; -0.24 mmol/L, $P < 0.001$ respectively) following a 3 week period of consuming matched fat-modified dairy products (51 g/100 g SFA, 39 g/100g cis-MUFA) (135). It is important to note, that in this particular study the resulting changes in fasting TC and LDL-C between the two diets did not consider in their statistical analysis the baseline lipid concentrations of the participants.

Additionally, studies have also looked at dairy products enriched with *cis*-9, *trans*-11 CLA (*c*-9, *t*11 CLA) and vaccenic acid (*trans*-11 18:1; *t*VA), and assessed their impact on the blood lipid profile (136, 137). CLAs are of interest due to the potentially beneficial effect on lipid risk markers. However, data from RCTs following supplementation with CLAs have been inconsistent, which may be explained by methodological variations pertaining to different mixtures of CLA isomers and doses (136). One cross-over study investigated the impact of a 6-week intake of modified dairy products (UHT cheese, milk and butter) naturally enriched with *cis*-9, *trans*-11 CLA and vaccenic acid in healthy men (136, 137). The modified dairy products provided 1.42 *c*-9, *t*-11 CLA g/d and 4.71 *t*VA g/d compared to control products which provided 0.17 *c*-9, *t*-11 CLA g/d and 0.31 *t*VA g/d (136, 137). The c9, t11 CLA and tVA content was increased in both plasma phosphatidylcholine, TAG, cholesteryl esters and peripheral mononuclear cells (136, 137). However, there were no significant changes in CVD risk markers as measured in lipid and inflammatory biomarkers, insulin resistance, LDL-P or the susceptibility of LDL to oxidation (137).

The methodology of these studies differed considerably when accounting for the type of supplement in the bovine diet, the number of subjects and study design, making it difficult to directly compare data and drawing firm conclusions (134). This makes it challenging to

evaluate well-powered estimations of CVD risk based on the available data from these dairy fat manipulation studies. It is also clear that in dietary intervention studies, the use of a single risk marker to assess potential benefits following fat manipulation may not be sufficient. Yet there is considerable evidence to suggest that partial replacement of SFA in the diet with an appropriate UFA will beneficially impact on CVD risk. The manipulation of dairy FA is most successful when MUFA replaces SFA in dairy fat. Considering the beneficial or neutral effect of *cis*-MUFA within the diet on markers ofCVD risk, more evidence from RCTs is needed to fully understand the impact of FA-modified dairy products on both traditional and novel CVD risk markers. This knowledge gap will be addressed in the study presented in the present thesis, which aims to investigate dairy fat manipulation with the use of a wider range of CVD risk markers. The results of this investigation may add further knowledge both on recommendations for dietary SFA replacement with unsaturated dietary fats and the potential beneficial effects of including FA-modified dairy products as the food vehicle to partially replace SFA with *cis*-MUFA in the diet.

1.7 Aims and objectives of the thesis

The overarching hypothesis, aim and chapter specific objectives of this thesis are stated below, with each chapter-specific question, hypothesis and objective pertinent to this thesis summarised at the beginning of each chapter. Additionally, my input to the data collection, analysis, statistics and writing will be detailed at the beginning of each chapter.

Hypothesis: Consumption of SFA-reduced, MUFA-enriched dairy products will beneficially impact markers of CVD risk in adults at moderate risk, compared to iso-energetic control dairy products with a FA profile typical of retail products. Additionally, in a subgroup of the study population retrospectively analysed for *APOE* SNP, the FA-modified dairy products

will beneficially impact on fasted and postprandial CVD risk markers in E4 carriers compared to *E3/E3* participants.

Aim: To determine the chronic and acute-within-chronic intake of SFA-reduced, MUFA-enriched dairy products on established and novel CVD risk markers in an adult population with moderate CVD risk and compared to conventional retail dairy products. Moreover, to explore the potential impact of APOE genotype polymorphisms on outcome measures in both the fasted and postprandial setting.

Objective 1: Assess the evidence to date of the effect of milk and dairy products on cardiometabolic health, with a particular focus on dairy fat composition (Chapter 1).

Objective 2: Describe the implementation and compliance to the food-exchange model with data derived from analysis of 4 day weighed food diaries and phospholipid fatty acid analysis (Chapter 2).

Objective 3: Determine in a randomized, double-blind controlled cross-over study the chronic impact of SFA-reduced, MUFA-enriched milk, cheese and butter on markers of cardiovascular risk in adults at moderate CVD risk (Chapter 3).

Objective 4: Evaluate the impact of chronic consumption of SFA-reduced, MUFA-enriched dairy products on postprandial lipaemia, insulin resistance and inflammatory markers relative to conventional dairy products in a sub-cohort of the main chronic study (Chapter 4).

Objective 5: Assess the impact of the *APOE* genotype on the responsiveness of the fasting and postprandial lipid responses to chronic dairy fat composition (Chapter 5).

Chapter II: Plasma Phospholipid Fatty Acid Profile Confirms Compliance to a Novel Saturated Fat-Reduced, Monounsaturated Fat-enriched Dairy Product Intervention in Adults at Moderate Cardiovascular Risk: a randomized controlled trial

Oonagh Markey, Dafni Vasilopoulou, Kirsty E. Kliem, Albert Koulman, Colette C. Fagan, Keith Summerhill, Laura Y. Wang, Alistair S. Grandison, David J. Humphries, Susan Todd, Kim G. Jackson, David I. Givens, and Julie A. Lovegrove

Published in Nutrition Journal; 2017; **16**:33

JAL, DIG and KGJ: designed the human study; OM, JAL and KGJ: designed the food-exchange model; CCF, ASG, DJH and DIG: designed and produced the modified dairy products; OM and DV: conducted the research (hands-on conduct of the experiment and data collection); OM, DV, KEK and AK: analyzed data and performed statistical analysis; KEK, KS and LW: performed fatty acid analysis; ST: provided statistical advice; OM and DV: wrote the manuscript, which was modified by all co-authors; and JAL had primary responsibility for final content. All authors have read and approved the final manuscript.

As this is the first chapter which will begin to describe the RESET intervention trial (Chapter II-VI) the following applies for all chapters:

Chapter input: I had primary responsibility of entering participants' diet diary data in DietPlan6 for analysis and subsequent extraction and statistical analysis and contributed to writing pertinent sections.

Throughout the intervention trial I shared responsibilities with Dr Oonagh Markey for the following: contacting, recruiting and screening potential participants who I then followed up during the timeframe of the trial; during study days I conducted vascular measurements, collected and processed blood samples, carried out the whole blood culture experiment,

supervised research students and assistants who assisted with the project in the laboratory. At the end of the human intervention trial I carried out most of the biochemical measures (ILAB with Dr O. Markey), Luminex assays, nitric oxide HPLC, analysis of cIMT.

Title: Plasma Phospholipid Fatty Acid Profile Confirms Compliance to a Novel Saturated Fat-Reduced, Monounsaturated Fat-enriched Dairy Product Intervention in Adults at Moderate Cardiovascular Risk: a randomized controlled trial

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Running head: Food-exchange model, saturated fatty acids

2.1 Abstract

Background: Dairy products are a major contributor to dietary SFA. Partial replacement of milk SFA with unsaturated fatty acids (FAs) is possible through oleic-acid rich supplementation of the dairy cow diet. To assess adherence to the intervention of SFA-reduced, MUFA-enriched dairy product consumption in the RESET (**RE**placement of **S**aturat**E**d fat in dairy on **T**otal cholesterol) study using 4-d weighed dietary records, in addition to plasma phospholipid FA (PL-FA) status.

Methods: In a randomised, controlled, crossover design, free-living UK participants identified as moderate risk for CVD (n = 54) were required to replace habitually consumed dairy foods (milk, cheese and butter), with study products with a FA profile typical of retail products (control) or SFA-reduced, MUFA-enriched profile (modified), for two 12-week periods, separated by an 8-week washout period. A flexible food-exchange model was used to implement each isoenergetic high-fat, high-dairy diet (38% of total energy intake (%TE) total fat): control (dietary target: 19%TE SFA; 11%TE MUFA) and modified (16%TE SFA; 14%TE MUFA).

Results: Following the modified diet, there was a smaller increase in SFA (17.2%TE vs. 19.1%TE; $p < 0.001$) and greater increase in MUFA intake (15.4%TE vs. 11.8%TE; $p < 0.0001$) when compared with the control. PL-FA analysis revealed lower total SFAs ($p = 0.006$), higher total *cis*-MUFAs and *trans*-MUFAs (both $p < 0.0001$) following the modified diet.

Conclusion: The food-exchange model was successfully used to achieve RESET dietary targets by partial replacement of SFAs with MUFAs in dairy products, a finding reflected in the PL-FA profile and indicative of objective dietary compliance.

Trial registration: ClinicalTrials.gov Identifier: NCT02089035, date 05-01-2014.

Key words: Cardiovascular disease, Dairy products, Dietary fat composition, Food-exchange model, Fatty acids, Monounsaturated fatty acids, Nutrition assessment, Phospholipids, Saturated fatty acids

2.2 Background

Cardiovascular diseases (CVD) are one of the leading causes of mortality in the UK (138). As a result of the clear link between a high intake of SFAs and elevated LDL-cholesterol concentrations, dietary guidelines for CVD prevention advocate reducing SFA intake to ≤10% of total energy (%TE) (9, 139). Despite recommendations, the UK adult population still exceeds the target for dietary SFA intake, with a mean intake of 12.1%TE (140).

Dairy products are major sources of dietary SFA and account for up to 35% of total UK SFA intake (140) and therefore reducing consumption of regular-fat dairy foods or replacing them with lower fat or fat-free alternatives is often advised (141, 142). However, prospective studies have not presented consistent evidence for an adverse link between higher consumption of milk and dairy products and increased risk of CVD, regardless of milk fat content (44, 143-145). Furthermore, this rationalisation does not acknowledge the complex nature of the dairy food matrix, which may be fundamental to cardiovascular health (146). Dairy fat contains a complex mixture of fatty acids (FA) including SFAs, MUFAs, PUFAs, *trans*-fatty acids (TFAs) and branched-chain FAs (147, 148). Furthermore, milk is rich in micronutrients and bioactive peptides, which have been reported to exert cardio-protective effects (149, 150).

While the findings of some recent epidemiological studies have challenged the traditional link between SFA and coronary heart disease risk, and mortality (20, 151, 152), it is important to consider the macronutrient that replaces energy from dietary SFA (20, 153). There is evidence from a randomized controlled trial (RCT) that replacement of SFA with PUFA had minimal effects on CHD mortality (29). However a more recent systematic review and meta-analysis of RCT suggested that lowering SFA intake could reduce cardiovascular events by 17%, with SFA replacement with PUFA estimated to reduce these events by 27% (154). Despite these apparent discrepancies, current US and EU public health dietary guidelines recommend reduction of SFA and replacement with PUFA and/or MUFA respectively (155, 156). In support of the latter review and dietary recommendations, a RCT illustrated that replacing SFA

with *n*-6 PUFA for 16-weeks induced a reduction in serum concentration of LDL-cholesterol (a major risk factor for CVD) by 13.6%, with a comparable reduction of 11.3% on replacement of SFA with MUFA (153).

It is well documented that partial replacement of milk SFA with unsaturated FAs, predominantly in the form of MUFA, through supplementation of the dairy cow diet with plant oils or oil seeds is feasible (157-159). At a population level, this initiative could provide a sustainable means of reducing SFA in dairy products, whilst limiting the entry of SFA into the wider food chain. While limited evidence from human studies suggests that consumption of dairy products with a modified FA profile may beneficially impact on CVD risk, there has been a heavy reliance on fasting lipid biomarkers as sole predictors of risk (160). Further research is needed to elucidate whether FA-modified dairy product consumption has a differential effect on more novel risk markers, including endothelial function, arterial stiffness, systemic inflammation and ambulatory blood pressure, when compared with dairy products of typical milk FA composition (150, 160).

The REplacement of SaturatEd fat in dairy on Total cholesterol (RESET) study was conducted to investigate the chronic and acute effects of two iso-energetic high-fat, high-dairy diets, which varied in FA composition, on traditional and novel cardiometabolic risk markers in free-living individuals. It is important to evaluate strategies employed for the achievement of dietary targets in controlled human intervention studies (161). The purpose of the current paper is to describe the RESET dietary exchange strategy that was developed to enable manipulation of the FA profile of the diet over two 12-week periods, through the use of SFA-reduced, MUFA-enriched (modified) dairy products and matched control dairy foods with a FA profile typical of retail products for the chronic study. Although it is recognised that specific plasma FA levels can be indicative of dietary FA consumption, the composition of the plasma phospholipid FA (PL-FA) fraction is believed to be a good biomarker of FA intake over recent days to weeks and an objective indicator of dietary compliance (162-164). Thus, this paper aims

to report the chronic dietary intervention food-exchange model and the compliance to the FA-modified and control dietary exchange periods using 4-d weighed dietary records, self-reported daily tick-sheets and PL-FA.

2.3 Materials and methods

2.3.1 Study participants and design

The RESET study was a double-blinded, randomised, controlled, crossover designed trial registered at Clinicaltrials.Gov as: NCT02089035. The study was given a favourable ethical opinion for conduct by the University of Reading's Research Ethics Committee (13/43) and was conducted according to the guidelines of the Declaration of Helsinki. All participants provided written informed consent prior to study entry.

Men and women aged 25 – 70 years were recruited from the Berkshire area of the UK in three cohorts between February 2014 and April 2016. A modified Framingham risk prediction algorithm was employed to identify individuals with moderate CVD risk (164, 165). To meet inclusion criteria, participants were required to have ≥ 2 CVD risk points, a score that suggested a 50% greater risk of CVD development than the population mean (165). Briefly, this score was calculated based on the existence of single or multiple CVD risk factors, including elevated fasting total cholesterol, glucose, systolic/diastolic blood pressure, low HDL-cholesterol, overweight/obesity or a family history of myocardial infarction. Potential participants were also required to meet the following inclusion criteria: BMI 19-32 kg/m^2; blood pressure <160/100 mmHg; total cholesterol <8mmol/L; haemoglobin: >125 g/L for women and 135 g/L for men; normal liver and kidney function; not pregnant or lactating; no dietary supplementation; no lactose or dairy intolerances/allergies; not taking medication for hyperlipidaemia, hypertension, hypercoagulation or inflammatory conditions; no diagnosis of myocardial infarction, stroke or diabetes; participating in <20 min x 3 times/week of vigorous aerobic activity and not consuming excessive amounts of alcohol (men: <21 units/week; women: <14 units/week). If

known not to interfere with study outcomes, participants continued to take their regular prescribed medication,without changes in dosage, for the duration of the study.

2.3.2 Study foods

The FA composition of the dairy products (including ruminant *cis* and TFAs), along with the methods for their production, will be described in detail elsewhere (166). Based on a similar feeding strategy, it was estimated that our bovine intervention would increase *cis*-MUFA in the milk from 20 to 30 g/100g FA, while reducing SFA from 70 to 55-60 g/100g FA (167). Briefly, the diet of recruited Holstein-Friesian cows was supplemented with approximately 1 kg high-oleic sunflower oil (AAK Ltd., Hull, East Yorkshire, UK) per cow per day for a ≥ 28-d period to produce milk which had a portion of SFA replaced with MUFA. Subsequently, raw milk was used to produce SFA-reduced, MUFA-enriched (modified) ultra high temperature (UHT) milk, Cheddar cheese and butter. Raw milk, provided by Arla UK Plc (Taw Valley Creamery, North Tawton, UK), was used to produce control UHT milk. Control Cheddar cheese and butter, with a FA profile typical of retail products, were also supplied by Arla UK Plc (Taw Valley Creamery, North Tawton, UK).

2.3.3 Dietary intervention

The technique of minimization, controlling for gender, age, BMI and total cholesterol was used to randomly allocate participants to one of two groups in the study, with Group 1 being assigned to receive Diet A (Modified) and then Diet B (Control) during their first and second dietary intervention periods, respectively and vice-versa for Group 2 (168). In each group, participants completed two 12-week dietary intervention periods, separated by an 8-week washout period. One dietary intervention was an iso-energetic high-fat daily dietary exchange (dietary target: 38 %TE total fat) which was achieved by replacing habitual dairy foods, cooking oil and snacks with SFA-reduced, MUFA-enriched UHT milk, Cheddar cheese and butter (modified). The second dietary intervention used matched products with a FA profile typical of retail products

(control). The dietary exchange periods were rich in dairy foods and were designed to give diets of equal fat content that varied in SFA and MUFA composition. The dietary target intake for total fat was 38 %TE in both diets, with specific dietary FA targets for the control (19 %TE SFA and 11 %TE MUFA) and modified diets (16 %TE SFA and 14 %TE MUFA).

Measurements of circulating total cholesterol, composed of LDL and HDL-cholesterol (primary outcome), and other established and novel CVD and cardiometabolic risk markers were assessed prior to and after each dietary intervention period (chronic study). This manuscript will present the 4-d weighed dietary records, tick-sheet records, anthropometric measures, and PL-FA analysis to assess dietary compliance. The other clinical outcome measures from the RESET study will be published elsewhere.

2.3.4 Food-exchange model

The RESET study food-exchange strategy for reducing SFA intake was designed based on a model adapted from both the Dietary Intervention and Vascular Function (DIVAS) study and the Reading, Imperial, Surrey, Cambridge, and Kings (RISCK) study (164, 169), which were based on the National Diet and Nutrition Survey (NDNS) for adults (aged 19 – 64 years) (170). The mean habitual energy, total fat, SFA, MUFA and PUFA intakes of participants from the RISCK study were used (169), with additional TFA data obtained from the DIVAS study (164), as these dietary data represented the intake of UK adults who were at increased or moderate CVD risk. Added oils, added fats (butter and spreads), milk, cheese as well as sweet and savoury snacks were identified as 'exchangeable dietary fat' sources which could be removed from the diet and replaced with study foods. The total contribution of these 'exchangeable fat' food groups to mean daily energy, fat and FA intake were estimated, based on mean population data from the 2000/2001 NDNS 'percentage contribution of types of foods' (Table 1). The total exchangeable fat was subtracted from the RISCK/DIVAS study habitual energy, total fat and FA intake to calculate non-modifiable fat intake. This was employed to form the backbone of

the food-exchange model, onto which the RESET study foods could be added to create two iso-energetic dietary exchange periods of dairy products that varied in FA composition (Table 2).

2.3.5 Implementation of intervention diets

After completing baseline visits (i.e. at the beginning of each intervention period), participants were provided with 1:1 dietary advice on how to replace dietary fat sources in their habitual diets (e.g. added oils, milk, cheese, sweet and savoury snacks) with the study dairy foods. They were also given dietary guidelines and recipe suggestions to take home. Care was taken to ensure that no study visits were arranged during or immediately after the Christmas period (mid-December to mid-January). Where it was not feasible to avoid holidays and business trips during intervention periods, participants were given instructions on how to travel with the study products. This was made more convenient by providing UHT milk that did not require refrigeration. Furthermore, participants were provided with frozen butter and were advised that they could keep butter in a frozen state during transit, with the use of ice packs. Products were provided in plain packaging and were only identifiable by a code (A or B), to ensure that participants and researchers remained blinded to the intervention arms.

At the beginning of each intervention period, participants were given adequate study dairy foods for a 4-week period. They attended the Hugh Sinclair Unit of Human Nutrition for a food collection visit at weeks 4 and 8. During this visit, adherence to the dietary intervention was assessed by reviewing completed tick-sheet records and any issues were discussed and resolved. In addition, investigators recorded the participant's weight and any changes $\geq \pm 1$ kg of baseline, were addressed through advice to alter snack, meat and/or carbohydrate intake. Participants were asked to maintain their habitual physical activity levels during each intervention period. At the end of the first intervention period, participants were asked to return unopened, leftover study products and were asked not to consume any leftover study products for the duration of the washout period.

Assessment of dietary intake. Participants received verbal and written instructions by the investigators for recording 4-d weighed dietary records approximately 2 week before the first study visit (week 0). Investigators also provided participants with examples of complete diaries, including how to record recipes, and digital scales for recording food intake. Exceptions for weighing included food consumed outside of the home. On these occasions, portion-size images were used to estimate consumed portions and subsequently quantified using published food portion tables (171). Participants completed diet diaries on four separate occasions (weeks 0, 11, 19 and 31): habitual diet intake was represented from baseline diet diaries (weeks 0 and 19), while diaries completed during weeks 11 and 31 represented participant compliance to the intervention diets. Prior to visit 1, food intake was recorded on three weekdays and one weekend day. The same days were repeated for subsequent diaries. Assessment of completion of the diaries was undertaken by the investigators during study visits. If necessary, additional information was requested to facilitate precise data entry.

Food diaries were analysed using the NDS Nutrient Database or McCance and Widdowson's (MW7) nutrient databank contained within the nutrient analysis software Dietplan 7 (Forestfield Software Ltd.). The nutritional content of the control and modified dairy products are previously given in Kliem et al. (166). Energy and macronutrient content (Group 1 nutritional analysis) was performed in duplicate by SGS UK Ltd. (Ealing, London, UK; ISO 17025 accredited laboratory). Analysis of sodium, calcium, magnesium and phosphorus content was conducted in duplicate by inductively coupled plasma-optical emission spectrometry at Quaternary Scientific (QUEST, School of Archaeology, Geography and Environmental Science, University of Reading, Reading, UK). Extracted lipids from milk, cheese and butter samples were analysed in triplicate for FA composition using a GC-flame ionisation detection method (172). A conversion factor of 0.933 was used to estimate the proportion of FAs in the total fat content of each dairy product (173). Subsequently, the quantity of SFA, MUFA, TFA and PUFA were calculated per daily portion of each study dairy product (g/d). Nutrient

composition of the study products was entered manually in Dietplan. For the purpose of analysis, the mean daily intakes of energy and macronutrients were recorded and the %TE was calculated to adjust for energy intake (EI). Dietary fibre intake was defined using the AOAC method (174).

2.3.6 Assessment of underestimation of energy intake

Determination of possible underestimation of dietary EI was assessed for each participant. Basal metabolic rate, based on age, gender and body weight, was estimated using the Henry equation (175). A sedentary lifestyle was represented by a physical activity level score of 1.2 (164, 176). The Goldberg lower 95% confidence limit was calculated as <1.132 using the CV recommended by Black (n = 51; 4 d) and was used to identify under-reporters of EI (176).

2.3.7 Assessment of dietary compliance

As outlined in Table 2, participants were required to consume the minimum daily portions of each of the following study products: 340 g/d milk, 45 g/d cheese and 21.5 or 25.1 g/d of control or modified butter, respectively; this ensured that the intervention diets were iso-energetic and contained equal quantities of dairy fat (38 %TE total fat; approx. 41 g/d). As a means of monitoring compliance with each study product type, participants were required to complete tick-sheet records on a daily basis throughout each 12-week dietary intervention period. Participants were given the option of marking the tick box if they had consumed the required portion size or could choose to record the actual weight of the product consumed. In order to calculate dietary compliance, one point was subtracted for each day that participants had not consumed a study product. These points were summed over each intervention period and were used to calculate percentage dietary compliance for each product type.

2.3.8 Assessment of anthropometrics

Participants were requested to fast overnight for 12 h before each clinical visit, following consumption of a standardised low-fat evening meal (< 1.46 MJ; < 7 g total fat) and low-nitrate water (Buxton Mineral Water, Nestlé Waters, Buxton, UK). At weeks 0, 12, 20 and 32, fasted measurements of BMI and waist circumference were recorded. Height was measured to the nearest 0.1 cm using a wall-mounted stadiometer (screening visit only). BMI was calculated using the Tanita BC-418 digital scale (Tanita Europe), under normal settings (standard body type and -1 kg for clothing) with participants wearing light clothing. Waist circumference was measured by a trained investigator, in triplicate, halfway between the iliac crest and the lowest rib margin to the nearest 0.5 cm (164).

2.3.9 Assessment of plasma phospholipid fatty acid status

Fasting blood samples were collected into lithium heparin tubes for determination of PL-FA status prior to and after each 12-week intervention period (week 0, 12, 20, 32). Chilled samples were centrifuged at 3000 rpm (1700 g) for 15 min at 4°C and plasma stored at -80°C until subsequent extraction and analysis.

Sequential multipurpose sampler systems (Gerstel GmbH & Co. KG, Mülheim an der Ruhr, Germany) were employed for automated sample preparation and derivatization of FAs from the phospholipid fraction at the MRC Human Nutrition Research, Cambridge, as previously described (177). The phospholipid fraction was isolated from plasma using solid phase extraction on Na_2SO_4 50 mg/NH_2 100 mg SPE cartridges (BE Gerstel; Agilent Technologies) and the FAME were produced from the phospholipid fraction according to the method published by Burdge et al (178). The FAMEs were separated using a 30 m capillary column (HP-88; Agilent Technologies, Cheshire, UK) and detected using flame ionization. Conditions of the GC analysis were as follows: helium carrier 1.5 ml/min, initial oven temperature profile 120°C for 1 minute, then ramped to 170°C at 10°C/min, held for 6 minutes,

and finally raised to 210°C at a rate of 3°C/min and held for 1 minute. The injector temperature was maintained at 250°C and the detector temperature maintained at 300°C. A post-run period (235°C for 3.5 minutes at 2.5 ml/min) was used to eliminate possible interferences. The total run time was approximately 30 minutes. Results were expressed as molar percentage of total PL-FAs (mol%).

2.3.10 Power calculation and statistical analyses

A total number of 54 participants were required for the chronic study to have sufficient power to detect a significant change in the primary and key secondary outcome measures. The primary outcome (serum total cholesterol) was predicted to result in a ~0.3 mmol/L reduction with a population mean of 4.54 ± 0.5 mmol/L, with a power of 80% at $P < 0.05$, allowing for a 15 % dropout rate. The chronic study was also powered to detect a 1.5% inter-group difference in the key secondary outcome measure, endothelial-dependent flow-mediated dilatation, with a power of 80% at $p < 0.05$ (179). However, this paper is reporting on the food-exchange model and compliance to our high-fat, high-dairy interventions, which varied in FA composition.

Weighed dietary records, anthropometrics and PL-FA were analyzed using mixed models. Change-from-baseline for each variable of interest were modelled. Fixed effects included in the model were baseline values of the assessed variable, period, treatment, age, gender and BMI. All data were checked for normality and log transformed, if necessary. Participants were included as a fixed effect. There were no effects of the period in the model for any outcome measure.

Paired *t*-tests were used to assess differences in mean dietary compliance scores, as assessed by tick-sheet records, and underestimation of EI between the control and modified dietary interventions. For confirmation that our randomization approach was effective, differences in baseline characteristics between participants randomly assigned to the control and modified dietary intervention periods were assessed using independent *t*-tests and Chi-

square tests for continuous and categorical variables, respectively. Statistical analyses were conducted using the SAS university edition statistical software (version 9.4; SAS Institute lnc., Cary, NC, USA). Results are presented as means and standard error of the mean (SEM). Differences were considered significant at $p \leq 0.01$ to account for multiplicity.

Orthogonal partial least squares discriminant analysis (PLS-DA) was applied to identify patterns between variables in our PL-FA dataset and summarised it by reducing the number of dimensions or components (180). Measured PL-FA concentrations of 36 individual FAs were subjected to orthogonal PLS-DA at: 1) baseline and 2) post-intervention using Metabolanalyst 3.0 (181). The R^2Y and Q^2 values represented the goodness of fit and predictability of the models, respectively. Significance of the models were tested using 1000 permutations.

2.4 Results

Participant flow through the study is illustrated in Fig. 1. Of the 74 participants who were randomly assigned and commenced the study, 54 (31 males and 23 females) completed the study. Participants continued to take their regular prescribed medication for the duration of the study (*n* = 2 levothyroxine for hypothyroidism; *n* = 1 proton pump inhibitor for heartburn; *n* = 1 hormone replacement therapy for menopausal symptom relief; *n* = 1 tricyclic antidepressant for depression; *n* = 2 fluticasone and salmeterol for mild asthma; *n* = 1 etonogestrel contraceptive implant and *n* = 1 levonorgestrel-releasing intrauterine contraceptive device). The breakdown of the ethnicity of the study group was as follows: White, 89% (*n* = 48); Asian, 4% (*n* = 2); Black, 4% (*n* = 2) and Chinese/Far Eastern, 4% (*n* = 2). There were no significant differences between participants randomly assigned to Groups 1 and 2 at baseline (Additional file 1: Table S1). Two participants were excluded from the dietary analysis due to insufficient data.

2.4.1 Dietary analysis

Recorded dietary intake at baseline and following diets that incorporated the control and modified dairy products (post-intervention) are shown in Table 3. In agreement with target intakes (Table 2), significant treatment effects were evident for dietary intakes of SFA and MUFA. Following introduction of the modified diet, there was a smaller increase in SFA and greater increase in MUFA and TFA, relative to baseline, when compared with the control.

2.4.2 Underestimation of energy intake

Based on the assumption that participants were in energy balance, it was estimated that recorded EI were underestimated for 35 and 22% of participants at baseline and post-intervention, respectively. Underestimation of EI was not considered further in the mixed model analysis.

2.4.3 Dietary compliance

On the basis of tick-sheet records, mean daily intake (± SEM) of the dairy products across each 12-week dietary exchange was as follows: milk (control: 343.5 ± 1.8; modified: 347.8 ± 2.8 g/d), cheese (control: 46.5 ± 0.6; modified: 45.9 ± 0.4 g/d) and butter (control: 22.0 ± 0.2; modified: 25.6 ± 0.3 g/d). Mean daily dietary compliance did not vary according to treatment: milk (control: 96.6 ± 0.01; modified: 96.5 ± 0.01%; p = 0.92), cheese (control: 96.6 ± 0.01; modified: 96.8 ± 0.01%; P = 0.83) and butter (control: 96.5 ± 0.01; modified: 97.0 ± 0.01%; p = 0.70).

2.4.4 Anthropometric measures

Relative to baseline, there was no significant treatment effect for BMI (control: 25.8 ± 0.5 vs. 26.2 ± 0.5 kg/m^2 for baseline vs. post-intervention; modified: 25.8 ± 0.5 vs. 25.9 ± 0.5 kg/m^2; p = 0.13) or waist circumference (control: 88.9 ± 1.4 vs. 89.8 ± 1.5 cm; modified: 89.3 ± 1.5 vs. 89.1 ± 1.5 cm; p = 0.53).

2.4.5 Plasma phospholipid FA status

After the 12-week intervention, there were significant differences in PL-FA composition between the modified and control groups ($p \leq 0.01$). Consumption of the modified dairy products led to a small but significant decrease in abundance of total SFAs from baseline (change: -0.60 ± 0.21 mol%) vs. control (change: 0.01 ± 0.17 mol%) (Table 4). Following the modified diet, there were significant increases in *cis*-MUFAs and *trans*-MUFAs from baseline vs. control (Table 4). There was a minor increase in the abundance of 16:0 from baseline following the control diet (change: 0.16 ± 0.13 mol%), while a decrease from baseline was evident following the modified diet (change: -0.46 ± 0.15 mol%) (Fig. 2.b). Compared with baseline, 18 : 1*cis*-9 and 18 : 1*trans*-9 increased following the modified diet vs. control (Fig. 2.b). Following the control diet, there was a significant increase in 20 : 3*n*-6 vs. the modified diet.

2.4.6 Orthogonal Partial Least Square Discriminant analysis of plasma phospholipid FA data

For the baseline PLS-DA, the first component representing the maximum differentiation between the two diets represented 4.7% of variation and was retained to interpret the FA profiles of the clusters on the score plots. PLS-DA of the PL-FA data revealed a lack of distinction between the control and modified intervention groups at baseline ($R^2Y = 0.142$ and $Q^2 = -0.35$, empirical *P*-values R^2Y: $p = 0.99$ (986/1000) and Q^2: $P = 0.76$ (763/1000), suggesting that the population was indistinguishable with regard to PL-FA profiles prior to commencement of the dietary exchange periods (Fig. 3.a). Additional file 2: Table S2 illustrates the loadings.

Following the intervention, the first component representing the maximum differentiation between the two diets represented 5.4 % of the variability in the data. The loadings are illustrated in Additional file 2: Table S2. In contrast to baseline, the post-intervention score plot identified a clear separation ($R^2Y=0.612$ and $Q^2=0.451$, empirical p-values R^2Y: $p < 0.001$ (0/1000) and Q^2: $p < 0.001$ (0/1000) in PL-FA profiles of participants when they were assigned

to the modified and control intervention diets (Fig. 3.b). The FAs that mainly contributed to this dietary status separation were: 18 : 1*trans*-*9*, 16 : 1*trans*, 18 : 1*cis*-9, that were higher following the modified diet, while 16 : 0, 14 : 1 *cis*, 14 : 0, 15 : 0 and 20 : 3*n*-6 were higher following the control diet.

2.5 Discussion

The food-exchange model was used for the implementation of two iso-energetic high-fat, high-dairy diets varying in FA composition in the RESET study, through the use of SFA-reduced, MUFA-enriched dairy products and control alternatives with a FA profile typical of retail dairy products. Specific dietary targets following treatments were largely achieved in a free-living population at moderate risk of CVD. Analysis of weighed dietary records confirmed that it was possible to lower the mean SFA intake by 2.5 %TE and increase MUFA intake by 3.7%TE following the diet containing modified dairy products compared with the control products. Previously, Noakes et al. reported differences of 2.2 %TE SFA and 2.8 %TE MUFA (described as oleic acid intake only) following consumption of dairy products that were produced following a protein-encapsulated lipid (rapeseed and soybean oil) bovine supplementation period, when compared to control dairy foods (135). There was a significant difference in recorded SFA intake between interventions in our study, however the reduction in SFA intake following the modified diet was slightly less than predicted by our food-exchange model. Compliance was further confirmed by assessment of dietary tick-sheet records.

To our knowledge, this is the first human study to assess the impact of modified milk, cheese and butter consumption on PL-FA concentrations, in comparison to control dairy products with a FA profile typical of retail products. In line with previous findings (164, 182-184), objective dietary compliance was confirmed by assessment of PL-FA profiles, with consumption of the control and modified dairy products leading to differential effects on total plasma phospholipid SFAs and MUFAs and their sub-classes. The consumption of the SFA-

reduced, MUFA-enriched dairy products led to a small decrease in the abundance of total SFAs, and increases in total *cis*-MUFAs, including 18 : 1*cis*-9 in the PL-FA profile, when compared to the control products. These changes are comparable to the proportion of PL-FA total SFAs and MUFAs observed in the DIVAS study following a 16-week MUFA-rich diet (9 %TE SFA; 19 %TE MUFA; 4 %TE *n*-6 PUFA) (164). Previous literature has suggested that SFAs and MUFAs with even numbered carbon chain length can be endogenously synthesised by humans and may be less affected by dietary intake (185). Hodson et al. suggested that this may be because it is difficult to alter the proportion of FAs that are already relatively abundant in the diet and that increases in SFA intake are not reflected in increases in the plasma FA profiles (163). However, changes in PL-FA concentrations observed in the RESET study mirrored the FA composition of our intervention dairy foods (166). PLS-DA provided a means for visualizing adherence to a dietary intervention. Despite using a relatively homogenous population and a modest dietary exchange, our PLS-DA plot highlighted some distinction between the PL-FA profile of the participants following the modified and control dietary exchange periods, suggesting a small but significant response to our intervention. This analysis only explained approximately 5 % of the overall variability in our dataset but the affected FAs, including 18 : 1*trans*-9 and 18 : 1*cis*-9 and 16 : 0, were relevant to our interevntions. Furthermore, it provided further evidence that PL-FA were illustrative of short to medium-term FA intake (162-164).

Supplementation of the dairy cow diet with unsaturated FA leads to increased levels of ruminant *trans*-fatty acids (rTFA) in the milk and dairy products, through ruminal biohydrogenation of unsaturated FA (150, 158, 160). There was a calculated dietary increase of 1.3 %TE in total TFA intake following the modified, when compared with the control diet. It was not possible to quantify our participants' voluntary intake of specific TFA isomers (i.e. ruminant or industrial TFA intake) from weighed dietary records using our nutrient analysis software. However, based on the differences in TFA composition between our control and

modified dairy products (166), it is apparent that the majority of the increased TFA intake recorded following the modified diet was derived from ruminant sources. In contrast to the recognised detrimental impact of industrially-produced TFA on cardiovascular health, consumption of rTFA may not be adversely linked to CVD risk (186), except possibly at high intakes (132, 187). It should be noted that the calculated dietary TFA intake following our modified intervention (2.5 ± 0.1 %TE) exceeded the recommended population maximum of 2 % food energy (139) which does not discriminate between ruminant and industrial sources, and is higher than the current mean TFA intake in UK adults (0.5 %TE and 0.5 % of food energy) (140). This may be in part explained by the high dairy fat content of our intervention diets. In support of this we also observed that consumption of the SFA-reduced, MUFA-enriched dairy products lead to significant increases in total *trans*-MUFA and 18:1 *trans-9* concentrations in the PL-FA. Whilst our PL-FA analysis approach was unable to identify specific *trans* isomers other than 18:1 *trans-9* (177), the detailed FA analysis of the dairy foods used in the RESET study (166) suggest there was likely to be a complex mixture of TFA in the phospholipid pool. It may be that feeding strategies that limit increases of TFA in milk fat following bovine supplementation with plant oils, such as the use of encapsulation protection technology may be advantageous (160, 188). However, further work is justified to determine whether rTFA are detrimental to cardiovascular health (160).

Our intervention presented some challenges. As outlined previously, our bovine supplementation strategy was successful in altering the FA profile of the milk (166). Alongside this, we observed a depression in milk fat content following supplementation of the bovine diet with high-oleic sunflower oil which has been reported previously (189). As a result of this, it was necessary to standardise the fat content of the raw control milk prior to UHT so that it was equivalent to that of the modified milk. Furthermore, our modified cheese had a lower fat content compared with conventional cheese and it was necessary for our participants to

consume an additional 3.6 g/d of butter during the modified dietary exchange period to standardize the fat intake of the two intervention periods.

A challenge faced by some participants was incorporating sufficient quantities of products, especially cheese, into their habitual diets on a daily basis. In line with recent NDNS data (140), some of our participants were not accustomed to consuming the quantities of dairy products that were prescribed in our food-exchange model (unpublished data obtained by food frequency questionnaire). Although it was not logistically feasible for us to include a wider range of dairy products in the RESET study, it is possible that a greater variety of items may have reduced the likelihood of 'product boredom' and minimized the potential for compliance issues (190). Compliance with the dietary exchange was cited as the main reason for dropout by nine of our participants, predominantly in the early stages of the intervention. It should be noted that a similar number of participants withdrew from the modified and control dietary exchange periods, suggesting that the two regimens were equal in terms of acceptability. For those who completed the study, our tick-sheet records suggested that compliance to the dietary regimens was excellent and our PL-FA data provided further objective evidence of dietary adherence. It is acknowledged that there may have been discrepancies in tick-sheet recordings as a result of 'desirability bias', i.e. participants may have recorded the portions that they were required to eat as opposed to what they had actually consumed (190), and this may have led to more modest differences in PL-FA profiles between the two dietary exchange periods.

We observed a moderately high amount of under-reporting assessed by EI at baseline, however, this was similar to that observed in previous free-living populations (164, 191). A high proportion of participants (61 %; $n = 33$) in the RESET study were classified as overweight (BMI of ≥ 25.0 kg/m^2). It is recognized that overweight or obese individuals may be more prone to selective bias and omission of foods with a negative health image, leading to potential under-reporting of dietary intake (192). We observed a lower degree of under-reporting following the intervention, when our replacement model predicted that the dairy products would contribute

to over 25 % of daily EI. It could be speculated that participants had a greater appreciation of the importance of giving an accurate account of their dietary intake whilst on the intervention. The present study has illustrated that through the incorporation of dairy products with an altered FA profile into the habitual diet, it was possible for free-living participants to successfully improve their dietary fat quality. This was in agreement with previous successful food-exchange models (164, 169, 190). Our dietary interventions were designed to contain a high quantity of dairy fat (~ 41 g/d). Both intervention periods increased relative intake of total fat and SFA over baseline. However, it is feasible that a similar, but more moderate, approach could be used for assisting in the reduction of dairy SFA intake at a population level, without reducing dairy consumption. Furthermore, we have previously reported that consumers generally accepted the SFA-reduced, MUFA-enriched dairy products, when tasted in a blinded manner (193).

2.6 Conclusions

We sucessfully implemented a high-fat, high-dairy food-exchange model that was suitable for replacing dairy products with a FA profile typical of retail products with SFA-reduced, MUFA-enriched alternatives over a 12-week period in a free-living population at moderate risk of CVD. Changes in the dietary intake of SFA and MUFA between our interventions was confirmed by changes in the concentrations of these FAs in the plasma phospholipid fraction, indicative of adherence to our dietary intervention.

List of Abbreviations

Ca: calcium; CVD: cardiovascular disease; DIVAS: Dietary Intervention and Vascular Function; FA: Fatty acid; Mg: magnesium; Na: sodium; NDNS: National Diet and Nutrition Survey; P: phosphorus; PC: principal component; PL-FA: plasma phospholipid FA; PLS-DA: partial least squares discriminant analysis; RCT: randomized controlled trial, RISCK: Reading,

Imperial, Surrey, Cambridge, and Kings; rTFA: ruminant *trans*-fatty acids; TFA: *trans*-fatty acid; UHT: ultra high temperature; %TE: percentage of total energy.

2.7 Declarations

Ethics approval and consent to participate

The study was approved by the University of Reading Research Ethics Committee (13/43) and written informed consent was obtained from all participants.

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

JAL is an expert on the UK Scientific Advisory Committee for Nutrition (SACN) Saturated Fats Working Group. There are no other competing interests.

Funding

This work was supported by funding from the Medical Research Council (United Kingdom; Ref: MR/K020218/1). Arla Foods UK and AAK UK supplied in kind the commercially available dairy products and oils according to our specification. Arla Foods UK and AAK UK had no role in the design, analysis or writing of this article.

Authors' contributions

The authors' contributions are as follows: JAL, DIG and KGJ: designed the human study; OM, JAL and KGJ: designed the food-exchange model; CCF, ASG, DJH and DIG: designed and produced the modified dairy products; OM and DV: conducted the research (hands-on conduct

of the experiment and data collection); OM, DV, KEK and AK: analyzed data and performed statistical analysis; KEK, KS and LW: performed fatty acid analysis; ST: provided statistical advice; OM and DV: wrote the manuscript, which was modified by all co-authors; and JAL had primary responsibility for final content. All authors have read and approved the final manuscript.

Acknowledgements

The authors gratefully acknowledge all volunteers who took part in the study. We thank S. Hargreaves, K. Jenkins, R. Mihaylova and the research students/assistants (C. Brilley, L. Bula, Y. Chatzidiakou, V. Clifton, H. Dong, A. Garcimartin, C. Holland, J. Guo, A. Kanneganti, T. Loughman, E. Mertens, J. Ryan, L. Sellem, T. Staff and H. Zhang) for their assistance.

Table 1 The RESET food-exchange model[a]

	Total energy (MJ/d)	Total fat (g/d)	SFA (g/d)	MUFA (g/d)	TFA (g/d)	PUFA (g/d)
Total habitual intake (including alcohol)[b]	8.25	80.1	29.6	26.6	1.0	13.1
Total habitual intake, %TE		36.6	13.5	12.1	0.4	6.0
Exchangeable fat intake						
Added oils, g/d	0.35	8.5	0.8	3.3		4.0
Added fats (butter and spreads), g/d	0.35	8.6	3.3	2.9	0.2	1.7
Milk, g/d	0.45	4.0	2.4	1.0	0.0	0.3
Cheese, g/d	0.25	4.5	2.8	1.0	0.1	0.1
Sweet and savoury snacks, g/d[c]	0.87	10.0	4.4	3.4	0.1	0.6
Total exchangeable fat intake, g/d	2.27	35.5	13.6	11.6	0.4	6.6
Total exchangeable fat intake adjusted for habitual intake, g/d	2.22	38.5	14.4	12.5	0.4	7.3

Non-exchangeable fat intake, g/d	6.03	41.7	15.3	14.1	0.6	5.9

TFA, *trans*-fatty acids; %TE, percentage of total energy. [a]Adapted from Weech et al. (164); [b]Mean daily dietary intakes (total energy, total fat, SFA, MUFA and PUFA) from a population with increased cardiovascular disease risk (169). Mean daily dietary TFA intake from a population at moderate cardiovascular disease risk (164); [c]Included buns, cakes, pastries, potato chips and chocolate confectionary.

Table 2 Replacement model for diets containing control and modified dairy products for use in the RESET study[a]

	Quantity (g/d)	Total energy (MJ/d)	Total fat (g/d)	SFA (g/d)	MUFA (g/d)	TFA[b] (g/d)	PUFA (g/d)
Non-exchangeable fat intake		6.03	41.7	15.3	14.1	0.5	5.9
Control dietary exchange							
Exchangeable fat intakes[c]							
Butter	21.5	0.65	17.4	11.1	4.5	0.7	0.6
Cheese	45.0	0.76	15.1	10.2	3.5	0.5	0.4
Milk	340.0	0.75	8.6	5.7	2.1	0.3	0.3
Total intake		8.19	82.8	42.3	24.2	2.0	7.2
Total intake, %TE			38.1	19.4	11.1	0.9	3.3
Target intake, %TE			38.0	19.0	11.0	0.9	
Modified dietary exchange							

Exchangeable fat intakes							
Butter	25.1	0.76	20.4	10.2	8.1	2.1	0.7
Cheese	45.0	0.69	12.6	6.3	5.0	1.4	0.4
Milk	340	0.79	8.8	4.4	3.6	0.9	0.3
Total intake		8.27	83.4	36.0	30.8	4.9	7.3
Total intake, %TE			38.0	16.4	14.0	2.1	3.3
Target intake, %TE			38.0	16.0	14.0	2.0	
Mean difference between dietary exchange periods			-0.6	6.2	-6.7	-2.9	-0.2

%TE, percentage of total energy. [a]Total intake is the sum of exchangeable and non-exchangeable intakes based on MJ/d for energy and grams/d for FAs; [b]Total TFA intake (%TE) is calculated based on recorded energy intake at baseline (MJ/d) in the DIVAS study (164); [c]Energy and fat content of the dairy products is based on nutritional analysis conducted by SGS United Kingdom Ltd. (Ellesmere Port, Cheshire). The FA composition of dairy products was determined using a GC-flame ionisation detection method (172), with a conversion factor of 0.933 used to estimate the proportion of FAs in the total fat content of each product (173, 194).

Table 3 Recorded dietary intake at baseline (week 0) and following diets that incorporated the control and modified dairy products (week 12) in adults at moderate cardiovascular disease risk and target FA intakes[a]

	Control				Modified				
	Baseline	Post	T[b]	Δ	Baseline	Post	T	Δ	*p*[c]
Energy, MJ/d	8.5 ± 0.4	9.0 ± 0.3		0.5 ± 0.3	8.2 ± 0.3	9.2 ± 0.4		1.0 ± 0.4	0.60
Total fat, %TE	36.5 ± 0.8	39.5 ± 0.7	38.0	3.0 ± 0.9	36.1 ± 0.8	41.1 ± 0.8	38.0	5.0 ± 1.1	0.03
SFA, %TE	13.9 ± 0.5	19.1 ± 0.4	19.0	5.2 ± 0.6	14.2 ± 0.5	16.9 ± 0.4	16.0	2.7 ± 0.6	<0.001
MUFA, %TE	11.9 ± 0.4	11.8 ± 0.3	11.0	-0.1 ± 0.3	11.7 ± 0.4	15.3 ± 0.4	14.0	3.6 ± 0.5	<0.0001
n-6 PUFA, %TE	4.6 ± 0.2	3.3 ± 0.2		-1.3 ± 0.2	3.9 ± 0.2	3.4 ± 0.2		-0.5 ± 0.2	0.08
n-3 PUFA, %TE	0.8 ± 0.1	0.6 ± 0.0		-0.2 ± 0.1	0.7 ± 0.0	0.6 ± 0.0		-0.1 ± 0.1	0.34
Total PUFA, %TE	5.8 ± 0.4	4.4 ± 0.5		-1.4 ± 0.3	4.6 ± 0.2	4.0 ± 0.2		-0.4 ± 0.2	0.14
TFA, %TE	1.0 ± 0.1	1.3 ± 0.1		0.3 ± 0.1	0.9 ± 0.1	2.5 ± 0.1		1.6 ± 0.1	<0.0001

Protein, %TE	16.3 ± 0.5	16.1 ± 0.4	-0.2 ± 0.5	16.9± 0.5	16.2 ± 0.3	-0.7 ± 0.6	0.80
Carbohydrates, %TE	46.3 ± 0.9	43.3 ± 0.9	-3.0 ± 0.9	46.6 ± 1.3	42.2 ± 1.0	-4.4 ± 1.5	0.19
Alcohol, %TE	3.1 ± 0.5	3.1 ± 0.5	0.0 ± 0.4	2.9 ± 0.5	2.7 ± 0.4	-0.2 ± 0.5	0.35
Dietary fiber (AOAC), g/d	20.4 ± 1.1	22.0 ± 1.1	1.6 ± 0.9	20.2 ± 1.1	19.4 ± 1.1	-0.8 ± 1.2	0.03
Sodium, g/d	2.7 ± 0.2	2.2 ± 0.1	-0.5 ± 0.1	2.5 ± 0.2	1.9 ± 0.1	-0.6 ± 0.1	0.03

AOAC; Association of Official Analytic Chemists; %TE, percentage of total energy. [a]Values are means ± SEM. Dietary intakes estimated from 4-d weighed dietary records at baseline (week 0) and after intervention (week 12); [b]Target FA intakes for the control and modified dietary exchange periods; [c]Overall effect of treatment based on change-from-baseline was calculated by mixed model analysis, with adjustments made for fixed effects of baseline values of the assessed variable, period, treatment, age, gender and BMI. Participant was included as a random effect. $p \leq 0.01$ deemed as significant.

Table 4 Plasma phospholipid fatty acids at baseline (week 0) and following diets that incorporated the control and modified dairy products (week 12) in adults at moderate cardiovascular disease risk[a]

	Control			Modified			
mol%	Baseline	Post	Δ	Baseline	Post	Δ	p^b
SFAs							
11:0	0.0000 ± 0.0008	0.0013 ± 0.0008	0.0000 ± 0.0011	0.0000 ± 0.0011	0.0002 ± 0.0000	0.0025 ± 0.0010	0.35
12:0	0.0230 ± 0.0018	0.0242 ± 0.0015	0.0012 ± 0.0019	0.0245 ± 0.0000	0.0225 ± 0.0000	0.0019 ± 0.0016	0.22
13:0	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.16
14:0	0.35 ± 0.01	0.42 ± 0.02	0.07 ± 0.02	0.35 ± 0.02	0.38 ± 0.02	0.03 ± 0.01	0.04
15:0	0.23 ± 0.01	0.26 ± 0.01	0.04 ± 0.01	0.22 ± 0.01	0.24 ± 0.01	0.02 ± 0.01	0.02
16:0	30.70 ± 0.16	30.86 ± 0.17	0.16 ± 0.13	30.78 ± 0.13	30.32 ± 0.13	-0.46 ± 0.15	<0.001
17:0	0.39 ± 0.01	0.40 ± 0.01	0.01 ± 0.01	0.38 ± 0.01	0.38 ± 0.01	0.00 ± 0.01	0.03
18:0	13.95 ± 0.18	13.67 ± 0.14	-0.29 ± 0.12	14.05 ± 0.12	13.83 ± 0.12	-0.22 ± 0.13	0.33
20:0	0.14 ± 0.00	0.13 ± 0.00	-0.01 ± 0.00	0.13 ± 0.00	0.12 ± 0.00	-0.01 ± 0.00	0.76
21:0	0.0128 ± 0.0037	0.0130 ± 0.0043	0.0001 ± 0.0036	0.0093 ± 0.0000	0.0147 ± 0.0000	0.0054 ± 0.0034	0.62
22:0	0.23 ± 0.01	0.22 ± 0.01	-0.01 ± 0.01	0.22 ± 0.01	0.22 ± 0.01	0.00 ± 0.01	0.69
23:0	0.10 ± 0.00	0.11 ± 0.00	0.01 ± 0.00	0.10 ± 0.00	0.11 ± 0.00	0.01 ± 0.00	0.34
24:0	0.25 ± 0.01	0.25 ± 0.01	0.00 ± 0.01	0.25 ± 0.01	0.24 ± 0.01	-0.01 ± 0.01	0.85
Total SFA[c]	46.37 ± 0.17	46.36 ± 0.13	0.01 ± 0.17	46.52 ± 0.21	45.92 ± 0.15	-0.60 ± 0.21	0.006
MUFAs							
18:1 *cis*-9	9.81 ± 0.15	9.98 ± 0.16	0.17 ± 0.15	9.99 ± 0.15	10.93 ± 0.15	0.93 ± 0.19	<0.0001

18:1 *trans*-9	0.08 ± 0.00	0.08 ± 0.00	0.01 ± 0.00	0.07 ± 0.00	0.17 ± 0.00	0.10 ± 0.01	<0.0001
22:1 *cis*-9	0.0202 ± 0.0037	0.0206 ± 0.0030	0.0004 ± 0.0045	0.0182 ± 0.0000	0.0201 ± 0.0000	0.0000 ± 0.0019	0.52
Total *cis*-MUFA[d]	11.02 ± 0.16	11.24 ± 0.17	0.21 ± 0.15	11.21 ± 0.18	12.20 ± 0.23	0.99 ± 0.20	<0.0001
Total *trans*-MUFA[e]	0.12 ± 0.00	0.12 ± 0.00	0.01 ± 0.00	0.10 ± 0.00	0.23 ± 0.01	0.12 ± 0.01	<0.0001
PUFAs							
18:2 n-6	22.26 ± 0.42	22.40 ± 0.37	0.14 ± 0.28	22.00 ± 0.28	22.29 ± 0.28	0.30 ± 0.29	0.87
18:3 n-6	0.08 ± 0.01	0.10 ± 0.01	0.02 ± 0.01	0.09 ± 0.01	0.09 ± 0.01	0.00 ± 0.01	0.51
18:3 n-3	0.31 ± 0.02	0.31 ± 0.01	0.00 ± 0.01	0.31 ± 0.01	0.29 ± 0.01	-0.02 ± 0.01	0.58
20:3 n-6	3.07 ± 0.08	3.39 ± 0.11	0.32 ± 0.07	3.12 ± 0.07	3.17 ± 0.07	0.06 ± 0.06	0.007
20:4 n-6	9.88 ± 0.27	9.53 ± 0.22	-0.35 ± 0.13	9.77 ± 0.13	9.41 ± 0.13	-0.36 ± 0.21	0.74
20:5 n-3	1.27 ± 0.08	1.25 ± 0.06	-0.02 ± 0.07	1.24 ± 0.07	1.14 ± 0.07	-0.10 ± 0.07	0.14
22:5 n-6	0.20 ± 0.01	0.20 ± 0.01	0.00 ± 0.01	0.20 ± 0.01	0.20 ± 0.01	0.00 ± 0.01	0.17
22:5 n-3	0.99 ± 0.03	1.02 ± 0.02	0.03 ± 0.02	1.00 ± 0.02	0.95 ± 0.02	-0.05 ± 0.03	0.55
22:6 n-3	3.77 ± 0.16	3.39 ± 0.13	-0.37 ± 0.09	3.79 ± 0.09	3.44 ± 0.09	-0.35 ± 0.10	0.74
Total n-3 PUFA[f]	5.35 ± 0.23	4.96 ± 0.17	-0.39 ± 0.14	5.34 ± 0.21	4.87 ± 0.17	-0.47 ± 0.14	0.58
Total n-6 PUFA[g]	36.15 ± 0.34	36.30 ± 0.27	0.15 ± 0.26	35.83 ± 0.32	35.86 ± 0.33	0.03 ± 0.33	0.32

[a]Values are given as means ± SEM; [b]Overall effect of treatment based on change-from-baseline was calculated by mixed model analysis, with adjustments made for fixed effects of baseline values of the assessed variable, period, treatment, age, gender and BMI. Participant was included as a random effect; [c]Total SFAs include: 11 : 0, 12 : 0, 13 : 0, 14 : 0, 15 : 0, 16 : 0, 17 : 0, 18 : 0, 20 : 0, 21 : 0, 22 : 0, 23 : 0 and 24 : 0; [d]Total *cis*-MUFAs include: 14 : 1*cis*, 15 : 1*cis*, 16 : 1*cis*, 17 : 1*cis*, 18 : 1*cis*-9, 20 : 1*cis*, 22 : 1*cis*-9 and 24 : 1*cis*. Where no bond position is listed it is unknown, as previously outlined in Wang et al. (177); [e]Total *trans*-MUFAs include: 16 : 1*trans* (bond position unknown) and 18 : 1*trans*-9 (177); [f]Total *n*-3 PUFAs include: 18 : 3*n*-3, 20 : 5*n*-3, 22 : 5*n*-3 and 22 : 6*n*-3; [g]Total *n*-6 PUFAs include: 18 : 2*n*-6, 18 : 2*trans*, 18 : 3*n*-6, 20 : 2, 20 : 3*n*-6, 20 : 4*n*-6 + 20:3*n*-3, 22 : 4 and 22 : 5*n*-6. Where no bond position is listed it is unknown (177). 20 : 4*n*-6 + 20 : 3*n*-3 co-eluted, but as 20 : 3*n*-3 concentration in human samples is negligible, this peak was identified as 20 : 4*n*-6 (177).

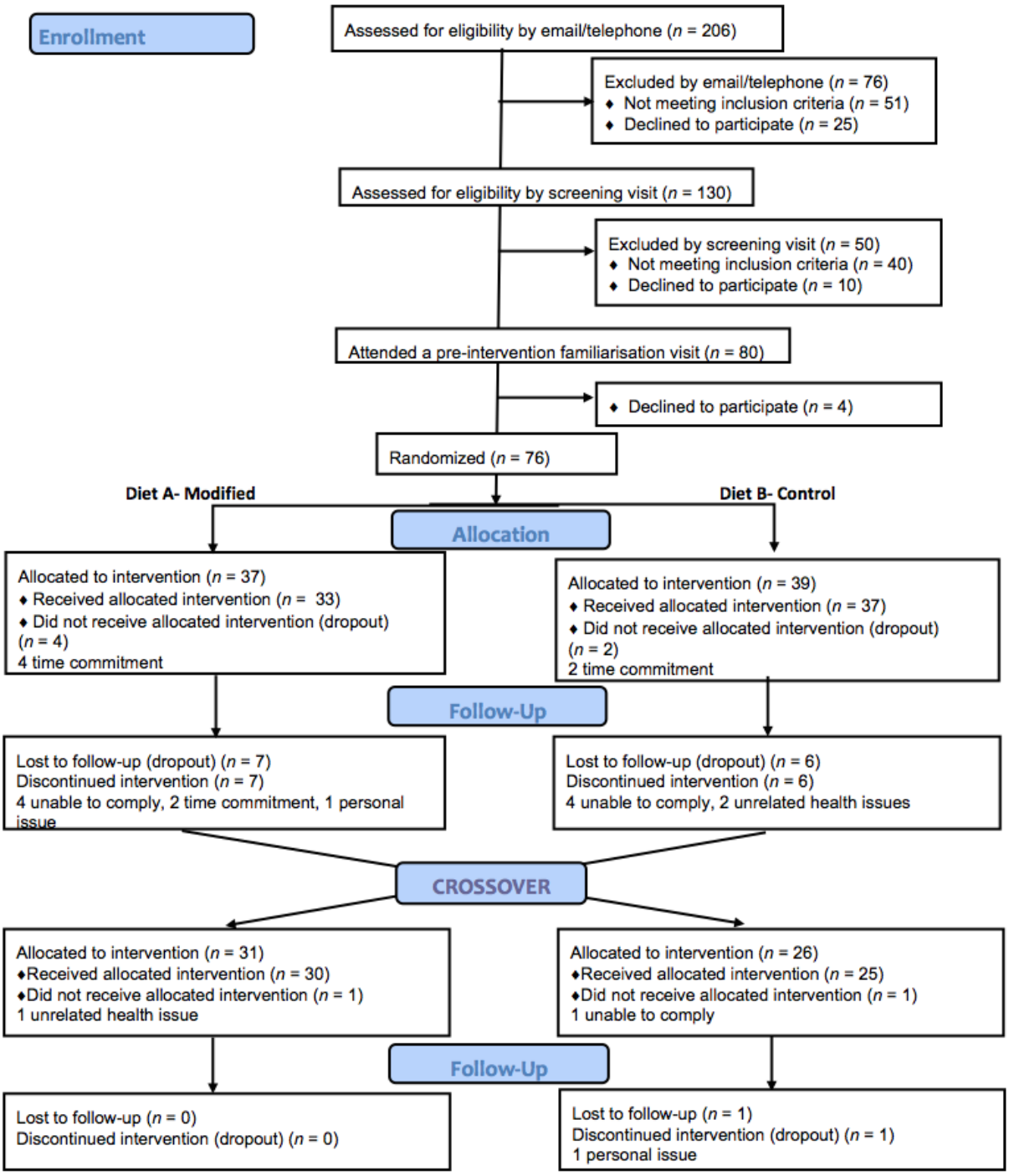
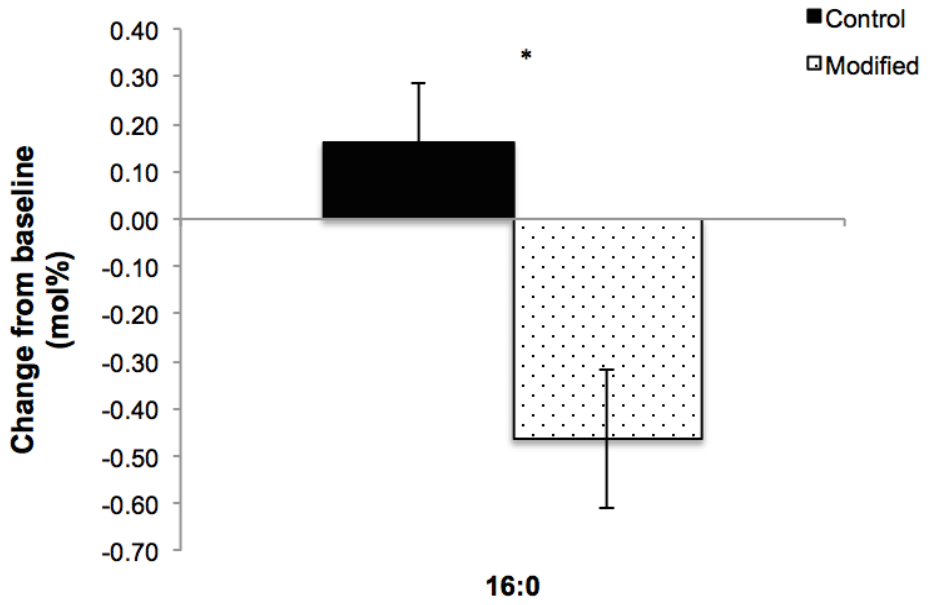


Figure 2.1 Flow of participants through the different stages of the RESET study.

(a)


Control
Modified
0.40
0.30
0.20
0.10
0.00
-0.10
-0.20
-0.30
-0.40
-0.50
-0.60
-0.70
*
Change from baseline (mol%)
16:0

(b)

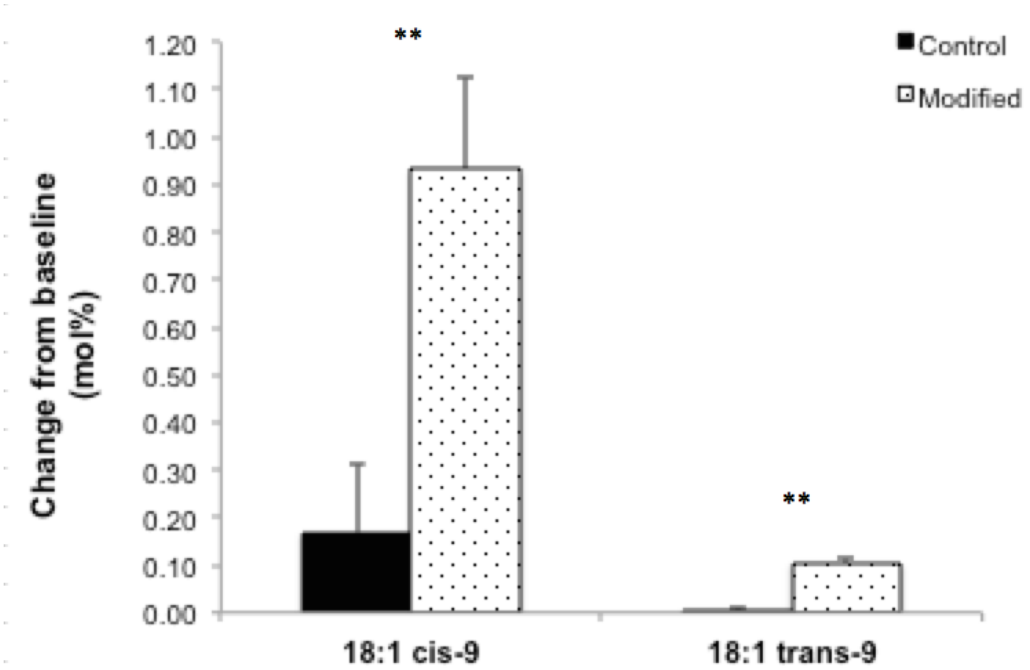

Control
Modified
1.20
1.10
1.00
0.90
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
**
**
Change from baseline (mol%)
18:1 cis-9
18:1 trans-9

Figure 2.2 Change-from-baseline in the plasma phospholipid profile of SFA: 16 : 0 (a) and MUFAs: 18 : 1*cis*-9 and 18 : 1*trans*-9 (b) following 12-week diets that incorporated control and modified dairy products. Values are means ± SEM, n = 54. Significance shown as * P <0.001, ** P < 0.0001.

(a) (b)

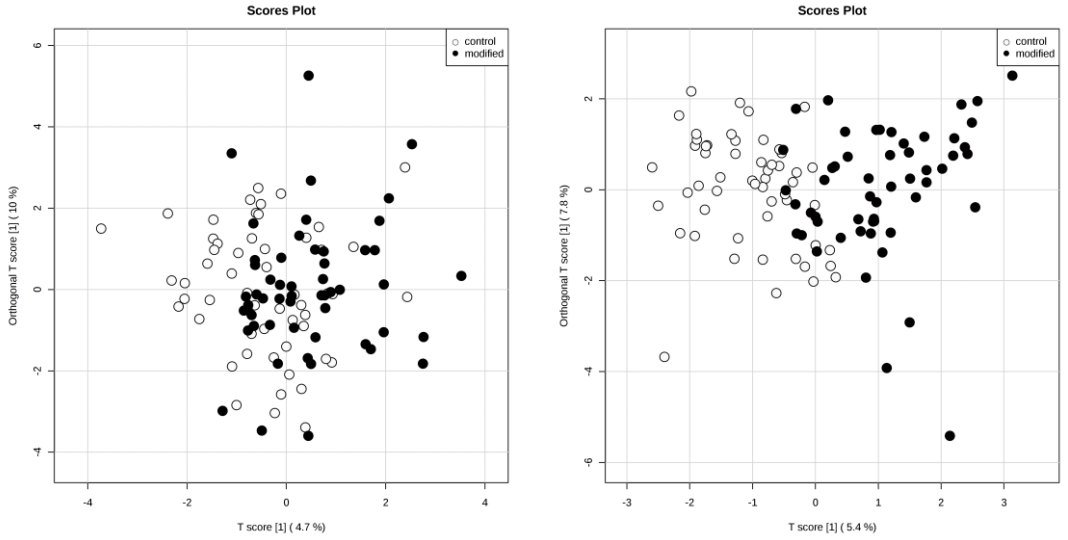


Figure 2.3 Orthogonal PLS-DA, score plots at baseline (a) and on post-intervention (b) calculated using plasma phospholipid FA concentrations in adults at moderate risk of cardiovascular disease (n = 54). FA, fatty acid; PLS-DA, partial least squares discriminant analysis.

Supplementary Table 2.1 Baseline characteristics for participants' based on the order of allocation the control and modified dietary exchange periods[a]

	Group 1[b] (*n* = 30)	Group 2 (*n* = 24)	*P*[c]
Age, *y*	52 ± 2	51 ± 3	0.41
Gender, *%M/%F*	53/47	63/38	0.50
BMI, *kg/m*	25.0 ± 0.6	25.8 ± 0.7	0.99
Waist circumference, *cm*	86.7 ± 1.9	90.2 ± 2.2	0.83
Fasting total cholesterol, *mmol/L*	5.60 ± 0.20	5.45 ± 0.18	0.80
Fasting HDL-cholesterol, *mmol/L*	1.46 ± 0.04	1.49 ± 0.08	0.92
Fasting glucose, *mmol/L*	5.37 ± 0.17	5.46 ± 0.22	0.84
Systolic blood pressure, *mmHg*[d]	119 ± 2	122 ± 2	0.67
Diastolic blood pressure, *mmHg*[d]	71 ± 1	74 ± 2	0.82
Family history of premature MI, *%*[e]	16.7	12.5	0.36
Risk Score[f]	3.2 ± 0.3	2.8 ± 0.2	0.28

[a]All values are given as mean ± SEM; [b]Group 1= participants were randomly allocated to consume control dairy products during their first dietary exchange period. Group 2= participants were randomly allocated to consume modified (SFA-reduced, MUFA-enriched)

dairy products during their first dietary exchange period; [c]Differences in baseline characteristics between participants randomly assigned to the control and modified dietary exchange periods were assessed using independent *t*-tests and Chi-square tests for continuous and categorical variables, respectively. $P \leq 0.01$ deemed as significant; [d]Measured over 24 h using ambulatory blood pressure monitors approximately 48 h before clinical visit 1 at wk 0; [e]Family history of prematurely diagnosed MI in parents or siblings (≤55 years for male relatives; ≤65 years for female relatives); [f]A score of ≥2 points relates to a 50% greater risk of CVD than the population mean.

Supplementary Table 2.2 Factor loadings identified by orthogonal partial least squares discriminant analysis of plasma phospholipid fatty acid profiles at baseline (week 0) and following diets that incorporated the control and modified dairy products (week 12) in adults at moderate cardiovascular disease risk.

	Baseline		Post-intervention	
	Loading (t1)	OrthoLoading (to1)	Loading (t1)	OrthoLoading (to1)
10:0	-0.008	0.153	-0.038	0.109
11:0	0.167	-0.076	-0.116	-0.193
12:0	0.013	0.158	-0.095	-0.055
13:0	0.089	0.111	-0.011	-0.312
14:0	-0.027	0.272	-0.238	0.224
14:1 *cis*	0.247	0.000	-0.255	0.005
15:0	-0.291	-0.107	-0.226	0.188
15:1 *cis*	0.292	-0.031	0.103	-0.285
16:0	0.105	0.129	-0.247	-0.085
16:1 *cis*	-0.451	-0.093	0.392	0.264
16:1 *trans*	0.036	0.233	-0.001	0.182
17:0	-0.216	-0.365	-0.147	0.115
17:1 *cis*	0.221	0.019	-0.123	0.174
18:0	0.222	0.026	0.088	0.033
18:1 *cis*-9	-0.383	0.053	0.579	0.235
18:1 *trans*-9	0.061	0.277	0.258	0.374
18:2 *cis*-6	-0.177	-0.152	0.070	0.038
18:2 *trans*-6	-0.148	-0.065	0.023	-0.101

18:3 n-6	0.118	0.241	-0.136	0.115
20:0	-0.181	-0.262	-0.053	-0.357
18:3 n-3	-0.038	0.182	-0.152	0.102
20:1 *cis*	-0.403	0.126	0.186	0.443
21:0	-0.094	-0.214	0.068	-0.123
20:2	-0.129	-0.026	-0.037	0.030
20:3 n-6	0.014	0.221	-0.158	0.214
22:0	-0.095	-0.423	0.087	-0.297
20:4 n-6	-0.037	-0.056	0.009	-0.132
22:1 *cis*-9	-0.078	0.023	-0.042	-0.003
23:0	-0.084	-0.427	-0.056	-0.240
20:5 n-3	-0.042	-0.094	-0.146	0.065
24:0	0.052	-0.220	0.040	-0.255
22:4	0.044	0.083	-0.062	-0.046
24:1 *cis*	-0.113	-0.432	-0.021	-0.495
22:5 n-6	0.063	0.052	0.023	0.070
22:5 n-3	0.105	-0.072	-0.201	0.045
22:6 n-3	0.065	-0.261	0.020	-0.217

Chapter III: Impact of dairy fat manipulation to replace saturated with unsaturated fatty acids in milk and dairy products on cardiovascular disease risk markers: findings from the RESET randomized controlled trial

Dafni Vasilopoulou, Oonagh Markey, Kirsty E. Kliem, Colette C. Fagan, Alistair S. Grandison, David J. Humphries, Susan Todd, Kim G. Jackson, David I. Givens, and Julie A. Lovegrove.

In preparation for submission to AJCN

JAL, DIG and KGJ designed the human study; CCF, ASG, DJH and DIG designed and produced the modified dairy products; DV, OM, KEK conducted the research and analysed data; ST provided statistical advice.

DV analysed the data presented in this chapter (with OM for biochemical ILAB and BP analysis and statistics), performed statistical analysis on all other outcome measures and wrote the manuscript for this thesis, which was approved by all authors.

DV was also responsible for finding and contacting the NMR company to analyse the samples.

Results of the FMD analysis will not be presented in this chapter.

Impact of dairy fat manipulation to replace saturated with unsaturated fatty acids in milk and dairy products on cardiovascular disease risk markers: findings from the RESET randomized controlled trial[1,2,3]

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List of all author's last names: Vasilopoulou; Markey; Kliem; Fagan; Grandison; Humphries; Todd; Jackson; Givens; Lovegrove

[1] Supported by funding from the Medical Research Council (Ref: MR/K020218/1). Arla Foods UK and AAK UK supplied in kind the commercially available dairy products and oils according to our specification.

[2] Author disclosures: J.A. Lovegrove is an expert on the UK Scientific Advisory Committee for Nutrition (SACN) and SACN's Saturated Fats Working Group.

[3] Supplemental Methods and Supplemental Tables [3] are available from the "Online Supporting Material" link in the online postings of the article.

[9] DV and OM contributed equally to this article.

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[11] Abbreviations used: BP, blood pressure; cIMT, carotid intima media thickness; CVD, cardiovascular disease; DBP, diastolic blood pressure; FA, fatty acid; FMD, flow-mediated dilatation; MUFA, monounsaturated fatty acids; RCT, randomized controlled trial; SBP, systolic blood pressure; SFA, saturated fatty acids; TC, total cholesterol; UHT, ultra-high temperature; Δ, change from baseline; %TE, percent total energy.

This trial was registered at www.clinicaltrials.gov as NCT02089035

Short running head: Dairy fat manipulation modulates CVD risk factors.

3.1 Abstract

Background: Modifying dairy fat composition by increasing the monounsaturated fatty acid (MUFA) content is a potential strategy to reduce dietary saturated fatty acid (SFA) intake for cardiovascular disease (CVD) prevention in the population.

Objective: To determine the effects of a high-dairy diet containing SFA-reduced, MUFA-enriched (modified) dairy products, versus conventional dairy products (control), on the fasting lipid profile, vascular function and other CVD risk markers in adults at moderate CVD risk.

Design: A double-blind, randomized, controlled cross-over intervention was conducted with an 8-wk washout period between the two 12-wk treatments. Participants (n = 76, 25-70 y) with a 1.5-fold higher CVD risk, replaced habitual dairy products with study products (milk, cheese and butter) to achieve a high-dairy iso-energetic daily dietary exchange (38% total energy intake (%TE) from fat: control (dietary target: 19%TE SFA; 11%TE MUFA) and modified (16%TE SFA; 14%TE MUFA) diet. Linear mixed model analyses determined the overall effect of dairy fat composition, based on change-from-baseline values during the modified and control interventions.

Results: Fifty-four participants (31 men; 23 women; 52 ± 3 y; BMI 25.8 ± 0.5 kg/m^2) completed both arms of the intervention. There was a borderline treatment effect on the change in fasting total cholesterol (P = 0.08), but not HDL-C. A significant attenuation in the rise of fasting LDL-C was observed following the modified diet (0.9%) compared to the control diet (5.5%; P = 0.03), reflected in the lower LDL-C:HDL-C ratio (P = 0.04). Additionally, fasting plasma nitrite increased after the modified, yet decreased after the control diet (P = 0.01). There were no significant differences in any of the other CVD risk markers.

Conclusion: A high daily intake of SFA-reduced, MUFA-enriched dairy products for 12-wk may have beneficial effects on fasting LDL-C and nitrite concentrations compared with conventional dairy products. This trial was registered at www.clinicaltrials.gov as NCT02089035.

Keywords: dairy fat, cardiovascular disease risk, cholesterol profile, flow-mediated dilatation, saturated fatty acids, monounsaturated fatty acids, vascular function.

3.2 Introduction

High intakes of dietary saturated fatty acids (SFA) have been associated with an increase in low density lipoprotein cholesterol (LDL-C) concentrations (9), an established risk factor in the development of atherosclerosis (195). Consumption of dairy products contribute up to 35% of total dietary SFA intake within the UK adult population (10) and reduced-fat dairy products are recommended as part of public health guidelines aimed at CVD prevention (196, 197). However, the adverse association between high SFA intake and CVD is not reflected in prospective cohort studies of dairy fat consumption, with the exclusion of butter (36, 47). This finding may reflect the complexity of the dairy food matrix, which contains other components such as micronutrients and bioactive peptides, both of which have been proposed to reduce the risk of cardiometabolic diseases (64, 198).

Despite recent meta-analyses questioning the link between high SFA intake and CVD risk and mortality (18, 19), there is increasing evidence that overall, a reduction in dietary SFA, iso-energetically replaced, particularly with PUFA, leads to reduction in CVD risk (199). The current evidence for a replacement with MUFA on CVD risk and mortality appears less clear, compared to replacement with PUFA (23). This discordance may be attributed to variations in sources of fatty acids (FA) within the study designs, and lack of RCTs with hard endpoints, as detailed in recent meta-analyses and reviews (23, 33, 199). Partial replacement of SFA in milk fat with unsaturated FA can be achieved by supplementing the dairy cow diet with plant oils or oil seeds (200). This alternative strategy could limit the entry of SFA from the food chain, without removing the beneficial aspects of dairy product consumption (150). Current evidence on the impact of SFA-reduced milk and dairy products on risk biomarkers of cardiovascular health from RCTs is limited, with most studies focusing on the use of FA-modified butter consumption as the study product and changes in fasting serum lipid levels as biomarkers of CVD risk (134). Additionally, more evidence is needed to investigate the

impact of FA-modified dairy consumption on novel CVD risk factors, including inflammatory markers, endothelial function and arterial stiffness(150).

In order to address this knowledge gap, the REplacement of SaturatEd fat in dairy on Total cholesterol (RESET) study was designed to investigate the impact of a high-dairy diet incorporating SFA-reduced, MUFA-enriched (modified) dairy products on established and novel CVD risk markers, compared to dairy foods with a FA profile typical of retail products (control), in adults at moderate CVD risk. We hypothesized that intake of the modified dairy products for 12-wk would result in an improvement in fasting TC (composed of LDL-C and HDL-C; primary outcome), flow-mediated dilatation (FMD) and other markers of CVD risk as secondary outcomes, compared to intake of the control dairy products.

3.3 Methods

3.3.1 Subjects and study design

The RESET trial was given a favourable ethical opinion for conduct by the University of Reading Ethics Committee (Project Reference 13/43), registered at clinicaltrials.gov as NCT02089035 and carried out in accordance with the Declaration of Helsinki. Men and women aged 25-70 y with moderate CVD risk were recruited from Berkshire, United Kingdom, from February 2014 to September 2015 in three cohorts. The study was completed in April 2016. All participants provided written informed consent. Details of the study inclusion and exclusion criteria are reported in Markey et al (201). In summary, a total of 130 potential participants attended a screening visit, resulting in 76 eligible for randomization (**Supplemental Figure 3.1** for participant flowchart). Data collected at the screening visit on fasting serum TC, HDL-C, glucose, blood pressure, BMI and family history of myocardial infarction were used to determine eligibility, whereby a score of ≥2 CVD risk points based on

a modified Framingham risk analysis suggested a 1.5-fold higher CVD risk compared to the population mean (164, 165, 201).

The RESET intervention was a double-blind, cross-over RCT with an 8-wk washout period between the two 12-wk dietary periods. A minimization technique stratifying by age, gender, BMI and fasting serum TC was used by a single researcher to randomise eligible participants to one of two dietary intervention groups, whereby Group 1 received the modified diet first (Diet A), followed by the control diet (Diet B) and vice-versa for Group 2 (202). In brief, participants received individual verbal and written instructions to incorporate the study products into their habitual diet following the baseline clinical visit (wk-0). Participants achieved an iso-energetic high-fat daily dietary exchange (38%TE from total fat) by replacing dairy products, oil/spreads and snacks habitually consumed with either the SFA-reduced, MUFA-enriched ultra-high temperature (UHT) milk, Cheddar cheese and butter (modified; dietary target: 16%TE SFA, 14%TE MUFA) or matched products with a FA profile typical of retail products (control; 19%TE SFA, 11%TE MUFA). The two intervention diets were equal in total exchangeable fat content (approx. 41 g/d). Relative to the control diet, consumption of the modified diet led to a mean decrease of total SFA intake of 2.5%TE and a parallel mean increase of total MUFA intake of 3.7%TE (201) in a diet that contained 38%TE as total fat. The nutritional composition of the study products is presented in **Table 3.1**.

3.3.2 Intervention diets

Full details of the production of the SFA-reduced, MUFA-enriched (modified) milk and dairy products have been published elsewhere (203). Briefly, following a 4-wk supplementation of the total mixed ration diet of multiparous Holstein-Friesian dairy cows with approximately 1 kg/d of high oleic sunflower oil (AAK Ltd, Hull, UK), the milk was collected for the purposes of manufacturing UHT milk, Cheddar cheese and butter. Control dairy products were provided by Arla Foods UK.

A food-exchange model was designed for the RESET study, based on the strategy for adults with increased CVD risk followed by the Dietary Intervention and Vascular Function (DIVAS) and the Reading, Imperial, Surrey, Cambridge and Kings (RISCK) studies (164, 204), both based on the National Diet and Nutrition Survey (NDNS) for adults (aged 19-64 y) (205) and is described in detail in Markey et al. (201). Participants were provided with the study products, in blinded packaging, to take home and were asked to consume 340 g/d of UHT milk, 45 g/d of Cheddar cheese and 21.5 g/d (control diet) or 25.1 g/d (modified diet) of butter (for nutrient composition, see **Supplemental Table 3.1**). Compliance was assessed using 4-d weighed food diet diaries (weeks 0, 12, 20 and 32), daily records of study product consumption and analysis of plasma phospholipid FA (PL-FA) which served as a short-term biomarker. To maintain constant body weight, participants were weighed every 4-wk by the investigators and any changes ±1 kg were addressed. Results from the analysis of the PL-FA composition, which showed a significant difference between the modified and control treatments following the two 12-wk interventions ($P \leq 0.01$), have been previously presented (for a summary of the results, see **Supplemental Table 3.2**) (201).

Additionally, self-reported physical activity was assessed using the International Physical Activity Questionnaire (IPAQ)-long form (206). A 100 mm visual analogue scale (VAS) questionnaire (207) was completed by participants at the end of each 12-wk intervention period to assess the visual appeal, smell, taste, palatability and aftertaste of the UHT milk, cheese and butter.

3.3.3 Study visits

Study visits were conducted in a temperature controlled-environment (22 ± 1 °C) at the Hugh Sinclair Unit of Human Nutrition, University of Reading, during weeks 0, 12, 20 and 32. Participants were asked to refrain from alcohol and aerobic exercise for 24 h prior to each visit and fast overnight for 12 h after consuming a low fat standard meal (< 1.46 MJ; < 7 g total fat) that was provided by the researchers. Participants drank only low nitrate mineral water (Buxton) during the 12 h fast and on the morning of the study visit. Following a 30 min rest in the supine position, non-invasive vascular function measurements were performed by the same trained researcher for all 4 visits for each study participant. Fasted venous blood samples were then collected into lithium heparin, EDTA or serum separator blood collection tubes (VACUETTE, Greiner Bio-One) and either kept briefly on ice (for plasma samples) or left to clot for a minimum of 30 minutes at room temperature (for serum samples) before centrifugation at 1700 x g for 15 min at 4°C. Plasma and serum were aliquotted prior to storage at -80°C for further analysis.

3.3.4 Biochemical analyses, risk scores and predictive equations

Serum lipids (TC, HDL-C and triacylglycerol), glucose, apolipoprotein (apo)B, C-reactive protein and non-esterified fatty acids (NEFA) were measured using an ILAB 600 autoanalyzer (TC, HDL-C, triacylglycerol, glucose and C-reactive protein reagents and analyzer: Werfen UK Ltd); apoB reagent: Randox laborarories Ltd; NEFA reagent: Alpha Laboratories Ltd.). The Friedewald equation was used to estimate fasting LDL-C (49). ELISA kits were used to analyze circulating serum insulin (Dako UK Ltd.), plasma vascular and intercellular adhesion molecules (VCAM, ICAM), E-selectin and P-selectin (R&D Systems Europe Ltd.). Insulin resistance was estimated by HOMA-IR, while insulin sensitivity was estimated with the revised quantitative insulin sensitivity check index (rQUICKI), using standard equations (106). Measurement of plasma nitrite and nitrate was determined by HPLC

(ENO-30, EiCom Corporation, San Diego) coupled with online reduction of nitrate to nitrite, and post-column derivatization with the Greiss reagent (ENO-30 Analyzer, EiCom Corporation, San Diego) (208). Mean inter-assay CVs were <10% for all assays.

Risk scores pre- and post-intervention for both diets were calculated using the modified Framingham CVD score (201, 209).

Furthermore, post-intervention changes in dietary total fatty acid intake as assessed by the 4-d weighed food diet diaries, were used to calculate the predicted change in TC and LDL-C with the use of published equations, which included change in FA intake of total SFA, MUFA and PUFA (210-214).

3.3.5 NMR Metabolomics

A high-throughput nuclear magnetic resonance (^{1}H-NMR) metabolomics platform (Nightingale Health Ltd., Finland) was used to quantify the particle size of LDL and HDL lipoprotein subclasses, as previously described (215). This manuscript will present the change from baseline following the two intervention diets of the LDL-particle (LDL-P; classified by their mean diameter size as small (S-LDL-P, 18.7 nm), medium (M-LDL-P, 23 nm) and large (L-LDL-P, 25.5 nm)) and HDL-particle (HDL-P; classified as small (S-HDL-P, 8.7 nm), medium (10.9 nm), large (12.1 nm) and very large (14.3 nm) concentrations.

3.3.6 Assessment of vascular function and 24h ambulatory blood pressure

Prior to assessing the vascular function measurements, clinic supine BP was measured on the left upper arm with the use of a validated BP monitor (Omron MX2 Digital Automatic Upper Arm Blood Pressure Monitor; OMRON, UK). Endothelial function was assessed using FMD as described elsewhere (83). Briefly, trained researchers used a CX50 CompactXtreme portable ultrasound system (Philips HealthCare, UK) to determine endothelial-dependent vasodilation of the brachial artery following defined guidelines. Electrocardiogram-gated

images were captures at 0.25 frames/s with the use of an image-grabbing software (Medical Imaging Applications LLC, USA) (83). A single researcher, blinded to diet allocation, analyzed the images in duplicate with the use of a wall-tracking software. The maximum change in post-occlusion brachial artery diameter relative to the baseline diameter represented the calculated FMD response, expressed as a percentage (%FMD). The carotid intima media thickness (cIMT), defined as the distance between the media-adventitia interface to the intima-lumen interface, was assessed using a 12-3 linear array transducer and CX50 ultrasound system (Philips HealthCare, UK) on the right and left common carotid artery (CCA), following published guidelines (216). For each side of the neck, a minimum of three images of the far-wall IMT of the artery were captured when the carotid vessel was at its widest diameter, reflecting artery expansion, using an automated edge-tracking software (Philips, QLAB) (84). The IMT value represents the average of left and right CCA images, when images of the carotid vessel were at its widest diameter.

Radial pulse wave analysis and carotid-femoral pulse wave velocity (m/s) were measured in triplicate using a SphygmoCor (AtCor Medical, Australia) (217). Pulse wave analysis determined the augmentation index corrected for a heart rate of 75 beats/min (%). The stiffness index (m/s) and reflection index (%) were determined by digital volume pulse (Pulse Trace PCA2; Micro Medical Ltd., UK) and provided measures of arterial stiffness and vascular tone, respectively (218).

Ambulatory blood pressure (ABP) and heart rate were measured using A/A grade automated oscillometric ABP monitors (A&D Instruments Ltd., UK) every 30 min from 0700 h to 2159 h and every 60 minutes from 2200 h to 0659 h. Participants were asked to wear the ABP monitors for at least 48 h prior to each of the four visits and repeat each measurement on the same selected day of the week. Sleep times were recorded by the participants and used to

estimate mean 24-h day and night measurements. Pulse pressure was calculated as the difference between systolic blood pressure (SBP) and diastolic blood pressure (DBP).

3.3.7 Statistical analysis

For our primary outcome (serum TC, composed of LDL-C and HDL-C) a total of 54 eligible participants were required to achieve a ~0.3 mmol/L predicted reduction in TC, calculated using Keys equations and taking into consideration a difference of 4.0%TE from SFA and a population mean of 4.54 mmol/L (SD 0.5), 80% power, 5% significance level, allowing for a 15% dropout rate. The same cohort size was also sufficient to detect a 1.5% inter-group difference in the FMD response (key secondary outcome), with a power of 80% at $P < 0.05$ (179). A $P < 0.05$ was considered significant for the primary and key secondary outcomes. For all other variables, a $P < 0.01$ was considered significant to adjust for multiple comparisons.

Statistical analyses were predominantly conducted using the SAS 9.4 University edition statistical software (SAS Institute Inc., Cary, NC, USA). All variables were checked for normality and data was logarithmically transformed where needed. Treatment effects were evaluated using a linear mixed model, with the differences from baseline (Δ; wk-12 – wk-0 and wk-32 – wk-20) as the dependent variable, adjusted for fixed effects of baseline values of the assessed variable, period, sequence, treatment, age, gender and BMI. Participant was included as a random effect. Results from the VAS questionnaire were analysed using paired *t*-test to assess differences between study product ratings. As this is a proof-of-concept study rather than a confirmatory trial, a per-protocol analysis approach was adopted. No period effects were observed for any outcome measure. Results in tables are presented as means ± SEMs.

3.4 Results

3.4.1 Study participation, anthropometric measures and product rating

A total of 76 eligible participants were randomly recruited to the intervention, 54 of whom completed both arms of the study successfully (Supplemental Figure 1). Baseline screening characteristics of the participants who completed the study, have been previously published (201). Briefly, 31 men and 23 women had the following characteristics (mean ± SEM): age 52 ± 3 y; BMI 25.8 ± 0.5 kg/m^2; waist 89.8 ± 1.4 cm; TC 5.49 ± 0.12 mmol/L; HDL-C 1.48 ± 0.04 mmol/L; glucose 5.37 ± 0.11 mmol/L; clinic SBP 123 ± 2 mm Hg and clinic DBP 74 ± 1 mm Hg). Based on screening parameters, participants had a CVD risk score of 3.0 ± 0.2, indicating a 1.5-fold higher risk. Participant activity scores, based on the IPAQ questionnaire, did not significantly differ between treatments (change post modified diet: 364 ± 128 min/week¹; change post control diet: -641 ± 247 min/week¹; $P = 0.17$). The VAS questionnaire, which assessed the visual appeal, smell, taste, aftertaste and palatability of the three dairy products in the modified and control diet, showed no significant treatment effect (data not shown).

3.4.2 Fasting serum lipids, biochemical analyses and predictive equations

There was a borderline impact on the change from baseline in serum TC between the two diets ($P = 0.08$). However, there was a significant attenuation in the rise of LDL-C following the modified diet, compared to the control diet (0.9% and 5.5% respectively, $P = 0.03$; **Table 3.2**). There was no significant difference in change from baseline in HDL-C between the two diets. There was a significant differential effect of the diets on the LDL-C:HDL-C ratio, which decreased by 2.5% following the modified diet, while it increased by 2.1% following the control diet ($P = 0.04$) (Table 3.2). No significant treatment effect was

observed for any other component of the lipid profile or indexes of insulin sensitivity/resistance (Table 3.2).

Using the published equations, greater changes in TC and LDL-C concentrations were predicted compared to the observed study results (Supplemental Table 3.3). Compared to baseline values, TC concentrations following the modified diet were predicted to increase by 3.1% (210-213) or 2.7% (214) compared to the observed 2.2%. Following the control diet, predicted increases in TC were calculated to increase by 6.7% (210-212), 5.7% (213) and 5.3% (214) respectively, compared to the observed 5.5%. Similarly, change from baseline in LDL-C concentrations following the modified diet were predicted to increase by 3.5% (211, 212), 2.3% (213) and 4% (214), whereas following the control diet values were predicted to increase by 7.6% (211, 212), 6.4% (213) and 8.2% (214) compared to the observed 0.9% and 5.5% of the modified and control diet, respectively.

3.4.3 NMR metabolomics

There were no significant differences in the concentrations in both LDL-P and HDL-P subclass distributions following the two intervention diets and compared to baseline values (Table 3.2).

3.4.4. Vascular function and 24-hour blood pressure, markers of endothelial activation, nitric oxide and inflammation

The additional measures of vascular function (cIMT) and arterial stiffness measured by PWA (augmentation index), PWV and DVP (stiffness index and reflection index) and blood and pulse pressure (**Table 3.3**) did not result in any significant differences following the two intervention treatments.

There was a differential effect of the dairy fat diets on fasting plasma nitrite concentrations, with a 15.4% increase observed relative to baseline after the modified diet and

a 26.7% decrease observed for the control diet (Table 3.3). There was no significant effect of the diets on plasma nitrate or any of the markers of endothelial activation or inflammation (Table 3.3).

3.4.5 Risk scores

Risk scores, calculated with the use of the modified Framingham risk score, did not significantly differ following the two intervention diets and relative to baseline values (Table 3.3).

3.5 Discussion

To our knowledge this is the first study to report on the long-term effects of partially replacing SFA with MUFA in a variety of dairy products on the fasting lipid profile, FMD and CVD markers, in adults at moderate CVD risk. We observed a tendency for a difference in TC compared to baseline values between the two diets, which did not reach statistical significance. However, we observed that the modified diet significantly attenuated the LDL-C and TC:HDL-C ratio, raising effect of the control diet with no impact on HDL-C or LDL-P and HDL-P size distribution. With the exception of a significant decrease in fasting nitrite concentrations following the modified diet, little impact on other vascular function and CVD risk markers were found.

Investigating how to optimally replace dietary SFA with a suitable macronutrient presents a number of challenges, as both the single nutrient and the food source should be considered within dietary patterns and risk markers. (23). The existing body of evidence, from RCTs and prospective cohort studies, which have investigated a replacement of SFA with *cis*-MUFA to reduce CVD mortality, is limited. Lack of clarity may in part be explained by limited studies with hard endpoints, variation in design of RCTs and ultimately the source of MUFA, as it appears that a beneficial outcome on risk markers is primarily observed from sources of plant oils and nuts, while other sources may lead to neutral results (23).

As dairy products represent the largest food source of dietary SFA intake in the UK (10), this food group provides a suitable vehicle for investigating partial SFA replacement with MUFA. A review of previously published human trials investigating the impact of FA-modified dairy products, indicated a tendency towards a beneficial effect on fasting lipid markers (134). In an 8-wk crossover study led by Noakes et al. (135), healthy adults (49 ± 10.3 y) consumed FA-modified milk, cheese, butter and ice-cream (16%TE SFA, 11.9%TE MUFA) or control dairy products (18.2%TE SFA, 9.1%TE MUFA). The study reported a significant decrease in TC (-4.3%) and LDL-C (-5.3%) following the modified dairy compared to the control diet. However, it is difficult to draw meaningful comparisons between the two studies, as baseline values were not included in the statistical analysis reported by Noakes et al (135).

Our results indicate that a high fat, high dairy diet including conventional dairy foods (control) led to an increase in LDL-C (5.5%), which was attenuated by the modified diet (0.9%). The observed LDL-C attenuation may be associated with the effects of dietary fatty acids on LDL-receptor expression and activity (56). There is increasing evidence suggesting that dietary SFA intake may differentially impact on LDL subclass concentrations, with small LDL-P recognised as more strongly associated with progression of atherosclerosis and CVD outcomes than larger LDL-P (219). In our study, the two intervention diets did not significantly impact on the particle size distribution of both LDL and HDL subclasses, which may be explained from both the dairy nature of the diets and the modest, partial substitution of SFA with MUFA in the modified compared to the control diet.

A comparison of predictive equations shows that our results appear more in line with the equations by Clarke et al. (213). It is worth noting that early predictive equations (210, 211) did not include a coefficient of change in dietary MUFA, as it was considered a neutral FA class with little impact on the lipid profile. Interestingly, the observed attenuation in LDL-

C following the modified diet was found even though the dietary total *trans* fatty acid (TFA) intake had increased (2.5%TE vs 1.2%TE) compared to the control and relative to baseline (201). Although it was not possible to adequately discriminate between the participants' intake of industrial and ruminant TFAs in the two diets using the dietary analysis software, supplementation of the bovine diet led to a greater proportion of ruminant TFA (rTFA) in the modified dairy products (203). The observed higher TFA intake following the modified diet exceeded the maximum 2% recommendation from food energy and the current mean TFA intake in UK adults (0.5%TE and 0.5% food energy) (10, 197). However, it is worth noting that the observed increased TFA intake following the modified diet did not appear to adversely affect fasting lipid biomarkers. This is in agreement with previous studies, which concluded that there were no significant adverse physiological effects of rTFA, unless consumed in high quantities (220). Furthermore, the observed increases in LDL-C following the control diet were also slightly lower than theoretically predicted. The attenuation in LDL-C concentrations following both diets compared to predicted changes, may be partly explained by the presence within the dairy matrix of bioactive compounds, such as proteins and micronutrients, which may mediate an attenuation in LDL-C concentrations through synergistic mechanisms (36). As the aim of our study was to provide diets high in dairy with a differential FA composition, the protein and micronutrient content of the study products was not significantly altered (201, 203). Considering the apparent effect on LDL-C, future investigation is warranted to explore potential mechanisms of other components within the matrix of the FA-modified dairy products.

Progression of endothelial dysfunction and arterial stiffness characterize vascular dysfunction, mediating CVD risk (150). Recently, a prospective study demonstrated that increased milk and dairy product (with the exception of butter) intake was associated with a decrease in arterial stiffness in men (94). In our study, we did not observe significant

differences in vascular function, vascular stiffness, blood pressure and biomarkers related to endothelial function and low-grade inflammation between the two diets. The only exception was a statistically significant increase in plasma nitrite concentrations following the modified diet, with respect to baseline values and the control diet. Nitric oxide (NO) is essential for maintaining vascular homeostasis and a reduced bioavailability may suggest endothelial dysfunction (221). These differences in nitrite concentrations between treatments should be interpreted with caution as they may reflect not only measures of vascular function but also the participants' dietary intake prior to study visits. Furthermore, the short half-life and rapid conversion to nitrate may also have affected the observed results (222).

Lastly, our results showed no significant treatment effect in fasting indexes of insulin, insulin sensitivity and glucose concentrations. This is in agreement with RCTs on modified dairy products (34, 223). Although a small number of studies on dairy consumption have proposed a beneficial impact on insulin sensitivity (77), well-designed and suitably powered RCTs are still needed to confirm effects of specific dairy products on insulin sensitivity.

Strengths of our study include its long-term, double-blind and randomized design. We employed a food chain approach to reducing SFA in dairy products, an agricultural-based reformulation initiative which has the potential to prevent movement of SFA into other food chain entry points, and results in clean label products that are favoured by consumers (224). Encapsulation technology could provide a potential strategy implemented in the bovine feed to limit the observed increase of TFA in the modified milk. Additionally, participant ratings by VAS of the study products was further supported by consumers generally accepting FA-modified milk and dairy products, when tasted in a blinded manner, as recently published (225). The implementation of high dairy diets with differential FA composition was used to explore traditional and novel biomarkers of CVD risk. This meant that participants consumed large quantities of dairy products, which may not reflect habitual dairy intake of the

participants (10). However, our findings suggest a favourable impact on fasting LDL-C following consumption of FA-modified dairy products compared with commercial retail dairy, warranting future investigation with lower, more representative dairy intakes to determine if similar effects are observed. Finally, as our participants were at moderate CVD risk, our results may not be comparable in a healthy adult population.

In conclusion, our study, which is the first to investigate the impact FA-modified UHT milk, Cheddar cheese and butter on established and novel CVD risk outcomes, indicates that high daily consumption of SFA-reduced, MUFA-enriched dairy products attenuated the rise of LDL-C concentration observed with conventional commercially available dairy products, without significantly impacting TC and HDL-C. Further research is warranted to explore the impact of FA-modified dairy products on CVD risk biomarkers in both healthy and at risk populations.

3.6 Acknowledgments

The authors gratefully acknowledge all participants who took part in the study. We thank S. Hargreaves, K. Jenkins, R. Mihaylova, the technical staff and research assistants/students (V. Bines, C. Brilley, L. Bula, Y. Chatzidiakou, V. Clifton, H. Dong, A. Garcimartin, C. Holland, J. Guo, A. Kanneganti, T. Loughman, E. Mertens, J. Ryan, L. Sellem, T. Staff and H. Zhang) for their assistance with recruitment, data collection, the blinding of intervention foods, data entry or food diary analysis. We also thank Dr Gunter Kuhnle, Dr Virag Sagi-Kiss and Mr Chris Humphries for technical assistance during the nitric oxide analysis.

J.A.L., D.I.G. and K.G.J designed the human study; C.C.F., A.S.G., D.J.H and D.I.G. designed and produced the modified dairy products; D.V and O.M conducted the research; D.V, O.M, and K.E.K analyzed data or performed statistical analysis; S.T. provided statistical advice; D.V and O.M wrote the manuscript, which was modified by all co-authors; and J.A.L. had primary responsibility for final content. All authors have read and approved the final manuscript. Arla Foods UK and AAK UK provided the control dairy study products and high oleic sunflower oil, respectively, and were not involved in the study design or data interpretation.

Table 3.1 Nutrient composition of the study products of the modified and control intervention diets[1]

	Modified diet			Control diet		
	UHT Milk	Cheese	Butter	UHT Milk	Cheese	Butter
Daily amount, g	340.0	45.0	25.1	340.0	45.0	21.5
Energy, MJ/d	0.79	0.69	0.76	0.75	0.76	0.65
Fat, g/d	8.8	12.6	20.4	8.6	15.1	17.4
SFA, g/d	4.4	6.3	10.2	5.7	10.2	11.1
MUFA, g/d	3.6	5.0	8.1	2.1	3.5	4.4
PUFA, g/d	0.3	0.4	0.7	0.3	0.4	0.6
TFA, g/d	0.9	1.4	2.1	0.3	0.5	0.7
CHO, g/d	16.0	1.2	0.2	14.9	1.4	0.4
Protein, g/d	11.1	12.1	0.1	10.2	10.6	0.0
Calcium, mg/d	404.0	410.0	4.49	428.0	340.5	3.65

[1] Values represent daily required intakes of the three study products. Adapted with permission from Markey et al. (201) and Kliem et al. (203).

CHO, carbohydrates; TFA, *trans* fatty acids; %TE, percent total energy.

Table 3.2 Fasting lipid profile, lipid ratios, particle distribution and indexes of insulin resistance at baseline (wk-0) and post-intervention (wk-12) of the modified and control diet[1].

	Modified diet			Control diet			*P*[2]
	Baseline	Post	Δ	Baseline	Post	Δ	
Fasting lipid profile							
TC, mmol/L	5.54 ± 0.13	5.66 ± 0.14	0.12 ± 0.07	5.47 ± 0.12	5.77 ± 0.13	0.29 ± 0.06	0.08
LDL-C, mmol/L	3.47 ± 0.11	3.50 ± 0.12	0.03 ± 0.06	3.43 ± 0.10	3.62 ± 0.11	0.19 ± 0.05	0.03
HDL-C, mmol/L	1.51 ± 0.04	1.55 ± 0.04	0.04 ± 0.02	1.50 ± 0.04	1.58 ± 0.05	0.07 ± 0.02	0.55
Triacylglycerol, mmol/L	1.24 ± 0.07	1.35 ± 0.10	0.11 ± 0.07	1.18 ± 0.06	1.24 ± 0.07	0.06 ± 0.05	0.32
Apolipoprotein B, g/L	1.01 ± 0.03	1.03 ± 0.03	0.02 ± 0.01	1.00 ± 0.03	1.03 ± 0.03	0.03 ± 0.01	0.47
NEFAs, μmol/L	567 ± 24	514 ± 23	-53 ± 27	556 ± 27	515 ± 23	-41 ± 27	0.84
Lipid ratios and particle distribution							
LDL-C:HDL-C	2.39 ± 0.09	2.33 ± 0.09	-0.06 ± 0.04	2.35 ± 0.09	2.40 ± 0.09	0.05 ± 0.04	0.04
TC:HDL-C	3.79 ± 0.12	3.76 ± 0.12	-0.03 ± 0.05	3.74 ± 0.11	3.78 ± 0.12	-0.04 ± 0.03	0.13
LDL-P (nmol/L)							
Small	172 ± 4	175 ± 5	3 ± 1	169 ± 4	176 ± 4	7 ± 2	0.36
Medium	278 ± 8	282 ± 8	5 ± 2	268 ± 9	284 ± 8	16 ± 8	0.34
Large	570 ± 16	581 ± 17	11 ± 5	551 ± 18	581 ± 16	38 ± 19	0.34
HDL-P (nmol/L)							
Small	10100 ± 109	10307 ± 128	207 ± 102	10080 ± 125	10354 ± 117	247 ± 112	0.85
Medium	3974 ± 102	4106 ± 111	132 ± 52	4028 ± 111	4118 ± 117	87 ± 55	0.46
Large	1592 ± 106	1652 ± 106	60 ± 53	1620 ± 91	1656 ± 101	36 ± 43	0.30
Very Large	229 ± 12	243 ± 14	14 ± 8	229 ± 11	238 ± 12	13 ± 7	0.33
Indexes of insulin resistance							
Glucose, mmol/L	5.38 ± 0.1	5.32 ± 0.1	-0.06 ± 0.07	5.4 ± 0.1	5.44 ± 0.14	0.04 ± 0.09	0.34

Insulin, pmol/L	41.3 ± 3.2	39.5 ± 2.9	-1.9 ± 1.9	39.8 ± 3.5	47.3 ± 6.1	7.5 ± 4.5	0.09
HOMA-IR	1.66 ± 0.13	1.55 ± 0.12	-0.11 ± 0.08	1.59 ± 0.14	1.88 ± 0.22	0.29 ± 0.12	0.08
rQUICKI	0.18 ± 0.00	0.19 ± 0.00	0.01 ± 0.00	0.18 ± 0.00	0.18 ± 0.00	0.00 ± 0.00	0.11

[1] Values are means ± SEMs. n = 54. Data not normally distributed were log transformed. HOMA-IR, homeostatic model of insulin resistance; NEFA, non-esterified fatty acids; rQUICKI, revised quantitative insulin sensitivity index; TC, total cholesterol; Δ, change from baseline.

[2] Linear mixed model analyses were used to calculate overall effect of treatment based on change-from-baseline values, with adjustments made for fixed effects of baseline values of the assessed variable, period, treatment sequence, gender, age and BMI. Participant was included as a random effect. For primary outcome measure (cholesterol profile), $P \leq 0.05$ was deemed significant. For all other secondary outcomes, $P \leq 0.01$ was deemed as significant.

Table 3.3 Vascular outcomes, blood pressure, endothelial and inflammatory biomarkers and CVD risk scores at baseline (wk-0) and post-intervention (wk-12) of the modified and control diet [1].

	Modified diet			Control diet			
	Baseline	Post	*Δ*	Baseline	Post	*Δ*	*P*[2]
Endothelial function							
% FMD							
Pre-occlusion artery diameter, mm							
Vascular function							
cIMT, mm	0.59 ± 0.02	0.59 ± 0.02	0.00 ± 0.00	0.58 ± 0.01	0.59 ± 0.02	0.01 ± 0.01	0.83
Arterial stiffness							
PWA, m/s							
AI at 75 beats/min, %	18.9 ± 1.6	19.1 ± 1.4	0.2 ± 0.7	17.1 ± 1.4	18.5 ± 1.4	1.4 ± 0.8	0.62
PWV, m/s	7.6 ± 0.3	7.4 ± 0.2	-0.2 ± 0.5	7.5 ± 0.2	7.8 ± 0.3	0.3 ± 0.44	0.21
DVP							
Stiffness index, m/s	7.6 ± 0.3	7.8 ± 0.4	0.2 ± 0.4	7.9 ± 0.4	8.2 ± 0.3.4	0.3 ± 0.33	0.96
Reflection index, %	69.1 ± 1.9	70.8 ± 1.9	1.7 ± 1.9	71.2 ± 1.8	70.3 ± 1.6	-0.9 ± 1.3	0.57
PPT, m/s	247 ± 10	241 ± 10	-6 ± 11	236 ± 10	228 ± 9	-8 ± 14	0.61
Clinic BP, mm Hg							
SBP	120 ± 2	119 ± 1	-1 ± 1	120 ± 2	119 ± 2	-1 ± 1	0.44
DBP	70 ± 1	69 ± 1	-1 ± 1	70 ± 1	70 ± 1	0 ± 1	0.24
Ambulatory BP, mm Hg							
24-h SBP	123 ± 2	124 ± 2	1 ± 1	123 ± 2	124 ± 2	1 ± 1	0.90
24-h DBP	74 ± 1	74 ± 1	0 ± 1	74 ± 1	75 ± 1.0	1 ± 1	0.60
24-h PP	49 ± 1	50 ± 1	1 ± 1	49 ± 1	49 ± 1	1 ± 1	0.64
Day SBP	127 ± 2	128 ± 2	1 ± 1	127 ± 2	128 ± 2	1 ± 1	0.92

Day DBP	77 ± 1	77 ± 1	0 ± 1	77 ± 1	77 ± 1	0 ± 1	0.51
Day PP	50 ± 1	51 ± 1	1 ± 1	50 ± 1	50 ± 1	0 ± 1	0.72
Night SBP	108 ± 2	110 ± 2	2 ± 2	108 ± 2	107 ± 2	-1 ± 1	0.15
Night DBP	63 ± 1	65 ± 1	2 ± 1	63 ± 1	64 ± 1	1 ± 1	0.29
Night PP	45 ± 1	45 ± 1	0 ± 1	44 ± 1	43 ± 1	-1 ± 1	0.26
Circulating biomarkers of endothelial activation and inflammation							
Nitric oxide, μmol/L	17.88 ± 1.60	17.40 ± 1.29	-0.48 ± 1.49	17.11 ± 1.04	16.74 ± 1.30	-0.37 ± 1.57	0.33
Nitrite, μmol/L	0.13 ± 0.02	0.15 ± 0.02	0.02 ± 0.01	0.15 ± 0.02	0.11 ± 0.02	-0.03 ± 0.02	0.01
Nitrate, μmol/L	17.74 ± 1.60	17.25 ± 1.29	-0.49 ± 1.49	16.96 ± 1.03	16.63 ± 1.30	-0.33 ± 1.56	0.51
VCAM-1, ng/mL	534.9 ± 29.7	537.9 ± 29.7	3.0 ± 20.7	542.8 ± 28.9	499.9 ± 28.3	-40.1 ± 1.7	0.08
ICAM-1, ng/mL	84.4 ± 6.6	79.2 ± 6.5	-5.1 ± 4.3	80.3 ± 8.6	87 ± 8.6	6.7 ± 5.5	0.64
E-selectin, ng/mL	27 ± 1.9	25.2 ± 1.7	0.6 ± 0.7	24.3 ± 1.7	25.2 ± 1.9	0.8 ± 0.6	0.84
P-selectin, ng/mL	25.2 ± 1.4	26.6 ± 1.6	1.5 ± 0.7	25.9 ± 1.5	26.1 ± 1.5	0.1 ± 0.7	0.30
C-reactive protein, mg/L	2.03 ± 0.76	1.59 ± 0.32	-0.44 ± 0.5	2.12 ± 0.60	1.75 ± 0.42	-0.37 ± 0.62	0.51
Risk score calculations							
CVD score[3]	2.7 ± 0.2	2.7 ± 0.2	0.0 ± 0.1	2.5 ± 0.2	2.8 ± 0.2	0.3 ± 0.1	0.09

[1] Values are means ± SEMs. $n = 54$ (except for DVP, where $n = 46$; PWA, $n = 50$; PWV, $n = 29$). Data not normally distributed were log transformed. AI, augmentation index; BP, blood pressure; cIMT, carotid intima media thickness; DBP, diastolic BP; DVP, digital volume pulse; FMD, flow-mediated dilation; ICAM-1, intercellular adhesion molecule 1; PP, pulse pressure; PWA, pulse-wave analysis; PWV, pulse-wave velocity; SBP, systolic BP; VCAM-1, vascular adhesion molecule 1; Δ, change from baseline.

[2] Linear mixed model analyses were used to calculate overall effect of treatment based on change-from-baseline values, with adjustments made for fixed effects of baseline values of the assessed variable, period, treatment sequence, gender, age and BMI. Participant was included as a random effect. For the key secondary outcome (FMD), $P \leq 0.05$ was deemed significant. For all other secondary outcomes, $P \leq 0.01$ was deemed as significant.

[3] Assessed with the use of a modified Framingham risk score, where a score of ≥ 2 points relates to a 1.5-fold higher risk of CVD than the population mean (164, 165, 201).

Supplemental Table 3.4 Target FA intakes and reported dietary intake at baseline (wk-0) and the change from baseline (*Δ*) following consumption of the modified and control dairy products for 12-wk in adults at moderate risk of cardiovascular disease[1].

	Modified diet			Control diet			
	Baseline	*Δ*	T[2]	Baseline	*Δ*	T[2]	*P*[3]
Energy, MJ/d	8.2 ± 0.3	1.0 ± 0.4		8.5 ± 0.4	0.5 ± 0.3		0.60
Total fat, %TE	36.1 ± 0.8	5.0 ± 1.1	38.0	36.5 ± 0.8	3.0 ± 0.9	38.0	0.03
SFA, %TE	14.2 ± 0.5	2.7 ± 0.6	19.0	13.9 ± 0.5	5.2 ± 0.6	16.0	<0.001
MUFA, %TE	11.7 ± 0.4	3.6 ± 0.5	11.0	11.9 ± 0.4	-0.1 ± 0.3	14.0	<0.0001
n-6 PUFA, %TE	3.9 ± 0.2	-0.5 ± 0.2		4.6 ± 0.2	-1.3 ± 0.2		0.08
n-3 PUFA, %TE	0.7 ± 0.0	-0.1 ± 0.1		0.8 ± 0.1	-0.2 ± 0.1		0.34
Total PUFA, %TE	4.6 ± 0.2	-0.4 ± 0.2		5.8 ± 0.4	-1.4 ± 0.3		0.14
TFA, %TE	0.9 ± 0.1	1.6 ± 0.1		1.0 ± 0.1	0.3 ± 0.1		<0.0001
Protein, %TE	16.9 ± 0.5	-0.7 ± 0.6		16.3 ± 0.5	-0.2 ± 0.5		0.80
Carbohydrates, %TE	46.6 ± 1.3	-4.4 ± 1.5		46.3 ± 0.9	-3.0 ± 0.9		0.19
Alcohol, %TE	2.9 ± 0.5	-0.2 ± 0.5		3.1 ± 0.5	0.0 ± 0.4		0.35
Dietary fiber (AOAC), g/d	20.2 ± 1.1	-0.8 ± 1.2		20.4 ± 1.1	1.6 ± 0.9		0.03
Sodium, g/d	2.5 ± 0.2	-0.6 ± 0.1		2.7 ± 0.2	-0.5 ± 0.1		0.03

Adapted with permission from Markey et al. (15).

[1] Values are given as means ± SEMs. Dietary intakes estimated from 4-d weighed dietary records at baseline (week 0) and change from baseline (*Δ*). AOAC, association of analytical communities; TE, total energy; TFA, t*rans* fatty acids.

[2] Target FA intakes for the modified and control dietary exchange periods.

[3] Mixed model analyses were used to calculate overall effect of treatment based on change-from-baseline values, with adjustments made for fixed effects of baseline values of the assessed variable, period, treatment sequence, gender, age and BMI. Participant was included as a random effect. $P \leq 0.01$ deemed as significant.

Supplemental Table 3.5 Proportion of plasma phospholipid fatty acids at baseline (wk-0) and the change from baseline (*Δ*) following consumption of the modified and control diets for 12-wk in adults at moderate cardiovascular risk[1]

	Modified diet		Control diet		*P*[2]
	Baseline	*Δ*	Baseline	*Δ*	
Total SFA	46.52 ± 0.21	-0.60 ± 0.21	46.37 ± 0.17	0.01 ± 0.17	0.006
Total *cis*-MUFA	11.21 ± 0.18	0.99 ± 0.20	11.02 ± 0.16	0.21 ± 0.15	<0.0001
Total *trans*-MUFA	0.10 ± 0.00	0.12 ± 0.01	0.12 ± 0.00	0.12 ± 0.00	<0.0001
Total n-3 PUFA	5.34 ± 0.21	-0.47 ± 0.14	5.35 ± 0.23	-0.39 ± 0.14	0.58
Total n-6 PUFA	35.83 ± 0.32	0.03 ± 0.33	36.15 ± 0.34	0.15 ± 0.26	0.32

Adapted with permission from Markey et al. (201).

[1] Values are means ± SEMs.

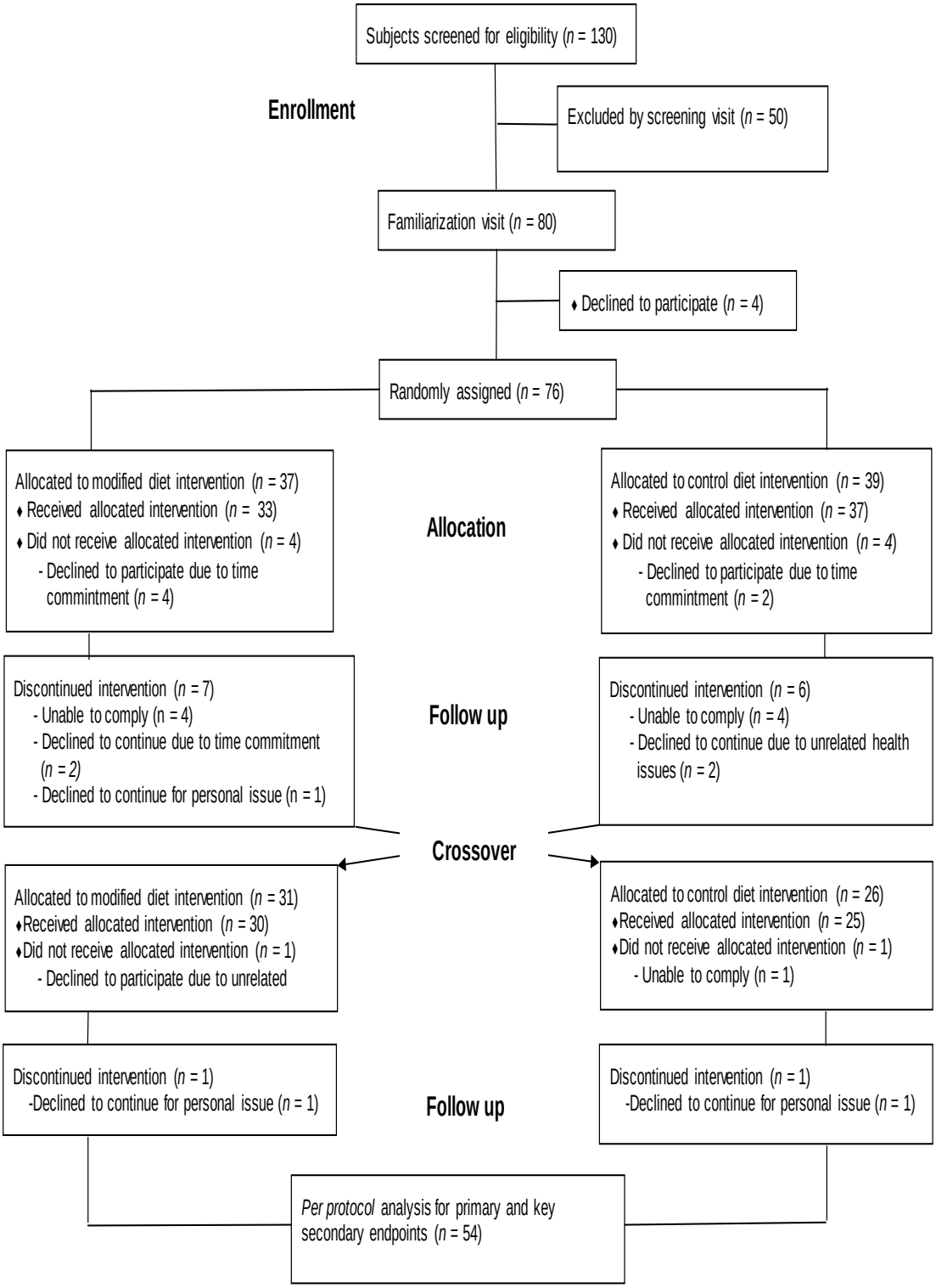
[2] Mixed model analyses were used to calculate overall effect of treatment based on change-from-baseline values, with adjustments made for fixed effects of baseline values of the assessed variable, period, treatment sequence, gender, age and BMI. Participant was included as a random effect. $P \leq 0.01$ deemed as significant.

Supplemental Table 3.6 Predictive equations for estimating changes in total cholesterol and LDL-C in response to changes in dietary fatty acids (as percent of energy)[1].

Predictive equations for estimating change in TC and LDL-C according to date of publication	Estimated change following the modified and control diet
Keys et al. 1965 (28) ΔTC = 0.0621ΔS - 0.0310ΔP	Modified: ΔTC = 0.17 mmol/L Control: ΔTC = 0.37 mmol/L
Hegsted et al. 1965 (29) ΔTC = 0.0543ΔS - 0.03115ΔP - 0.00318ΔM ΔLDL-C = 0.0449ΔS - 0.0198ΔP	Modified: ΔTC = 0.17 mmol/L; ΔLDL-C = 0.13 mmol/L Control: ΔTC = 0.37 mmol/L; ΔLDL-C = 0.26 mmol/L
Mensink and Katan 1992 (30) ΔTC = 0.0556ΔS - 0.0031ΔM - 0.015ΔP ΔLDL-C = 0.033ΔS - 0.006ΔM - 0.014ΔP	Modified: ΔTC = 0.17 mmol/L; ΔLDL-C = 0.13 mmol/L Control: ΔTC = 0.37 mmol/L; ΔLDL-C = 0.26 mmol/L
Clarke et al. 1997 (31) ΔTC = 0.052ΔS - 0.026ΔP + 0.005ΔM ΔLDL-C = 0.036ΔS - 0.022ΔP - 0.008ΔM	Modified: ΔTC = 0.17 mmol/L; ΔLDL-C = 0.08 mmol/L Control: ΔTC = 0.31 mmol/L; ΔLDL-C = 0.22 mmol/L
Howell et al. 1997 (32) ΔTC = 0.0496ΔS - 0.0233ΔP ΔLDL-C = 0.0468ΔS - 0.0128ΔP	Modified: ΔTC = 0.15 mmol/L; ΔLDL-C = 0.14 mmol/L Control: ΔTC = 0.29 mmol/L; ΔLDL-C = 0.28 mmol/L

[1]Summarized predictive equations according to author and publication year are presented, followed by the predictive change in total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-C) for the modified and control diets, as previously published. M; monounsaturated fatty acids. P, polyunsaturated fatty acids; S, saturated fatty acids; Δ, change from baseline.

Supplemental Figure 3.1 RESET Participant flowchart



Chapter IV: Impact of chronic consumption of SFA-reduced, MUFA-enriched dairy products on postprandial endothelial, lipid and inflammatory responses: results from the randomized, cross-over, double-blinded, controlled RESET study

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In preparation for submission to AJCN

JAL, DIG and KGJ designed the human study; O.M., K.G.J. and J.A.L, designed the test meal protocol; CCF, ASG, DJH and DIG designed and produced the modified dairy products; DV and OM conducted the research and analysed data; KEK analysed samples for total lipid fatty acids; ST provided statistical advice.

DV analysed the data presented in this chapter (with OM for biochemical ILAB analysis and statistics), performed statistical analysis on all other outcome measures and wrote the manuscript for this thesis, which was approved by all authors.

Results of the FMD analysis will not be presented in this chapter.

Impact of chronic consumption of SFA-reduced, MUFA-enriched dairy products on postprandial endothelial, lipid and inflammatory responses: results from the randomized, cross-over, double-blinded, controlled RESET study [1, 2, 3]

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[1] Supported by funding from the Medical Research Council (Ref: MR/K020218/1). Arla Foods UK and AAK UK supplied in kind the commercially available dairy products and oils according to our specification.

[2] Author disclosures: J.A. Lovegrove is an expert on the UK Scientific Advisory Committee for Nutrition (SACN) and SACN's Saturated Fats Working Group.

[3] Supplemental Methods and Supplemental Figures [1] are available from the "Online Supporting Material" link in the online postings of the article.

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[11] Abbreviations used: BP, blood pressure; CVD, cardiovascular disease; DBP, diastolic blood pressure; FA, fatty acid; FMD, flow-mediated dilatation; RCT, randomized controlled trial; SBP, systolic blood pressure; rTFA, ruminant *trans* fatty acids; TC, total cholesterol; TFA, *trans* fatty acids; TRL, triglyceride-rich lipoprotein; UHT, ultra-high temperature; WBC, whole blood culture; Δ, change-from-baseline; %TE, percent total energy.

This trial was registered at www.clinicaltrials.gov as NCT02089035

Short running head: Postprandial responses to dairy fat manipulation

4.1 Abstract

Background: Chronic dietary fat manipulation has been shown to impact on postprandial lipaemia, an independent cardiovascular disease (CVD) risk marker.

Objective: To determine the impact of long-term consumption of dairy products with a different dietary fatty acid (FA) profile on postprandial endothelial function (primary outcome) and other CVD risk markers to sequential meal ingestion.

Design: In a randomized, double-blinded, crossover, controlled design, participants (n = 76; 25-70 y) with a 1.5-fold higher CVD risk were assigned to receive a high-fat, high-dairy diet (38% total energy intake (%TE) from total fat) by incorporating either conventional (control; dietary target: 19%TE SFA; 11%TE MUFA) or SFA-reduced, MUFA-enriched (modified; 16%TE SFA; 14%TE MUFA) dairy products for 12-wk in random order, with an 8-wk washout period between diets. A sequential two-meal challenge was performed at the beginning and end of each intervention period using the same dairy products as the assigned intervention diet. Linear mixed model analyses determined overall treatment effects based on change in postprandial summary response measures from the pre-intervention (wk-0) to the post-intervention study (wk-12) visit on measurements of endothelial function, lipaemia, inflammation and plasma total lipid fatty acids FA.

Results: Fifty-two participants (31 men, 21 women; 53 ± 2 y; BMI 25.8 ± 0.5 kg/m^2) completed the study. The change in the postprandial apolipoprotein (apo)B incremental area under the curve (iAUC) to the modified meals was significantly lower compared to following the control meals (P = 0.004). Significant changes in specific plasma total FA were observed which reflected the dairy fat composition of the dietary intervention and test meal. There were no significant differences in any of the other postprandial CVD risk markers.

Conclusion: Our findings suggest that chronic consumption of SFA-reduced, MUFA-enriched dairy products led to an attenuating response to the postprandial iAUC of apoB, compared to

iso-energetic control test meals, suggestive of a beneficial impact on triacylglycerol-rich lipoprotein metabolism.

Keywords: dairy fat, cardiovascular disease risk, sequential test meal protocol, postprandial lipaemia, apoB, flow-mediated dilatation, saturated fatty acids, monounsaturated fatty acids, vascular function.

4.2 Introduction

Postprandial lipaemia is recognised as an independent risk factor of cardiovascular disease (CVD)[11]. In this context, the fatty acid (FA) composition of meals plays an important factor in modulating postprandial lipaemia, determining magnitude and clearance of circulating triacylglycerol (TAG), glucose and insulin (107, 108).The consumption of moderate to high-fat meals (36 – 80 g total fat), has been shown to transiently impair endothelial function (218, 226), characterized in part by a decreased bioavailability of nitric oxide (NO), raised TAG-rich lipoproteins (TRL) and an increased expression of pro-inflammatory cytokines and cellular adhesion molecules (227-229).

A reduction in total saturated fatty acid (SFA) intake and a parallel substitution with unsaturated FAs remains a key dietary recommendation aimed at reducing CVD risk (10). However, most of the evidence is based on intervention trials which have investigated the impact of SFA substitution on fasting outcome measures of CVD risk (23, 199). Although there is a wealth of evidence from postprandial investigations on the modulatory effects of dietary FA composition, fewer studies have considered the long-term dietary fat substitution followed by a postprandial investigation implemented with test meals reflective of the dietary intervention (108). Additionally, evidence from intervention trials evaluating the incorporation of MUFA in dietary interventions on both fasted and postprandial outcomes have observed improvements for CVD risk factors (23, 230, 231). However, the source of MUFA incorporated in test meals has been primarily of plant origin and postprandial investigations have mainly focused on postprandial lipaemia in isolation from other risk factors (232-236). Furthermore, there is evidence of a beneficial effect of MUFA, as olive oil, and FMD response in the fasting state (237). Therefore, further studies are needed to determine the mechanisms underlying the effects of MUFA on established and novel risk markers, including endothelial function and postprandial lipaemia, in response to dietary FA manipulation.

Supplementation of the dairy cow diet is a feasible strategy leading to partial replacement of SFA in milk fat, whilst maintaining the beneficial properties related to dairy consumption and removing SFA from the food chain (150, 203). Dairy products, contributing up to 35% of mean total SFA intake in the UK population (10) have not been linked to detrimental effects of CVD risk (with the exclusion of butter) (36, 47). Initial findings from the RESET study have shown that a 12-wk daily intake of SFA-reduced, MUFA-enriched dairy products attenuated the rise in fasting LDL-cholesterol (LDL-C) and increased fasting nitrite concentrations, compared to conventional matched dairy products (238). However, little is known of the impact of long term consumption of modified dairy products on postprandial markers of vascular function, lipemia and inflammatory markers.

The aim of the REplacement of SaturatEd fat in dairy on Total cholesterol (RESET) acute-within-chronic study was to investigate the effects of modified UHT milk, Cheddar cheese and butter consumption for 12-wk on postprandial endothelial function, lipaemia and other secondary markers of CVD risk, following a two-meal challenge and compared to control products. We hypothesized that long-term consumption of SFA-reduced, MUFA-enriched dairy products would beneficially impact on postprandial endothelial function (measured as %FMD response, primary outcome measure), lipaemia and inflammatory markers (secondary outcome measures).

4.3 Methods

4.3.1 Study design and participants

The RESET trial was given a favourable ethical opinion for conduct by the University of Reading Ethics Committee (Reference no. 13/43), registered at clinicaltrials.gov as NCT02089035 and carried out in accordance with the Declaration of Helsinki. The trial was a double-blind, randomized, controlled crossover 12-wk dietary intervention study with an 8-

wk washout period between the two intervention arms. The study was conducted at the the Hugh Sinclair Unit of Human Nutrition, University of Reading. Between February 2014 and September 2015, men and women aged 25-70 y with 1.5-fold higher CVD risk were recruited from the Berkshire area (United Kingdom) in three cohorts. The study was completed in April 2016. All participants gave their written informed consent prior to starting the study.

Details of the chronic study design including participant recruitment, inclusion and exclusion criteria and dietary intervention are presented in detail elsewhere (201, 238). Briefly, fasted screening values of serum TC, HDL-C, glucose, blood pressure (BP), BMI and family history of CVD were used to assess eligibility. Based on a modified Framingham risk analysis, a score of $\geq$2 CVD risk points indicated a 1.5-fold higher CVD risk compared to the population mean (164). Participants were randomly allocated to one of two dietary intervention groups, whereby Group 1 received the modified diet first (wk-0 – wk-12), followed by the control diet (wk-20 – wk-32) and vice-versa for Group 2. Randomization was performed using a minimization technique which stratified by age, gender, BMI and fasting serum TC (202).. An iso-energetic high dairy daily dietary exchange (38% TE from total fat) was achieved through replacement of habitually consumed dairy products, oil/spreads and snacks with either the SFA-reduced, MUFA-enriched ultra-high temperature (UHT) milk, Cheddar cheese and butter (modified dietary target: 16%TE SFA, 14%TE MUFA) or matched conventional dairy products (control dietary target: 19% SFA, 11%TE MUFA) (201). At pre- and post-intervention visits for each dietary period a sequential meal postprandial study was performed, incorporating dairy products from the assigned dietary intervention.

The 480 min postprandial study visits took place at the beginning (pre-intervention: wk-0; wk-20) and end (post-intervention: wk-12; wk-32) of the two dietary interventions. A total of 52 participants completed the acute-within-chronic study (see **Supplemental Figure 1** for flowchart).

4.3.2 Visits and test meals

Production of the SFA-reduced, MUFA-enriched (modified) milk and dairy products and RESET food exchange model have been described in detail elsewhere (201, 203). As previously described (239), during the 12-wk of each intervention period, participants consumed daily amounts of the study products, provided in blinded packaging: 340 g UHT milk, 45 g Cheddar cheese and 21.5 g (control diet) or 25.1 g (modified diet) butter. Compliance was monitored using 4-d food diaries (pre- and post-intervention), daily consumption records and plasma phospholipid FA analysis, the results of which have been published (201).

Participants refrained from alcohol and aerobic exercise 24 h prior to each postprandial study visit (239). For standardization purposes, a low-fat meal (< 1.46 MJ; < 7 g total fat) and low nitrate mineral water (Buxton) were provided for consumption on the evening prior to each study visit before fasting overnight for 12 h drinking only water during this time. All visits were conducted in a temperature controlled room (22 ± 1 °C) whereby on arrival, participants were required to rest for 30 min in the supine position before a cannula in the antecubital vein of the forearm was inserted, to allow frequent blood sampling. Fasted blood samples were collected and measurement of endothelial function by flow mediated dilatation (FMD) was performed. After a second fasted blood draw (0 min), the breakfast test meal was provided and consumed within 20 min. The breakfast test meal contained 50 g total fat and consisted of a milkshake prepared with study UHT milk (330 g) and strawberry syrup (19 g), Cornflakes (38g, Kellogg's) with UHT study milk (195 g), two slices of toasted bread (75 g; Kingsmill white medium) with study cheese (32.6 g) and butter (29.4 g for the control and 32.6 g for the modified meals respectively). The test lunch meal provided at 330 min, contained 30 g total fat and was consumed within 20 min. It consisted of a milkshake prepared with UHT study milk (control: 350 g; modified: 352 g) and 27 g strawberry syrup and two toasted slices of bread (60 g) with 15 g study cheese and butter (control: 18.6 g;

modified: 19.8 g). Both modified and control test meals contained dairy products identical to those assigned in the specific dietary intervention. The nutrient profile of the test meals is presented in **Table 4.1**. During the acute study visit, frequent blood samples were drawn, for: TAG, non-esterified fatty acids (NEFA), insulin, glucose at 0, 30, 60, 90, 120, 180, 240, 300, 330, 360, 390, 420, 480 min; apolipoprotein (apo)B similar time points, with the exclusion of 30, 90, 330 min time points. Endothelial function, measured by FMD, was assessed at 180, 300 and 420 min postprandially. At the FMD timepoints, blood samples were also collected for the assessment of plasma cellular adhesion molecules, whole blood culture (WBC) for determination of LPS-stimulated cytokines, plasma nitrite/nitrate and total lipid FA.

4.3.3 Biochemical analyses

Blood samples were collected into lithium heparin, EDTA or serum separator blood collection tubes (VACUETTE, Greiner Bio-One) and subsequently processed as previously detailed (239). Blood samples collected for the determination of LPS-stimulated cytokines in WBC were not centrifuged and were stored at 4 °C until processing.

TAG, NEFA, apoB and glucose were measured using an ILAB 600 clinical chemistry autoanalyzer (TAG and glucose reagents and analyzer: Werfen UK Ltd; apoB reagent: Randox Laborarories Ltd; NEFA reagent: Alpha Laboratories Ltd). A HPLC (ENO-30, EiCom Corporation, San Diego) coupled with an online reduction of nitrate to nitrite and post-column derivatization with the Greiss reagent (ENO-30 Analyzer, EiCom Corporation, San Diego) was used for the determination of plasma nitrite and nitrate. Insulin, vascular and intercellular adhesion molecules (VCAM, ICAM), E-selectin and P-selectin were analyzed with ELISA kits (Dako Ltd. and R&D Systems Europe Ltd., respectively). Mean inter-assay CVs were <10% for all assays.

4.3.4 Whole blood cultures

Whole blood samples collected in K2EDTA tubes were diluted 1:9 with RPMI 1640 medium (Sigma, UK) supplemented with 1% antibiotics, 1% L-glutamine and 1% non-essential amino acids (Bioscience, UK). Diluted blood samples were cultured in 12-well plates (Greiner bio-one, UK) with 0.5 μg/ml of bacterial lipopolysaccharide (LPS; *E. coli* 026:B6, Sigma, UK), leading to a final concentration of 0.05 μg/ml. WBC were subsequently incubated at 37 °C for 24 h before centrifugation at 700 x g (1000 rpm) to isolate the supernatant, which was stored at -20 °C until analysis. Measurement of the monocyte count of each sample was performed by the Pathology Department at the Royal Berkshire Hospital (Reading, UK). A human cytokine premixed 5-Plex Panel (IL-6, IL-8, IL-1β, IL-10, TNF-α; R&D Systems Europe Ltd) and a Luminex 200 with xPONENT software was used to measure the concentrations of cytokines in the WBC supernatant in a 1:2 dilution. Cytokine production was corrected for the number of monocytes in the whole blood sample.

4.3.5 Total lipid fatty acid analysis

Total lipid was extracted from plasma samples isolated from blood samples collected at 0, 180, 300 and 420 min on each study visit using the method described by Burdge et al (178). Briefly, lipid was extracted using using chloroform-methanol (2:1, v/v), redissolved in toluene and methylated (2% H_2SO_4 in methanol, incubation for 2 h at 50 °C). Resulting FAME were resolved on 100 m fused silica capillary column (CP-SIL 88, Agilent Technologies, Cheshire, UK) using a gas chromatograph (Bruker 350, Bruker, Germany), with a flame ionisation detector. GC conditions were as previously described (188). Plasma FAME were identified based on retention time comparisons with an authentic standard (GLC #463, Nu-Chek-Prep Inc, Elysian, MN) and cross referencing with previously published chromatograms (240). Carbon deficiency in the flame ionization detector response for FAME containing 4- to 10-carbon atoms was accounted for using a combined correction factor which also converted FAME to FA (241). Results were expressed as g/100 g total FAs.

4.3.6 Statistical analysis

Power calculations were performed for the study's primary outcome measure: change in FMD response. At 80% power and 5% significance, the minimum number of participants required to detect a 1.4% inter-intervention difference in FMD with an SD of 2.3% was calculated as $n = 45$ (179). With the allowance for a 15% dropout rate we aimed to recruit a minimum of 52 participants.

Statistical analyses were conducted using the SAS 9.4 University edition statistical software (SAS Institute Inc., Cary, NC, USA). All variables were checked for normality and data was logarithmically transformed where needed. The total area under the curve (AUC) for the postprandial period was calculated for all variables using the trapezoidal rule, which was subtracted from the fasting value to derive incremental AUC (iAUC). The inclusion of the iAUC summary response measure provides an accurate indication of the change in the postprandial response to the sequential meal ingestion (242). For postprandial variables with 10 (apoB) or 13 (TAG, NEFA, insulin, glucose) time points, maximum concentration (CMax) and time to reach maximum concentration (TTMax) were also calculated. Percentage NEFA suppression was also calculated since there is initial drop in NEFA concentration following meal ingestion. Using a linear mixed-model, treatment effects were evaluated, with differences in the postprandial summary measures from the pre-intervention to the post-intervention study visit calculated (e.g. AUC at wk-12 minus AUC at wk-0) as the dependent variable, adjusted for fixed effects of the baseline pre-intervention values of the assessed variable, period, sequence, treatment, age, gender and BMI. Participant was included as a random effect. A per-protocol analysis approach was adopted due to the proof-of concept nature of the study. No period effects were observed for any outcome measure. Postprandial summary measures are presented in tables as mean ± SEMs. For the primary outcome measure, %FMD response, a $P < 0.05$ was taken as significant. For all other outcome measures, $P \leq 0.01$ was considered significant to adjust for multiple comparisons.

4.4 Results

4.4.1 Participants

Baseline characteristics of the 52 completing participants are presented in **Table 4.2**. All test meals were well tolerated by the participants.

4.4.2 Biochemical analyses

Postprandial responses in apoB, TAG, NEFA, glucose and insulin expressed as AUC, iAUC, CMax and TTMax are presented in **Table 4.3.** We observed a significantly greater reduction in the iAUC for the postprandial apoB response following the modified test meals, compared to the control test meals ($P = 0.004$). The AUC, CMax and TTMax for the apoB response were not differentially affected by the two dairy test meals. Additionally, the change in the postprandial summary measures of TAG, NEFA, glucose and insulin following the control and modified test meals were not significantly different.

4.4.3 Nitric oxide and cellular adhesion molecules

There was a tendency for a reduction in the iAUC for the nitrite response following the modified test meals, compared to an increase in iAUC following the control test meals ($P = 0.05$). The two dairy test meals did not significantly impact on the postprandial responses for plasma nitrate or any of the cellular adhesion molecules (**Table 4.4**).

4.4.4 Whole blood culture

As shown in Table 4.4, there were no significant changes in the postprandial summary measures of LPS-stimulated cytokines following the modified and control test meals.

4.4.5 Postprandial total lipid fatty acids

Following the two 12-wk dietary interventions, there were significant differences in the change in the iAUC summary measure for total FA response between the modified and

control test meals (**Table 4.5**). The iAUC response for total SFA significantly decreased after consumption of the modified test meals, and compared to control ($P = 0.0001$). In particular, the iAUC of 15:0 and 18:0 were significantly lower following the modified test meals and compared to control test meals ($P = 0.0001$ and $P = 0.01$, respectively).

Change in iAUC response of total *cis*-MUFA was not significantly different between the two diets. However, the iAUC change in total *trans*-MUFA and total *trans* FA response was significantly lower following the modified test meals ($P = 0.009$ and $P = 0.008$ respectively), compared to control.

For total PUFA, change in iAUC was significantly lower following the modified test meals, compared to the control ($P = 0.001$). Similarly, change iAUC response for both n-3 and n-6 PUFA reflected the iAUC change in total PUFA ($P = 0.002$ and $P = 0.001$, respectively). For all other FAs presented in Table 5.4, postprandial iAUC summary response measures were not influenced by the intervention test meals.

4.5 Discussion

Using an acute-within-chronic design, the aim of this novel study was to compare the effect of long-term consumption of FA-modified dairy products and conventional counterparts on postprandial changes in endothelial function, lipaemia and inflammation in adults at moderate CVD risk. We observed a lower response in the iAUC for apoB response following a sequential two-meal challenge of the FA-modified dairy products. Additionally, our total lipid FA results reflected the current dietary intervention and test meals fat composition.

ApoB is considered a marker of the number of circulating TRLs (243). Several studies have investigated the long-term consumption of diets with a differential FA composition and the subsequent impact on the postprandial state following ingestion of test meals representative of the dietary intervention. However, it should be noted that most have focused

on an enrichment of dietary interventions with PUFA, while sources of MUFA have mainly originated from plant oils, particularly olive oil, which is in contrast to our study design (232, 233). In an 8-wk cross-over study, Roche et al. (232) investigated the long-term effect of two dietary interventions (41%TE total fat) rich in either SFA (51% SFA; 38% MUFA) or MUFA (39% SFA; 56% MUFA) in the form of spreads and oils, which was followed by a postprandial investigation in healthy men. The MUFA-rich diet led to a significant beneficial impact in fasting LDL-C and an attenuation in the postprandial activation of factor VII (232). An additional study used spreads and cooking oils to implement a chronic substitution of a dietary SFA (16% SFA; 12% MUFA) with either a moderate (13% SFA; 15% MUFA) or high (10% SFA; 18% MUFA) MUFA diet for 8-wk in 51 healthy adults (244). A sub-cohort of the main study's population completed a postprandial intervention which included a standard test meal and resulted in a 30-40% reduction in the postprandial number of circulating intestinally derived lipoproteins following the moderate and high MUFA diets respectively (244). It is worth noting, that in addition to the implementation of MUFA-rich test meals sourced from plant oils, the aforementioned studies also present a degree of heterogeneity in study populations which precludes from making definite comparisons with our study. However, the evidence from these studies suggests an impact in the kinetics of postprandial responses following MUFA-rich diets, influencing the number and size of TRLs and increasing the rate of clearance in the circulation (107). The decrease in postprandial iAUC of apoB may therefore be related to the observed fasting LDL-C attenuation we observed following intake of the SFA-reduced, MUFA-enriched dairy products in the 12-wk dietary intervention, compared to conventional dairy products (239). Furthermore, the findings from a previously published *in vitro* study investigating the consumption of a single SFA-rich meal, suggested greater competition for LDL-C uptake between circulating TRLs in HepG2 liver cells, compared to circulating TRLs following MUFA-rich meals(245). This may

in turn suggest a potential mechanism to explain higher circulating LDL-C observed following ingestion of SFA-rich diets (245).

Postprandial studies which have investigated the impact of FA-modified dairy products following modifications of the dairy cow diet, have primarily focused on implementing a dietary intervention using modified butter fat. Tholstrup et al. (223) compared the effects of 4-wk consumption of Danish butter (40%TE from fat; 37% palmitic acid; 15% oleic acid) and a FA-modified butter (21% palmitic acid; 25% oleic acid) in 18 healthy men in a cross-over design. The postprandial investigation following each standard meal resulted in no significant changes in postprandial lipid markers, which the authors suggest may be attributed to a 5-fold increase in *trans* 18:1 in the modified butter compared to the control diet (223). However, in contrast to our study design, Tholstrup et al. (223) used only butter fat in the chronic intervention and a standard test meal not representative of the dietary intervention in a small number of participants, which may explain null findings.

The FA composition of study meals have specific characteristics, which may influence digestion rate and absorption (108). In the current study, we observed significant changes in postprandial iAUC responses in the plasma concentration of total lipid FA classes, which appear reflective of the FA composition of the test meals. In particular, there was a marked difference in the postprandial iAUC response of total *trans* FA (TFA) and *trans* MUFA following the modified diet, reflecting the observed increase in ruminant TFA (rTFA) of the dairy products following supplementation of the dairy cow feed (201, 203). Although there is less evidence for a detrimental effect of rTFA compared to their industrial counterparts (220), the recommended TFA intake from all sources should not exceed 2% total food energy (10). The FA modified study meals had a higher (2.9 g/d) rTFA content compared to the control study meals, which do not appear to have detrimental effects postprandially. Similarly, differences in the iAUC postprandial response of total and specific classes of SFA is in line with the observed partial replacement of SFA in the modified dairy products (201, 203).

Inflammation and endothelial function in the postprandial state have also been attributed to a progressive detrimental effect on CVD risk, in particular following high SFA intake (98). In our previous publication, we observed a significant increase in fasted nitrite concentrations following 12-wk consumption of the modified diet, and compared to control diet (239). Postprandial TAG and NEFA following, high-fat meals, have been shown to reduce concentrations of NO, impacting on postprandial NO-dependent FMD of the brachial artery (246, 247). In the current study, we observed a tendency for a non-significant decrease in the postprandial iAUC for the plasma nitrite response following the modified test meals, compared to control. Interestingly, this response supports the change in fasted nitrite concentrations, which significantly increased following 12-wk consumption of the modified diet , relative to baseline, when compared with the control (238).

The two dairy test meals did not significantly impact on the postprandial summary response measures of the cellular adhesion molecules. This is in agreement with a study which compared the postprandial effects of 3 iso-caloric high fat milkshakes (95 g total fat), either high in SFA (54%TE), MUFA (83%TE) or *n*3 PUFA (40%TE) in lean and obese middle aged men (248). Previous studies have demonstrated a triggering of postprandial, pro-inflammatory cytokine production following high-fat meal intake with a moderate to high SFA content (249-251). It is therefore possible that the moderate reduction in total SFA and parallel increase of MUFA in the modified dairy products, compared to the control (201, 238), did not elicit a differential impact on adhesion molecules or LPS-stimulated cytokines. Additionally, the evidence to date on inflammatory postprandial biomarker response to meal FA composition remains inconclusive (252). This may allude to variations in measurements observed in both healthy and at risk individuals, linked to additional participant characteristics which may affect the concentration of inflammatory markers (including age, overall diet and genetics) (98).

A strength of the current study is the double-blinded, crossover, randomized and long-term design. Additionally, the sequential two-meal challenge and 480 min postprandial investigation more closely reflected Western dietary patterns. Furthermore, the breakfast and lunch test meals of the postprandial intervention contained representative quantities of the dairy study products which were used in the chronic dietary intervention. Our study population was at moderate CVD risk and evidence supports that compared with healthy subjects, individuals at risk may be more sensitive to meal challenges (108). However, as the eligibility of participants was based on an overall scoring ≥2 CVD risk markers, baseline fasting characteristics of all participants were not homogenous with respect to CVD risk factors. This may potentially have led to the lack of an impact on the change in postprandial responses of specific markers of postprandial lipaemia, such as TAG and NEFA. Lastly, as described in our previous publication (238), there were modest differences in the SFA and *cis*-MUFA content between the FA-modified and control dairy products and test meals which may also explain a lack of differential postprandial responses in some of our secondary outcome measures.

In conclusion, the present study indicates that 12-wk consumption of SFA-reduced, MUFA-enriched dairy products, led to a reduction in the postprandial iAUC response of apoB, following sequential test meals representative of the dietary intervention, suggesting an effect on TRL metabolism. The results of the postprandial FMD response, particularly in light of the postprandial nitrite response, may further add to a better understanding of the impact of FA-modified dairy consumption

4.6 Acknowledgements

The authors gratefully acknowledge all participants who took part in the study. We thank S. Hargreaves, K. Jenkins, R. Mihaylova, the technical staff and research assistants/students (V. Bines, C. Brilley, L. Bula, Y. Chatzidiakou, V. Clifton, H. Dong, A. Garcimartin, C. Holland, J. Guo, A. Kanneganti, T. Loughman, E. Mertens, J. Ryan, L. Sellem, T. Staff and H. Zhang) for their assistance with recruitment, data collection, the blinding of intervention foods, data entry or food diary analysis. We also thank Dr Gunter Kuhnle, Dr Virag Sagi-Kiss and Mr Chris Humphries for technical assistance during the nitric oxide analysis.

J.A.L., D.I.G. and K.G.J designed the human study; O.M., K.G.J. and J.A.L, designed the test meal protocol; C.C.F., A.S.G., D.J.H and D.I.G. designed and produced the modified dairy products; D.V and O.M conducted the research; D.V, O.M, and K.E.K analyzed data or performed statistical analysis; S.T. provided statistical advice; D.V and O.M wrote the manuscript, which was modified by all co-authors; and J.A.L. had primary responsibility for final content. All authors have read and approved the final manuscript. Arla Foods UK and AAK UK provided the control dairy study products and high oleic sunflower oil, respectively, and were not involved in the study design or data interpretation.

Table 4.1 Nutritional composition of the sequential test breakfast and lunch meals consumed by participants at baseline (wk-0) and post-intervention (wk-12) of the modified and control high-fat, high dairy diets[1]

	Energy MJ	Protein g (%TE)	CHO g (%TE)	Fat g (%TE)	SFA g (%TE)	MUFA g (%TE)	PUFA g (%TE)	Trans MUFA g (%TE)
Breakfast (0 min)								
Modified diet	4.3	36.1 (14.1)	105.9 (41.3)	50.6 (44.3)	24.5 (21.5)	20 (17.5)	2.9 (2.5)	3.9 (3.4)
Control diet	4.1	39.7 (16.1)	101.4 (41.0)	49.9 (45.5)	31.7 (28.8)	12.3 (11.2)	2.8 (2.5)	2.2 (2.4)
Lunch (330 min)								
Modified diet	2.6	20.9 (13.5)	64.6 (41.6)	30.6 (44.3)	14.8 (21.4)	12.1 (17.5)	1.8 (2.7)	2.6 (3.8)
Control diet	2.5	19.6 (13.1)	63.3 (42.4)	30.3 (45.6)	19.1 (28.8)	7.4 (11.1)	1.8 (2.8)	1.4 (2.0)

[1]Values represent total energy and macronutrient quantities of each test meal according to modified and control diet. CHO, carbohydrates, TE, total energy.

Table 4.2 Baseline screening characteristics of participants who completed the acute-within-chronic RESET study[1]

	Value
Total (men/women) *n*	31/21
Age, y	53 ± 2
Weight, kg	76 ± 2
BMI, kg/m²	26 ± 1
Waist circumference, cm	90 ± 1
SBP, mm Hg	129 ± 2
DBP, mm Hg	75 ± 1
TC, mmol/L	5.71 ± 0.12
HDL-C, mmol/L	1.59 ± 0.04
TAG, mmol/L	1.18 ± 0.06
Glucose, mmol/L	5.53 ± 0.10
Risk score[2]	3 ± 1

[1]Values are means ± SEMs, *n* = 52. DBP, diastolic blood pressure; TC, total cholesterol; TAG, triacylglycerol; SBP, systolic blood pressure.

[2]Assessed with the use of a modified Framingham risk score, where a score of ≥ 2 points relates to a 1.5-fold higher risk of CVD than the population mean (164, 165, 201)

Table 4.3 Postprandial summary measures of apoB, lipids, glucose and insulin responses to sequential modified and control dairy test[1]

	Modified diet			Control diet			
	Pre	Post	Δ	Pre	Post	Δ	P^2
ApoB							
AUC (g/L x 480 min)	475 ± 14	477 ± 15	4 ± 7	467 ± 14	487 ± 14	19 ± 6	0.06
iAUC (g/L x 480 min)	-16 ± 3	-20 ± 3	-4 ± 3	-11 ± 2	-9 ± 2	3 ± 3	0.004
Cmax (g/L)	1.04 ± 0.03	1.05 ± 0.03	0.01 ± 0.02	1.03 ± 0.03	1.07 ± 0.03	0.04 ± 0.02	0.29
TTmax (min)	209 ± 23	208 ± 22	-1 ± 31	210 ± 22	224 ± 23	14 ± 29	0.36
TAG							
AUC (mmol/L x 480 min)	953 ± 52	1018 ± 72	72 ± 55	879 ± 41	906 ± 54	55 ± 40	0.26
iAUC (mmol/L x 480 min)	354 ± 25	388 ± 30	34 ± 28	321 ± 23	332 ± 27	19 ± 20	0.14
Cmax (mmol/L)	2.8 ± 0.1	2.9 ± 0.2	0.1 ± 0.1	2.6 ± 0.1	2.6 ± 0.2	0.0 ± 0.1	0.62
TTmax (min)	359 ± 9	330 ± 10	-25 ± 11	334 ± 9	324 ± 12	-10 ± 25	0.43
NEFA							
AUC (mmol/L x 300 min)	92 ± 4	95 ± 4	2 ± 4	97 ± 4	97 ± 3	1 ± 3	0.37
iAUC (mmol/L x 300 min)	-108 ± 8	-87 ± 10	18 ± 9	-103 ± 11	-86 ± 9	18 ± 10	0.65
Cmax (μmol/L)	565 ± 25	523 ± 20	-42 ± 28	555 ± 24	564 ± 24	9 ± 28	0.12
TTmax (min x 300 min)	172 ± 24	203 ± 24	31 ± 29	227 ± 24	177 ± 23	-50 ± 25	0.34
suppression (%)	43 ± 2	39 ± 2	-4 ± 2	41 ± 2	38 ± 2	-4 ± 2	0.98
Glucose							
AUC (mmol/L x 480 min)	2937 ± 61	2925 ± 63	-15 ± 32	2866 ± 59	2888 ± 85	44 ± 42	0.43
iAUC (mmol/L x 480 min)	345 ± 39	358 ± 49	14 ± 50	283 ± 38	302 ± 38	25 ± 31	0.61
Cmax (mmol/L)	8.3 ± 0.2	8.4 ± 0.2	0.1 ± 0.2	8.0 ± 0.2	8.1 ± 0.2	0.1 ± 0.2	0.21
TTmax (min)	223 ± 24	258 ± 25	31 ± 27	251 ± 24	287 ± 23	16 ± 25	0.90
Insulin							
AUC (nmol/L x 480 min)	122 ± 10	120 ± 12	-2 ± 6	114 ± 8	111 ± 7	-3 ± 3	0.47
iAUC (nmol/L x 480 min)	102 ± 9	101 ± 11	-1 ± 6	95 ± 7	89 ± 6	-6 ± 4	0.28
Cmax (nmol/L)	0.57 ± 0.05	0.59 ± 0.06	0.03 ± 0.02	0.52 ± 0.04	0.50 ± 0.03	-0.02 ± 0.03	0.21

TTmax (min)	165 ± 24	203 ± 24	20 ± 30	227 ± 24	171 ±23	-63 ± 25	0.34

[1]Values are means ± SEMs; Data not normally distributed was log transformed. ApoB, apolipoprotein B; AUC, incremental area under the curve; Cmax, maximum concentration; NEFA, non-esterified fatty acids; iAUC, incremental AUC; TAG; triacylglycerol; TTmax, time to reach maximum concentration; Δ, change-from-baseline.

[2] Linear mixed model analyses were used to calculate overall treatment effect based on change in postprandial summary measures from the pre-intervention to the post-intervention study visit, with adjustments made for fixed effects of baseline values of the assessed variable, period, treatment sequence, gender, age and BMI. Participant was included as a random effect. For all outcome measures, $P \leq 0.01$ was deemed as significant.

Table 4.4 Postprandial summary measures of circulating biomarkers of endothelial activation and inflammation before (pre) and after (post) the modified and control postprandial dairy diets[1]

	Modified diet			Control diet			
	Pre	Post	*Δ*	Pre	Post	*Δ*	*P*[2]
Nitrite							
AUC (μmol/L x 420 min)	64 ± 8	65 ± 8	1 ± 5	64 ± 8	60 ± 10	-4 ± 8	0.50
iAUC (μmol/L x 420 min)	4 ± 5	-2 ± 3	-6 ± 5	-1 ± 5	10 ± 5	11 ± 7	0.05
Nitrate							
AUC (μmol/L x 420 min)	5688 ± 519	5622 ± 335	-66 ± 399	5321 ± 270	5633 ± 462	322 ± 370	0.57
iAUC (μmol/L x 420 min)	-1683 ± 303	-1791 ± 338	-108 ± 381	-1530 ± 310	-1197 ± 307	333 ± 456	0.37
Adhesion molecules							
VCAM-1							
AUC (μg/mL x 420 min)	226 ± 15	220 ± 14	-6 ± 5	235 ± 16	222 ± 16	-13 ± 7	0.38
iAUC (μg/mL x 420 min)	-1 ± 4	-7 ± 7	-6 ± 9	2 ± 6	7 ± 6	5 ± 9	0.11
ICAM-1							
AUC (μg/mL x 420 min)	38 ± 4	35 ± 3	-2 ± 1	36 ± 4	37 ± 3	1 ± 2	0.22
iAUC (μg/mL x 420 min)	1 ± 2	0.4 ± 2	-0.6 ± 3	1 ± 2	-1 ± 72	-2 ± 3	0.15
E-selectin							
AUC (μg/mL x 420 min)	10 ± 1	10 ± 1	0 ± 0	10 ± 1	11 ± 1	1 ± 0	0.68
iAUC (μg/mL x 420 min)	-0.3 ± 0.2	-0.4 ± 0.3	-0.1 ± 0.3	-0.3 ± 0.2	-0.1 ± 0.3	0.2 ± 0.3	0.25
P-selectin							
AUC (μg/mL x 420 min)	11 ± 1	11 ± 1	0 ± 0	11 ± 1	11 ± 1	0 ± 0	0.66
iAUC (μg/mL x 420 min)	-0.1 ± 0.2	-0.4 ± 0.3	-0.3 ± 0.4	-0.6 ± 0.2	-0.1 ± 0.2	0.5 ± 0.3	0.32
LPS-stimulated cytokines							
TNFα							
AUC (x 420 min)	4847 ± 328	5393 ± 303	536 ± 228	4904 ± 290	5447 ± 295	543 ± 341	0.85
iAUC (x 420 min)	-267 ± 213	-216 ± 213	51 ± 306	169 ± 239	-305 ± 164	475 ± 258	0.70

IL-6							
AUC (x 420 min)	32034 ± 1675	35199 ± 1983	3165 ± 1119	32835 ± 1709	34972 ± 1765	2138 ± 1596	0.43
iAUC (x 420 min)	-2879 ± 1086	-2347 ± 1094	532 ± 1752	285 ± 1337	-2660 ± 213	2944 ± 1474	0.57
IL-1β							
AUC (x 420 min)	11577 ± 562	11914 ± 570	337 ± 404	12222 ± 606	13489 ± 700	1266 ± 631	0.10
iAUC (x 420 min)	60 ± 381	449 ± 329	388 ±533	1442 ± 400	1242 ± 406	-200 ± 578	0.63
IL-8							
AUC (x 420 min)	48211 ± 3795	53918 ± 5429	5707± 3279	51440 ± 5533	49782 ± 5604	-1659 ± 4031	0.15
iAUC (x 420 min)	-2698 ± 2841	-1320 ± 4144	1378 ± 4717	-839 ± 4374	-4881 ± 3504	-4041 ± 3850	0.91
IL-10							
AUC (x 420 min)	288 ± 23	302 ± 26	14 ± 23	356 ± 34	321 ± 30	-44 ± 29	0.34
iAUC (x 420 min)	-61 ± 22	-65 ± 24	-4 ± 33	-19 ± 25	-84 ± 20	-65 ± 34	0.76

[1]Values are means ± SEMs. All LPS-stimulated cytokines are expressed as μg x 10^3 monocytes. For all variable, $n = 50$; except TNF-α, IL-1β, IL-6, IL-10, $n = 49$ and IL-8, $n = 47$. Data not normally distributed was log transformed. AUC, area under the curve; iAUC, incremental AUC; ICAM-1, intercellular adhesion molecule-1; TNFα, tumor necrosis factor alpha; VCAM-1, vascular adhesion molecule 1; Δ, change-from-baseline (pre-intervention visit).

[2] Mixed model analyses were used to calculate overall treatment effect based on change in postprandial summary measures from the pre-intervention to the post-intervention study visit, with adjustments made for fixed effects of baseline values of the assessed variable, period, treatment sequence, gender, age and BMI. Participant was included as a random effect. For all outcome measures, $P \leq 0.01$ was deemed as significant.

Table 4.5 Postprandial iAUC summary measures of plasma total lipid fatty acid responses after the modified and control postprandial test meals at baseline and following the 12-wk dietary interventions (post)[1]

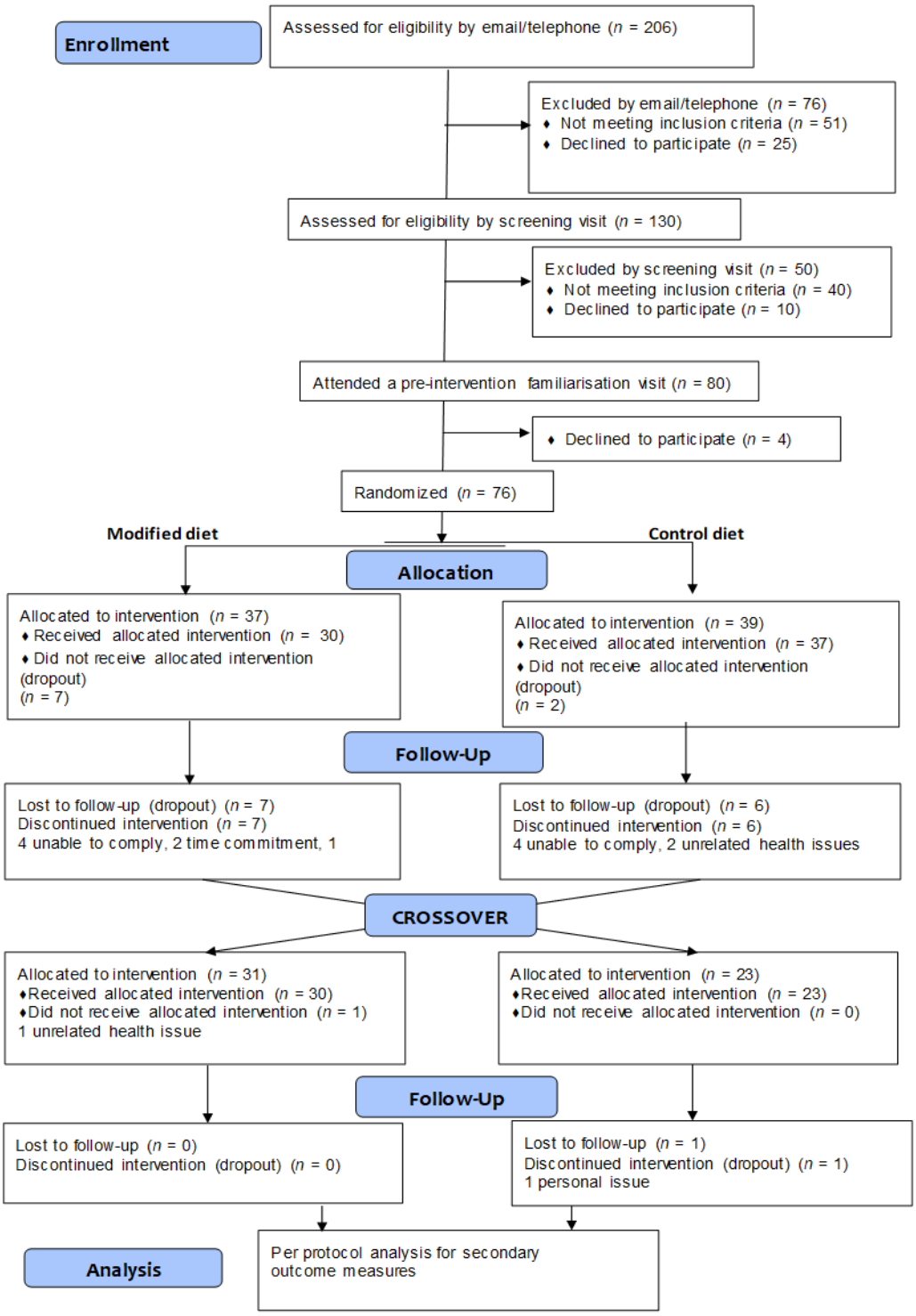
	Modified diet			Control diet			
FA (g/100g x 420 min)	Baseline	Post	*Δ*	Baseline	Post	*Δ*	*P*[2]
SFAs							
14:00	72 ± 20	19 ± 20	-53 ± 22	96 ± 30	94 ± 30	-3 ± 30	0.02
15:0	23 ± 2	20 ± 2	-3 ± 2	38 ± 3	39 ± 3	0 ± 3	<0.0001
15:0 antiso	5 ± 1	3 ± 1	-3 ± 1	6 ± 2	5 ± 1	-1 ± 1	0.03
16:0	-115 ± 59	-375 ± 142	-260 ± 157	-33 ± 120	196 ± 204	229 ± 250	0.02
17:0	2 ± 3	0.1 ± 3	-2 ± 3	12 ± 4	11 ± 4	-1 ± 4	0.06
17:0 iso	-1 ± 3	-5 ± 4	-4 ± 5	0.1 ± 3	0 ± 5	-0.1 ± 5	0.34
18:0	99 ± 33	-94 ± 69	-192 ± 72	-35 ± 57	122 ± 66	157 ± 92	0.01
Total SFA	827 ± 250	497 ± 160	-330 ± 285	1566 ± 293	1704 ± 262	138 ± 378	<0.0001
MUFAs							
14:1 *trans 9*	2 ± 1	0 ± 1	-2 ± 1	4 ± 1	3 ± 1	-1 ± 1	0.01
18:1	742 ± 174	391 ± 133	-350 ± 214	-181 ± 192	24 ± 170	205 ± 258	0.16
18:1 *cis*	326 ± 167	-10 ± 117	-336 ± 204	-293 ± 189	-80 ± 165	-213 ± 253	0.81
18:1 *trans*	416 ± 23	402 ± 28	-14 ± 22	112 ± 14	104 ± 12	-8 ± 13	0.02
18:1 *cis 9*	329 ± 200	-8 ± 139	-336 ± 234	-421 ± 102	-140 ± 220	-413 ± 176	0.60
18:1 *trans* 6-8	35 ± 4	11 ± 4	-25 ± 5	6 ± 2	7 ± 1	1 ± 2	0.04
18:1 *trans 9*	19 ± 3	4 ± 4	-14 ± 4	1 ± 4	6 ± 3	5 ± 5	0.31
18:1 *trans 10*	74 ± 10	24 ± 10	-50 ± 10	5 ± 4	8 ± 3	3 ± 4	0.04
Total *cis*-MUFA	399 ± 179	55 ± 127	-343 ± 219	-182 ± 202	57 ± 177	239 ± 272	0.94
Total *trans*-MUFA	419 ± 24	402 ± 29	-17 ± 25	116 ± 16	112 ± 15	-3 ± 17	0.009
CLAs							
CLA *cis9, trans11*	20 ± 2	5 ± 3	-15 ± 3	12 ± 2	7 ± 3	-4 ± 3	0.42
Total CLA	39 ± 3	33 ± 3	-6 ± 3	25 ± 3	22 ± 2	-2 ± 3	0.40

Total *trans*	439 ± 26	418 ± 31	-21 ± 28	124 ± 18	122 ± 18	-3 ± 21	0.008
PUFAs							
n-3	-84 ± 36	-182 ± 15	-97 ± 38	-120 ± 53	-79 ± 36	41 ± 64	0.0023
n-6	-1035 ± 284	-1744 ± 174	-709 ± 290	-1200 ± 280	345 ± 414	-182 ± 20	0.001
Total PUFA	-1064 ± 313	-1880 ± 184	-817 ± 319	-1287 ± 327	-925 ± 353	362 ± 472	0.0013

[1]Values are means ± SEMs. For all variables, $n = 49$. Data not normally distributed was log transformed. CLA, conjugated linoleic acid; iAUC, incremental area under the curve; Δ, change-from-baseline.

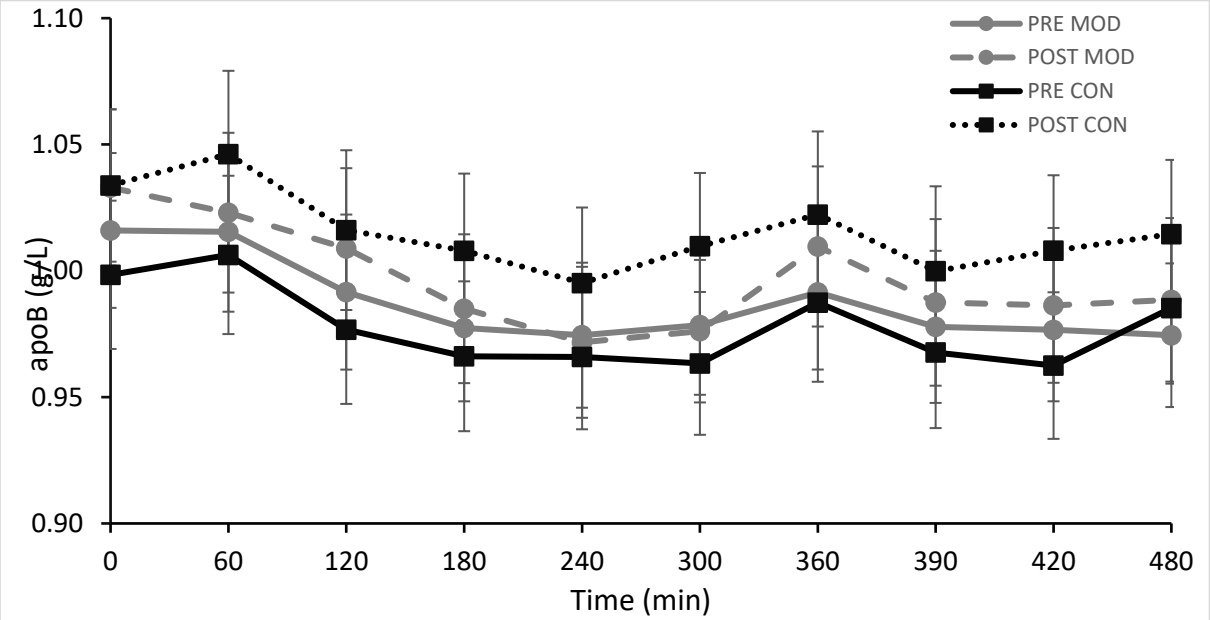
[2] Linear mixed model analyses were used to calculate overall treatment effect based on change in postprandial summary measures from the pre-intervention to the post-intervention study visit, with adjustments made for fixed effects of baseline values of the assessed variable, period, treatment sequence, gender, age and BMI. Participant was included as a random effect. For all outcome measures, $P \leq 0.01$ was deemed as significant.

Supplemental Figure 4.1 RESET participant flowchart for the acute-within-chronic study

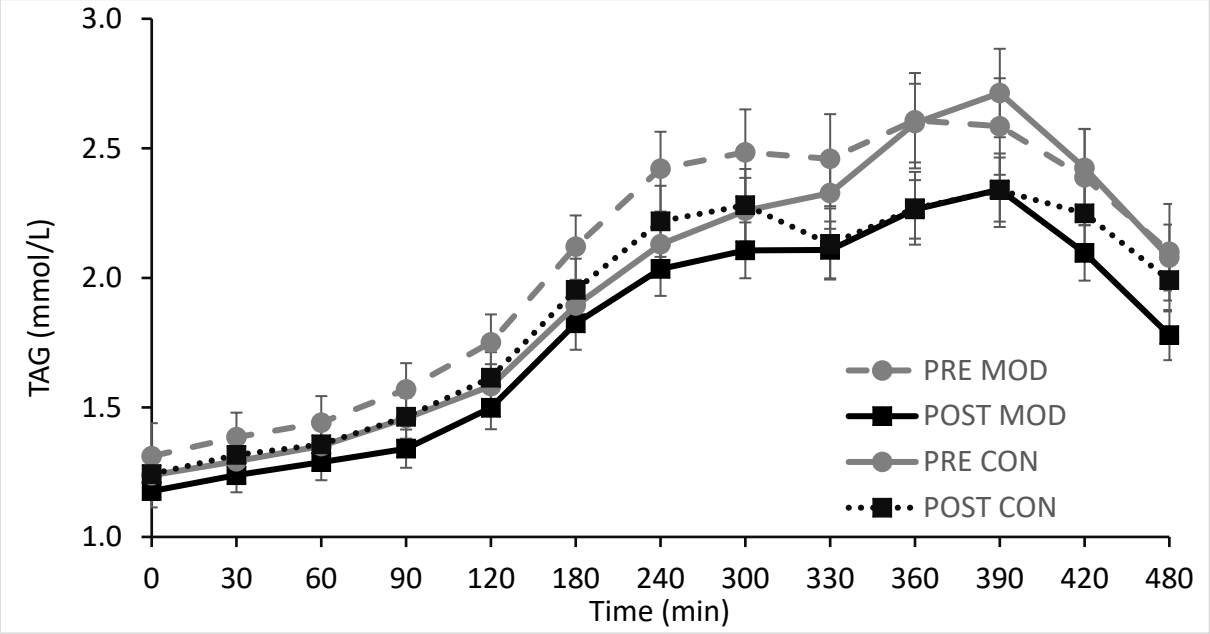


Supplemental Figures

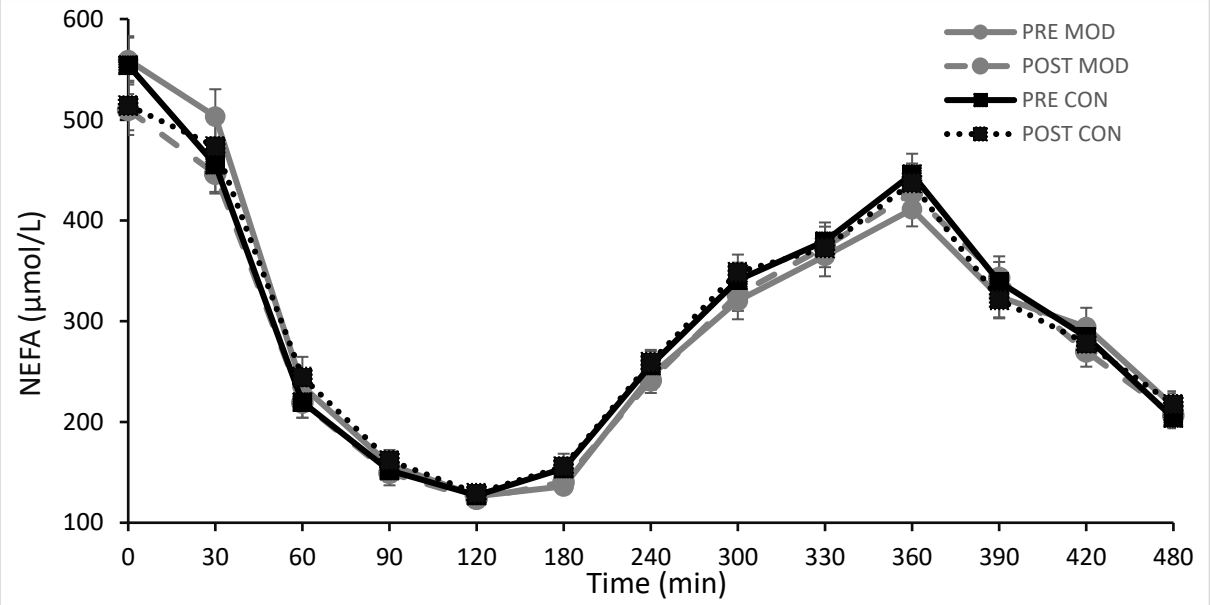
a)



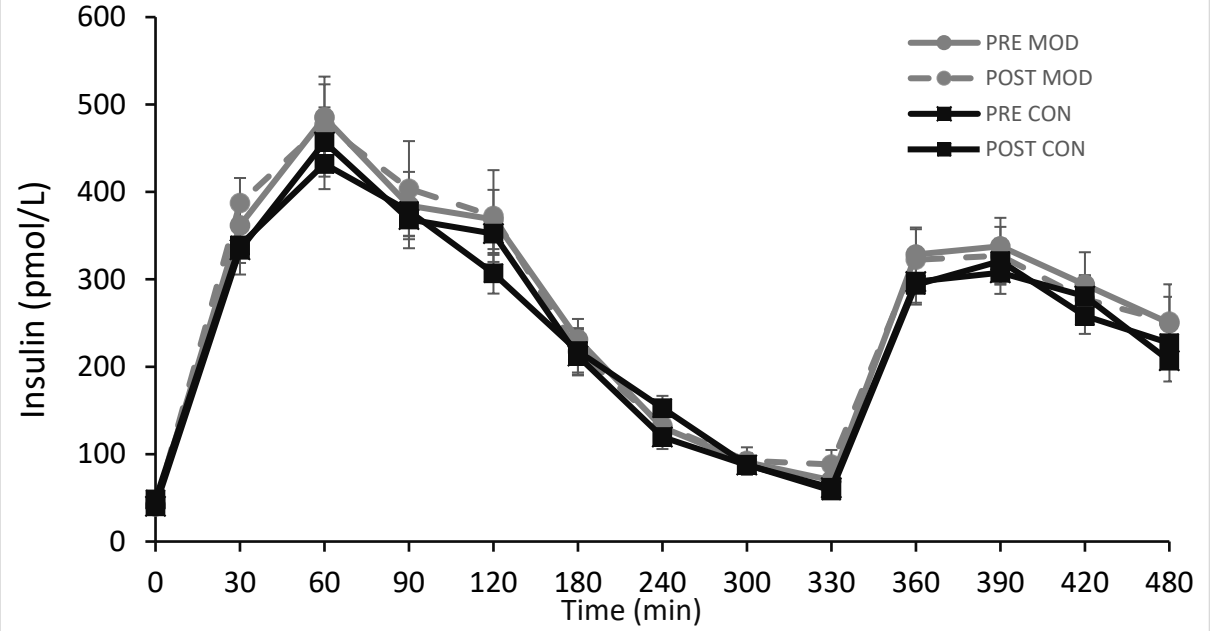
b)



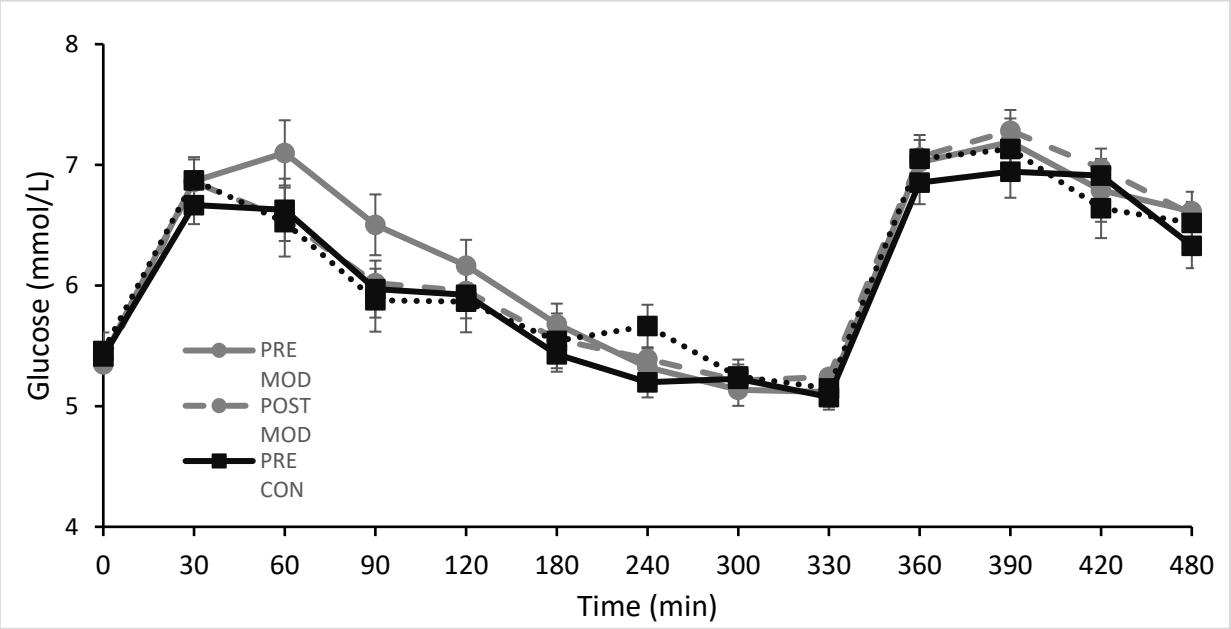
c)


PRE MOD
POST MOD
PRE CON
POST CON
NEFA (µmol/L)
600
500
400
300
200
100
0
30
60
90
120
180
240
300
330
360
390
420
480
Time (min)

d)


PRE MOD
POST MOD
PRE CON
POST CON
Insulin (pmol/L)
600
500
400
300
200
100
0
0
30
60
90
120
180
240
300
330
360
390
420
480
Time (min)

e)



Postprandial time response curve of: a) apolipoprotein B (apoB); b) triacylglycerols (TAG); c), non-esterified fatty acids (NEFA); d) insulin and e) glucose following 12-week intake of the FA-modified dairy diets and two meal challenge. Values are ± SEMs. Con, control diet; Mod, modified diet; Pre, relates to postprandial investigation study prior to the assigned 12-week dairy diet; Post, relates to the postprandial investigation at the end of each assigned 12-week dairy diet

Chapter V: Impact of dairy fat composition and *APOE* genotype on fasting and postprandial CVD risk markers: insights from the RESET study

Dafni Vasilopoulou, Oonagh Markey, Kirsty E. Kliem, Colette C. Fagan, Alistair S. Grandison, David J. Humphries, Susan Todd, Kim G. Jackson, David I. Givens and Julie A. Lovegrove.

In preparation for submission as a short communication to Mol Nutr Food Res

JAL, DIG and KGJ designed the human study; CCF, ASG, DJH and DIG designed and produced the modified dairy products; DV, OM, KEK conducted the research and analysed data; ST provided statistical advice.

KGJ conducted the APOE genotyping of the RESET participants

DV prepared samples for PCR analysis, analysed the data, conducted statistical analysis on all outcomes and wrote the manuscript for this thesis, which was approved by all authors.

Impact of dairy fat composition and *APOE* genotype on fasting and postprandial CVD risk markers: insights from the RESET study

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Short running title: Dairy fat, *APOE* genotype and CVD risk markers

5.1 Abstract

Scope: To determine whether apolipoprotein (*APO)E* genotype influences fasting and postprandial responses to long term consumption of saturated fatty acid (SFA)-reduced, monounsaturated fatty acid (MUFA)-enriched dairy products (modified) compared to conventional dairy products (control).

Methods and results: Adults (52 ± 2 y) at 1.5-fold higher CVD risk, retrospectively genotyped for *APOE* (*n* = 31 *E3/E3*, *n* = 16 *E4* carriers), were assigned to a high dairy, iso-energetic daily dietary exchange with varying fatty acid composition (38% total energy (%TE) from total fat; dietary targets: modified, 16%TE SFA, 14%TE MUFA; control, 19%TE SFA, 11%TE MUFA) in a random order, with an 8 week wash out between diets. At the beginning and end of each 12-wk dietary period, a postprandial investigation was conducted using dairy products from the assigned intervention diet. At baseline, high-density lipoprotein cholesterol (HDL-C) and non-esterified fatty acids (NEFA) concentrations were higher in *E4* carriers, with a greater proportion of small and medium sized HDL particles compared to the *E3/E3* genotype group ($P < 0.05$). No genotype x treatment interactions were observed for the postprandial CVD risk biomarkers.

Conclusion: *APOE* genotype only influenced baseline fasted lipids, with little impact on the fasting and postprandial CVD risk markers in response to the dairy intervention diets and test meals of varying fat composition.

Key words: Dairy fat, *APOE* genotype, dietary fat manipulation, saturated fatty acids, monounsaturated fatty acids

5.2 Introduction

Genetic factors are known to play a key role in the progression of cardiovascular diseases (CVD) (116). Common genetic variants of the apolipoprotein E (*APOE*) gene have been extensively investigated in association with CVD risk, which accounts for up to 7% variance observed in the fasting total (TC) and low-density lipoprotein cholesterol (LDL-C) concentrations (111, 119, 253). Of particular interest are carriers of the *APOE4* allele, representing just over 30% of the UK population (254), associated with a higher CVD risk relative to the wild type variant *E3/E3*, partially due to increased fasted LDL-C concentrations, and a potentially more pro-oxidative and pro-inflammatory phenotype (116, 255).

A greater responsiveness to dietary fatty acid (FA) composition has also been reported in *E4* carriers compared to the wild type *E3/E3* variant (116, 256). However, studies which have investigated the impact of *APOE* genotype following dietary fat manipulation have primarily focused on lipid responses in the fasted state (116, 119, 256, 257). Fewer studies have investigated responses to meal FA composition in the postprandial state, particularly when comparing meals rich in monounsaturated (MUFA) and saturated fatty acids (SFA) (258-260). Current evidence suggests a modest beneficial impact on the lipid profile following a replacement of SFA with unsaturated fatty acids, although most studies have primarily focused on long chain n-3 FAs (116, 119). Additionally, limited studies have investigated the impact of *APOE* genotype on novel risk markers, such as biomarkers of endothelial function and inflammation, which may also be modulated by dietary and meal FA composition (102, 261, 262).

Current UK dietary recommendations advocate for a reduction of total SFA intake to ≤10% of total energy intake, a target which is still exceeded by 27% of the adult population (10, 263). Therefore, reducing SFA entry into the food chain and the subsequent impact on

CVD risk markers is an important public health strategy aimed at reducing the prevalence of CVD in the population. The RESET trial (NCT02089035), examined the effects of partially replacing the SFA content of milk and dairy products with unsaturated FAs, predominantly in the form of MUFA, through supplementation of the dairy cow diet (203) on traditional and novel CVD risk factors in the fasted and fed (postprandial) state (201, 238, 264). To the best of our knowledge, no other study has investigated the impact of the *APOE* genotype on CVD risk biomarkers responses to long-term consumption of the FA modified dairy products, produced as a result of supplementation of the dairy cow feed with plant oils. The current paper is a secondary analysis which aimed to explore the potential role of the *APOE* genotype on the fasting and postprandial risk markers following two iso-energetic, high dairy diets with a differential fatty acid profile. We hypothesised that fasted and postprandial risk markers of CVD would be significantly impacted in the E4 carriers group following the two dairy diets and compared to the wild type *E3/E3* participants.

5.3 Materials and methods

5.3.1 Study design and subjects

The RESET study was a 12-wk double-blind, randomized, controlled crossover intervention study conducted with an 8-wk washout period between the two dietary treatments.

The intervention trial was conducted at the Hugh Sinclair Unit of Human Nutrition (University of Reading, UK), between February 2014 and April 2016 in accordance with the Declaration of Helsinki and was given a favourable ethical opinion by the University of Reading Research Ethics Committee (Project reference 13/14). All participants gave their written informed consent before starting the intervention.

A total of 54 participants (31 males; 23 females), were screened for fasting and anthropometric parameters and a scoring system was used, based on the modified Framingham

risk score, to recruit participants with a 1.5-fold higher CVD risk (164, 201). A flexible food-exchange model (201) was developed to implement two separate iso-energetic high dairy diets (38% of total energy intake (%TE) from total fat): modified (dietary target: 16%TE SFA, 14%TE MUFA) and control (19%TE SFA, 11%TE MUFA) which were consumed in a randomised cross-over design, separated by an 8-wk washout period.

Before each study visit at weeks 0, 12, 20 and 32, participants refrained from alcohol and aerobic exercise for 24 h and fasted for 12 h, after consuming a low fat standard meal (< 1.46 MJ; < 7 g total fat), drinking only low nitrate mineral water during this time. Prior to fasting blood sampling and vascular function measurement, participants were cannulated in the antecubital vein of the forearm, to allow multiple blood samples to be collected throughout the day. Of the 54 participants, only 52 completed the postprandial intervention study following fasted measurements, with test meals containing dairy products with a FA composition representative of the assigned dietary intervention. The breakfast test meal (50 g total fat) was provided at 0 min and consisted of a toasted sandwich with study cheese and butter, UHT study milk with cornflakes and milkshake prepared with study UHT milk and strawberry syrup. At 330 min, the lunch test meal (30 g total fat) was provided, consisting of a toasted study cheese and butter sandwich and strawberry UHT study milkshake drink. A total of 13 blood samples were collected at regular intervals until 480 min after the breakfast meal (264) (see Supplemental Table 5.1 for test meal composition).

5.3.2 Biochemical analyses

Serum lipids (total cholesterol, TC (fasting only); HDL-cholesterol (HDL-C; fasting only); and triacylglycerol, TAG), glucose, apolipoprotein (apo)B, C-reactive protein (fasting only) and non-esterified fatty acids (NEFA) were measured using an autoanalyzer (reagents and analyzer: Instrumentation Laboratory Ltd. ILAB600, Werfen,UK); apoB reagent: Randox Laborarories Ltd; non-esterified fatty acid reagent: Alpha Laboratories Ltd.). The Friedewald

equation was used to estimate fasting LDL-C (49). ELISA kits were used to analyze circulating serum insulin (Dako UK Ltd.).

5.3.3 NMR metabolomics

Quantification of the particle size distribution of the LDL and HDL subclasses in the fasted state, was performed through a high-throughput nuclear magnetic resonance (^{1}H-NMR) metabolomics platform (Nightingale Health Ltd., Finland) as previously described (215). Change from the pre-intervention visit (baseline) following each intervention diet for the number of LDL-particle (LDL-P; classified by their mean diameter size as small, (18.7 nm), medium (23 nm) and large (25.5 nm)) and HDL-particle (HDL-P; classified as small (8.7 nm), medium (10.9 nm), large (12.1 nm) and very large (14.3 nm) within each subclass distribution will be presented.

5.3.4 Stimulation of whole blood cultures

LPS-stimulated cytokine production from whole blood samples were determined as previously described (262). Briefly, K2EDTA whole blood cultures (WBC), diluted 1:9 in RPMI medium (Sigma, UK) were cultured in 12-well plates (Greiner bio-one, UK) with 0.5 μg/ml of bacterial lipopolyssacharide (LPS; *E. coli* 026:B6, Sigma, UK), with the addition of 1% antibiotics, 1% L-glutamine and 1% non-essential amino acids (Bioscience, UK). Following 24 h incubation at 37 °C, cultures were centrifuged at 700 x g (1000 rpm) and the supernatant stored at -20 °C. Measurement of the monocyte count of each sample was performed by the Pathology Department at the Royal Berkshire Hospital (Reading, UK). A human cytokine premixed 5-Plex Panel (IL-6, IL-8, IL-1β, TNF-α; R&D Systems Europe Ltd) and Luminex 200 with xPONENT software was used to measure the concentrations of cytokines in the WBC supernatant (1:2 dilution). Cytokine production was corrected for the number of monocytes in the whole blood sample.

5.3.5 Genotyping

The Qiagen DNA Blood Mini Kit (Qiagen Ltd., Crawley, UK) was used to extract DNA from the buffy coat samples, collected into K3EDTA-coated tubes at week 0. *APOE* genotyping was performed retrospectively using "Assay-on-Demand" single nucleotide polymorphism genotyping assays (rs7412 and rs429358) (Life Technologies, UK).

5.3.6 Statistical analysis

All data were checked for normality and log transformed where necessary prior to statistical analysis. The genotype distribution of the two SNPs at the *APOE* genes were in Hardy-Weinberg equilibrium ($p > 0.05$). Differences in participant baseline characteristics (prior to starting the dietary interventions) according to *APOE* genotype, were assessed by independent *t-test*. For fasted outcome measures, differences from baseline (Δ; wk-12 – wk-0 and wk-32 – wk-20) were calculated. Summary measures of change in postprandial responses were calculated using the trapezoidal rule for area under the curve (AUC) and, by subtracting the fasted value, the incremental AUC (iAUC). Percentage NEFA suppression was also calculated to represent an initial drop in NEFA concentration following the breakfast meal (0 – 120 min). Therefore, the postprandial summary measures of NEFA AUC and iAUC were calculated from 120 min to the end of the postprandial period (480 min). Maximum concentration (CMax) and time to reach maximum concentration (TTMax) were calculated for variables with 10 (apoB) and 13 (TAG, NEFA, glucose and insulin) time points only. Genotype x treatment effects were evaluated for *E3/E3* and *E4* carriers, using a linear mixed-model run through the SAS 9.4 University edition statistical software (SAS Institute Inc., Cary, NC, USA). Differences in fasted baseline values and summary measures of change of postprandial responses were inputted as the dependent variable, adjusted for fixed effects of baseline values of the assessed variable, period, sequence, treatment, age, gender, BMI and genotype.

Participant was included as a random effect. No genotype effects were observed for any of the fasting outcome measures or postprandial summary responses. Data are presented in the tables as the mean change ± SEM. Values of $P \leq 0.05$ were taken as significant.

5.4 Results

Genotyping for *APOE* resulted in the following frequencies: 57% *E3/E3* (*n* = 31; 19 males and 12 females); 30% *E4* carriers (*n* = 15 *E3/E4*; n = 1 *E4/E4;* 6 males and 10 females) and 13% *E2* carriers (*n* = 7; 6 males and 1 female). Due to the low numbers of *E2* carriers, this group of participants was excluded from the data analyses, resulting in a total of 47 participants in this genotyping dataset. Additionally, *E3/E4* and *E4/E4* carriers were combined for the analysis.

Subject characteristics for the complete genotyping dataset (*n* = 47) and by *E3/E3* (*n* = 31) and *E4* carriers (*n* = 16) are presented in Table 5.1. There was a significant difference between the two genotype groups in fasting concentrations of HDL-C, which was 16% higher in the *E4* carriers compared to the *E3/E3* group (*P* = 0.03). Additionally, fasting small and medium sized HDL particle (HDL-P) concentrations were 5% and 14% higher in the *E4* carriers, compared to *E3/E3* (*P* = 0.03 and *P* = 0.04 respectively). Fasting NEFA concentrations were also 28% higher in the *E4* carriers compared to the wild type genotype group (*P* = 0.04). There were no other significant differences observed for any of the other baseline characteristics according to *APOE* genotype.

There were no genotype or treatment effects and no genotype x treatment interactions on the fasting concentrations or postprandial summary measures of CVD risk biomarkers, as measured by AUC, iAUC, CMax and TTMax (Tables 5.2 and 5.3). Similarly, genotype and treatment effects and genotype x treatment interactions were not observed for the postprandial WBC LPS-stimulated cytokine production (IL-1β, IL-6, IL-8, IL-10 and TNFα), following the two dietary interventions (Table 5.4).

5.5 Discussion

As far as the authors are aware, this is the first study to determine the effects of *APOE* genotype on the lipid responses following manipulation of the dairy fat composition. Significant differences in certain pre-intervention (baseline) parameters between *E4* carriers and *E3/E3* groups were observed. Yet, although *APOE* genotype has been shown to influence the lipid response to dietary intervention and meal intake, our study found no effect following consumption of dairy products in which SFA was partially replaced by MUFA.

We observed higher baseline fasting NEFA concentrations in the *E4* carriers, compared to *E3/E3* which is in line with previous observations and have been recognized as a CVD risk marker (112, 113, 119, 265). We also observed higher baseline fasted HDL-C concentrations in the *E4* carriers, compared to the wild type *E3*, which is in contrast to previously published studies (266, 267). Interestingly, this difference appears to be reflected in a higher proportion of small and medium sized HDL-P in the *E4* carrier group. The heterogeneity of the HDL-P, in terms of size, density and functionality is well recognized (268). The association of each HDL-P sub-class to CVD risk is unclear, with some studies suggesting that larger, more buoyant HDL-P present a stronger inverse association with CVD risk compared to smaller, dense HDL-P (269). If true, this would imply that the *E4* carriers in our study may be at higher CVD risk. However, our observed baseline differences should be interpreted with caution, due to retrospective genotyping and small number of *E4* participants and would need confirmation in further studies.

Although a limited number of studies have investigated the impact of replacing SFA with MUFA on fasted or postprandial CVD risk markers according to *APOE* genotype, the majority have used commercially available foods, such as spreads, snacks and oils (predominantly olive oil) to implement the dietary fat manipulation (270). In the LIPGENE study, individuals with the metabolic syndrome (n = 416) were randomized to one of four iso-

energetic dietary interventions (high SFA, high MUFA, two low fat, high complex carbohydrate supplemented with 1g long chain n-3 FA (EPA and DHA) or placebo oil) (271). No interaction between *APOE* genotype was observed when comparing the high SFA diet (38%TE total fat; 16%TE SFA, 12%TE MUFA) to the high MUFA diet (38%TE total fat; 20%TE MUFA; 8%TE SFA) (119, 271). Furthermore, the SATgene study (n = 88), which investigated the impact of *APOE* genotype following a low fat (24% E total fat, 8% as SFA, 59% CHO), high SFA (HSF; 38% total fat, 18% SFA, 45% CHO) and high SFA with added 3 g/d DHA diets in prospectively genotyped normolipidaemic adults, found a modest impact on TAG and C-reactive, suggesting a greater sensitivity to dietary fat composition in *E4* carriers (118, 257, 258). Similar to our study design, the SATgene postprandial study also used test meals representative of the macronutrient content of the dietary intervention (258). A variable postprandial TAG response was reported according to genotype and following the HSF-DHA diet compared to the other three diets, whereby *E4* carriers exhibited a greater TAG-lowering effect (258). In a further postprandial study, which explored the responses to SFA-rich, MUFA-rich (both from vegetable oil sources) and SFA with fish oil in mixed meals, no significant differences in outcome measures were observed, when comparing *E4* carriers to the *E3/E3* group (260). However, both postprandial studies were carried out in healthy men, which is in contrast to our study cohort.

Dietary fat manipulation may impact inflammatory responses, with evidence suggesting that the *APOE* genotype affects macrophage cytokine secretion and fasted CRP concentrations (116, 118, 255). In our study, we observed no differences in baseline concentrations of *ex vivo* LPS-stimulated cytokines. This is in contrast to *in vitro* and the small number of human studies which report higher concentrations of pro-inflammatory cytokines and lower concentrations of anti-inflammatory cytokines in *E4* carriers, compared to *E3* homozygotes (255). This may be explained in part by the small number of *E4* carriers in our cohort, which was lower than the *E3/E3* group. Additionally, few studies have investigated the interaction of genotype on

modulation of the inflammatory response following dietary fat manipulation. In agreement with our results, a subset of the SATgene study which investigated *ex vivo* LPS-stimulated cytokine production in whole blood cultures, did not observe an interaction of *APOE* genotype following the sequential dietary fat interventions (262). The authors concluded that dietary fat composition, rather than *APOE* genotype, influenced *ex vivo* cytokine production (262). Taking into account that inflammatory markers are also affected by other factors, such as age and gender, more studies are needed to investigate the impact of *APOE* genotype on fasting and postprandial inflammatory markers following dietary fat modification.

A strength of the current study is the investigation of a genotype effect in a chronic and acute-within-chronic design. Additionally, the test meals incorporated dairy with a FA composition representative of the dietary interventions. Furthermore, the *ex vivo* production of LPS-stimulated cytokines used whole blood cultures, considered more physiologically informative for the assessment of low grade inflammation, compared to measurement of circulating plasma cytokines (272). Lastly, the genotype frequency in our sub-cohort appears in line with data from the literature for the UK population (254).However, a limitation of the current hypothesis generating study was the use of a retrospective genotype approach, which resulted in unequal group sizes and gender distribution of *E3/E3* homozygotes and *E4* carriers. Due to this approach, the number of participants was relatively small compared to studies which have previously investigated dietary fat manipulation and *APOE* genotype.

In conclusion, this study investigated the interaction of *APOE* genotype on fasting and postprandial CVD risk markers following long-term consumption of dairy products with a differential fatty acid composition. The observed baseline differences in HDL particle size number between the two genotype groups is a novel finding worth exploring in future studies. No outcome measures were significantly modulated by *APOE* genotype. However, there is considerable interest to determine sensitivity of *APOE* polymorphisms to dietary SFA

replacement with unsaturated FAs, as UK dietary recommendations advocate for a reduction of current SFA intakes for CVD risk reduction (10). Future studies are therefore needed to evaluate this interaction in larger, prospectively recruited cohorts.

5.6 Acknowledgments

The authors gratefully acknowledge all participants who took part in the study. We thank S. Hargreaves, K. Jenkins, R. Mihaylova, the technical staff and research assistants/students (V. Bines, C. Brilley, L. Bula, Y. Chatzidiakou, V. Clifton, H. Dong, A. Garcimartin, C. Holland, J. Guo, A. Kanneganti, T. Loughman, E. Mertens, J. Ryan, L. Sellem, T. Staff and H. Zhang) for their assistance with recruitment, data collection, the blinding of intervention foods, data entry or food diary analysis.

J.A.L., D.I.G. and K.G.J designed the human study; C.C.F., A.S.G., D.J.H and D.I.G. designed and produced the modified dairy products; D.V. and O.M. conducted the research; D.V., O.M., and K.G.J. analyzed data or performed statistical analysis; S.T. provided statistical advice; D.V. wrote the manuscript, which was modified by all co-authors; and J.A.L. had primary responsibility for final content. All authors read and approved the final manuscript. Arla Foods UK and AAK UK provided the control dairy study products and high oleic sunflower oil, respectively, and were not involved in the study design or data interpretation.

Table 5.1 Pre-intervention baseline characteristics of the group as a whole and according to *APOE* genotype

	All	*E3/E3* carriers	*E4* carriers	p^1
n (M/F)	47 (25/22)	31 (19/12)	16 (6/10)	
Age (years)	52 ± 2	51 ± 2	55 ± 3	0.35
BMI (kg/m2)	26 ± 0	26 ± 1	25 ± 1	0.27
Systolic BP (mm Hg)	121 ± 2	121 ± 3	122 ± 2	0.76
Diastolic BP (mm Hg)	70 ± 1	70 ± 2	72 ± 1	0.30
TC (mmol/L)	5.58 ± 0.13	5.46 ± 0.16	5.79 ± 0.19	0.23
LDL-C (mmol/L)	3.54 ± 0.11	3.48 ± 0.14	3.67 ± 0.17	0.43
HDL-C (mmol/L)	1.50 ± 0.05	1.42 ± 0.05	1.65 ± 0.09	0.03
TC:HDL-C ratio	3.85 ± 0.12	3.97 ± 0.16	3.61 ± 0.17	0.17
TAG (mmol/L)	1.18 ± 0.07	1.25 ± 0.10	1.04 ± 0.08	0.19
ApoB (g/L)	1.02 ± 0.03	1.02 ± 0.04	1.02 ± 0.05	0.94
NEFA (μmol/L)	602 ± 30	551 ± 28	703 ± 62	0.04
Glucose (mmol/L)	5.38 ± 0.11	5.47 ± 0.16	5.20 ± 0.08	0.13
Insulin	36.2 ± 2.8	34.8 ± 3.4	38.7 ±4.9	0.53
HOMA-IR	1.42 ± 0.12	1.39 ± 0.15	1.50 ± 0.20	0.67
CRP (mg/L)	1.64 ± 0.40	1.60 ± 0.44	1.73 ± 0.81	0.89
Particle distribution				
LDL-P (nmol/L)				
Small	173 ± 4	170 ± 6	179 ± 4	0.31
Medium	280 ± 8	275 ± 1	290 ± 13	0.40
Large	579 ± 16	562 ± 20	611 ± 24	0.15
HDL-P (nmol/L)				
Small	9966 ± 117	9782 ± 141	10310 ± 177	0.03
Medium	3969 ± 114	3783 ± 123	4318 ± 205	0.02

Large	1647 ± 114	1518 ± 131	1889 ± 206	0.13
Very Large	235 ± 13	224 ± 16	256 ± 24	0.27
LPS-stimulated cytokines				
IL-6 (μg x 10^3 monocytes)	77 ± 4	78 ± 5	74 ± 4	0.53
IL-8 (μg x 10^3 monocytes)	140 ± 15	123 ± 15	178 ± 31	0.14
IL-1β (μg x 10^3 monocytes)	27 ± 1	27 ± 2	28 ± 3	0.87
IL-10 (μg x 10^3 monocytes)	29 ± 3	27 ± 3	33 ± 2	0.07
TNF-α (μg x 10^3 monocytes)	11 ± 1	11 ± 1	10 ± 1	0.22
Risk score				
CVD risk score[2]	3.1 ± 0.2	3.0 ± 0.2	3.2 ± 0.3	0.88

Values are mean ± SEM. ApoB, apolipoprotein B; BP, blood pressure; CRP, C-reactive protein; HDL-C, HDL-cholesterol;

HDL-P, HDL particle size; LDL-C, LDL-cholesterol; LDL-P, LDL particle size; NEFA, non-esterified fatty acids; TC, total cholesterol; TAG, triacylglycerol.

[1] Independent *t test* was used to assess differences between fasting baseline characteristic of the genotype groups.

[2] Assessed with the use of a modified Framingham risk score, where a score of ≥ 2 points relates to a 1.5-fold higher risk of CVD than the population mean (164, 165, 201).

Table 5.2 Change in fasting lipid profile, particle size distribution, glucose, insulin and C-reactive protein following the modified and control diet and according to genotype[1]

	Modified diet		Control diet		*p*[2]		
	E3/E3	*E4* carriers	*E3/E3*	*E4* carriers	Treatment	Genotype	Genotype x treatment
TC (mmol/L)	0.24 ± 0.09	0.15 ± 0.14	0.23 ± 0.12	0.26 ± 0.08	0.11	0.91	0.47
LDL-C (mmol/L)	-0.05 ± 0.08	0.08 ± 0.12	0.17 ± 0.07	0.21 ± 0.08	0.07	0.44	0.65
HDL-C (mmol/L)	0.05 ± 0.03	-0.01 ± 0.04	0.08 ± 0.03	0.07 ± 0.05	0.34	0.25	0.81
TC:HDL-C ratio	-0.07 ± 0.06	0.10 ± 0.09	0.03 ± 0.05	0.04 ± 0.09	0.76	0.14	0.34
TAG (mmol/L)	0.16 ± 0.10	0.18 ± 0.09	0.12 ± 0.07	-0.11 ± 0.08	0.08	0.45	0.10
ApoB (g/L)	4 ± 18	52 ± 30	39 ± 17	34 ± 22	0.86	0.53	0.35
NEFA (μmol/L)	-47 ± 29	-92 ± 59	-31 ± 28	-82 ± 59	0.62	0.25	0.60
Glucose (mmol/L)	0.02 ± 0.08	-0.02 ± 0.06	0.11 ± 0.10	-0.09 ± 0.21	0.16	0.17	0.61
Insulin (pmol/L)	-2 ± 2.4	-4.5 ± 4.5	11.5 ± 6.4	1.1 ± 3.3	0.75	0.21	0.68
HOMA-IR	0.02 ± 0.07	-0.20 ± 0.19	0.35 ± 0.25	0.13 ± 0.23	0.16	0.61	0.68
CRP (mg/L)	0.14 ± 0.42	-0.08 ± 0.25	0.09 ± 0.23	-0.64 ± 0.57	0.73	0.21	0.42
Particle distribution							
LDL-P (nmol/L)							
Small	2.2 ± 3.2	6.2 ± 3.2	6.2 ± 2.3	4.8 ± 3.6	0.74	0.45	0.31
Medium	0.7 ± 4.7	12.7 ± 6.0	9.2 ± 4.1	7.6 ± 6.4	0.76	0.82	0.21
Large	-3.0 ± 11.4	30.1 ± 13.6	46.2 ± 30.3	24.0 ± 14.2	0.50	0.21	0.29
HDL-P (nmol/L)							
Small	241.5 ± 151.0	165.8 ± 136.6	385.0 ± 140.8	19.3 ± 227.8	0.85	0.21	0.56
Medium	136.0 ± 81.0	49.4 ± 62.7	276.4 ± 149.1	27.7 ± 117.0	0.83	0.49	0.64
Large	95.5 ± 63.1	-79.2 ± 111.6	85.5 ± 63.1	36.2 ± 67.9	0.98	0.64	0.46
Very Large	10.1 ± 8.3	14.2 ± 21.6	12.6 ± 9.5	17.7 ± 11.5	0.69	0.10	0.64

[1] Values represent change-from-baseline in fasted parameters following the modified and control diets ± SEM. *n* = 31 *E3/E3*; *n* = 16 *E4* carriers. ApoB, apolipoprotein B; CRP, C-reactive protein; HDL-C, HDL cholesterol; HDL-P, HDL particles; HOMA-IR, homeostatic model assessment of insulin resistance; LDL-C, LDL cholesterol; LDL-P, LDL particles; NEFA, non-esterified fatty acids; TAG, triacylglycerol; TC, total cholesterol.

[2] Mixed model analyses were used to calculate overall effect of genotype x treatment based on change-from-baseline values, with adjustments made for fixed effects of baseline values of the assessed variable, period, treatment sequence, gender, age, BMI and genotype. Participant was included as a random effect.

Table 5.3. Change in postprandial summary response measures of lipids, glucose and insulin following consumption of the modified and control test meals, according to genotype[1]

	Modified diet		Control diet		*p*[2]		
	E3/E3	*E4* carriers	*E3/E3*	*E4* carriers	Genotype	Treatment	Genotype x treatment
TAG							
AUC (mmol/l x 480min)	117 ± 76	121 ± 88	91 ± 59	-23 ± 59	0.79	0.74	0.56
iAUC (mmol/l x 480min)	38 ± 35	48 ± 48	-8 ± 36	40 ± 36	0.90	0.14	0.30
Cmax (mmol/l)	0.2 ± 0.2	0.3 ± 0.3	0.1 ± 0.2	-0.1 ± 0.2	0.83	0.72	0.28
TTmax (min)	-22 ± 15	-13 ± 22	-14 ± 20	-10 ± 28	0.72	0.77	0.53
NEFA							
% suppression (120min)	52 ± 31	42 ± 51	13 ± 33	8 ± 61	0.85	0.89	0.83
AUC (mmol/l 120-480min)	-0.4 ± 0.1	11 ± 11	-0.5 ± 4.2	0.8 ± 9.3	0.70	0.97	0.76
iAUC (mmol/l 120-480min)	10 ± 13	20 ± 16	14 ± 11	23 ± 29	0.99	0.68	0.76
Cmax (mmol/l)	-0.73 ± 0.32	-0.04 ± 0.64	0.2 ± 0.32	-0.53 ± 0.62	0.31	0.16	0.47
TTmax (min)	7 ± 43	3 ± 41	-70 ± 31	-16 ± 61	0.97	0.25	0.96
ApoB							
AUC (mg/l x 420min)	-3 ± 9	15 ± 14	18 ± 7	21 ± 10	0.33	0.18	0.71
iAUC (mg/l x 420min)	-4 ± 4	1 ± 7	4 ± 4	6 ± 8	0.58	0.09	0.57
Cmax (mg/l)	10 ± 22	37 ± 37	40 ± 22	55 ± 26	0.34	0.36	0.92
TTmax (min)	-1 ± 42	72 ± 44	0 ± 48	46 ± 48	0.74	0.49	0.10
Glucose							
AUC (mmol/l x 480min)	-15 ± 36	27 ± 64	137 ± 58	-86 ± 45	0.13	0.44	0.11
iAUC (mmol/l x 480min)	-34 ± 51	18 ± 56	35 ± 43	-36 ± 48	0.49	0.59	0.97
Cmax (mmol/l)	-0.1 ± 0.2	0.3 ± 0.3	0.1 ± 0.4	-0.5 ± 0.2	0.35	0.5	0.27
TTmax (min)	41 ± 36	30 ± 52	-6 ± 31	63 ± 59	0.75	0.96	0.51
Insulin							
AUC (pmol/l x 480min)	-2 ± 5	13 ± 16	2 ± 4	-8 ± 8	0.54	0.73	0.44
iAUC (pmol/l x 480min)	-7 ± 7	15 ± 18	-9 ± 7	-7 ± 7	0.38	0.82	0.83
Cmax (pmol/l)	29 ± 27	94 ± 69	2 ± 30	-105 ± 71	0.19	0.14	0.15
TTmax (min)	7 ± 43	55 ± 11	-67 ± 30	-39 ± 64	0.81	0.67	0.67

[1] Values represent post-intervention change from the pre-intervention visit following the sequential test meals of the modified and control diets ± SEM. *n* = 31 *E3/E3*; *n* = 16 *E4* carriers. ApoB, apolipoprotein B; AUC, area under the curve; Cmax, maximum concentration; iAUC, incremental area under the curve; NEFA, non-esterified fatty acids; TAG, triacylglycerol; TTMax, time to reach maximum concentration.

[2] Mixed model analyses were used to calculate overall effect of genotype and genotype x treatment effect based on change-from-baseline values, with adjustments made for fixed effects of baseline values of the assessed variable, period, treatment sequence, gender, age, BMI and genotype. Participant was included as a random effect.

Table 5.4 Change in fasting and postprandial summary response measures of LPS-stimulated cytokine production in whole blood cultures following the modified and control test meals, according to genotype[1]

	Modified		Control		p^2		
	APOE3/E3	*APOE4* carriers	*APOE3/E3*	*APOE4* carriers	Genotype	Treatment	Genotype x treatment
IL-6							
fasting	10 ± 6	-3 ± 9	9 ± 6	-0.4 ± 7	0.69	0.26	0.31
AUC (x 420 min)	3857 ± 1613	3439 ± 2100	2420 ± 2170	755 ± 2181	0.26	0.25	0.68
iAUC (x 420 min)	-739 ± 2454	-582 ± 1974	-2450 ± 2097	-2790 ± 2438	0.40	0.62	0.27
IL-8							
fasting	45 ± 38	10 ± 18	2 ± 20	4 ± 23	0.90	0.54	0.98
AUC (x 420 min)	7201 ± 3430	2549 ± 8448	-5476 ± 4506	3467 ± 11069	0.84	0.12	0.23
iAUC (x 420 min)	268 ± 6709	-1645 ± 6226	-4475 ± 5677	-429 ± 6096	0.74	0.44	0.18
TNFα							
fasting	2 ± 2	2 ± 2	2 ± 2	2 ± 2	0.19	0.99	0.95
AUC (x 420 min)	457 ± 329	598 ± 427	591 ± 464	127 ± 468	0.29	0.73	0.38
iAUC (x 420 min)	10 ± 445	403 ± 537	-382 ± 386	-510 ± 372	0.63	0.74	0.52
IL-1β							
fasting	0 ± 2	-2 ± 2	3 ± 2	4 ± 4	0.88	0.42	0.47
AUC (x 420 min)	564 ± 611	-84 ± 609	1149 ± 869	1670 ± 1130	0.94	0.13	0.86
iAUC (x 420 min)	21 ± 733	721 ± 1018	-130 ± 771	318 ± 1047	0.77	0.19	0.96
IL-10							
fasting	0.0 ± 0.1	0.0 ± 0.2	0.0 ± 0.1	0.0 ± 0.3	0.82	0.45	0.32
AUC (x 420 min)	9 ± 34	-9 ± 36	-34 ± 36	-99 ± 58	0.71	0.18	0.38
iAUC (x 420 min)	-4 ± 41	-2 ± 79	-52 ± 29	-85 ± 98	0.21	0.97	0.87

[1] Values represent change in fasting values and change in postprandial summary response measures following the modified and control diets ± SEM. All cytokines are expressed in μg x 10^3 monocytes. For fasted parameters: *n* = 30 *APOE3/E3*; *n* = 15 *APOE4* carriers. For postprandial parameters: *n* = 28 *APOE3/E3*; *n* = 14 *APOE4* carriers (IL-6, TNFα, IL-1β, IL-10); *n* = 28 *APOE3/E3*; *n* = 12 *APOE4* carriers (IL-8). AUC, area under the curve; iAUC, incremental area under the curve.

[2] Mixed model analyses were used to calculate overall effect of genotype x treatment effect based on change-from-baseline values, with adjustments made for fixed effects of baseline values of the assessed variable, period, treatment sequence, gender, age, BMI and genotype. Participant was included as a random effect.

Supplemental Table 5.1 Macronutrient composition of the breakfast and lunch test meals consumed by participants at baseline (wk-0) and post-intervention (wk-12) of the modified and control diets.

	Energy MJ	Protein g	CHO g	Fat g	SFA g	MUFA g	PUFA g	*trans* MUFA g
Breakfast (0 min)								
Modified diet	4.3	36.1	105.9	50.6	24.5	20.0	2.9	3.9
Control diet	4.1	39.7	101.4	49.9	31.7	12.3	2.8	2.2
Lunch (330 min)								
Modified diet	2.6	20.9	64.6	30.6	14.8	12.1	1.8	2.6
Control diet	2.5	19.6	63.3	30.3	19.1	7.4	1.8	1.4

CHO, carbohydrates. Adapted from Vasilopoulou et al (264).

Chapter VI: General discussion and future perspectives

The following chapter summarises the studies presented in this thesis, providing an overview and concluding remarks of the findings to date. Additionally, recommendations for future research are presented.

6.1 General discussion

Milk and dairy products are a widely consumed food group and a source of several important nutrients in the diet. The impact of dairy products on human health remains of significant importance, considering their use in a wide variety of composite foods, ranging from pastries to ready meals. Cardiovascular disease (CVD) remains one of the major causes of death and morbidity worldwide, despite declining rates in mortality following prevention strategies and improved treatments (5). In the UK, CVD represents the second most common cause of mortality, responsible for almost a third of all deaths (4). High intakes of dietary saturated fatty acids are regarded as one of the key risk factors associated with the development and progression of CVD, mediated by increased levels of circulating LDL-cholesterol (LDL-C) (9). Therefore, most national dietary guidelines advocate for a reduction of total SFA intake. In the UK, current recommendations aim for a reduction of SFA to <10% total energy intake, a target which is still exceeded by the majority of the adult population (10). Milk and dairy products (including butter) contribute on average up to 35% of total dietary SFA in the UK and are regarded as the greatest contributor to dietary SFA intake (10). Dietary recommendations for dairy products advise for a limitation of total dairy product consumption and a preference for low-fat alternatives, compared to full-fat counterparts (263). These recommendations were also described in the UK's former Eatwell Plate, which advised for dairy consumption to contribute up to 15% of the overall diet. In 2016, the new Eatwell Guide replaced the Eatwell Plate and presented a reduced target of dairy consumption of up to 8% of the overall diet, which includes dairy alternatives such as soya drinks (273). As presented in the literature review (**Chapter 1**), these dietary recommendations do not seem to be in accordance with current evidence from prospective cohort studies and RCTs, which have not shown a detrimental effect of dairy consumption on CVD risk, with the exception of butter intake (36, 48, 150). In contrast to current UK advice on dairy intake, other countries such as

Denmark and USA, advocate for the consumption of at least 3 portions of dairy products per day. As described in **Chapter 1**, a number of potential mechanisms have been proposed to explain the beneficial impact of milk and specific dairy product consumption on CVD risk (68). Specifically, bioactive compounds such as dairy proteins, amino acids and micronutrients may, beneficially impact on CVD risk markers. Furthermore, increasing evidence suggests that focus should shift toward investigating the food matrix of dairy products (the sum of dairy nutrients within the specific dairy food structure), rather than single nutrients, to evaluate their impact on health (21, 25).

Recently, three meta-analyses of prospective studies reported no association between dietary SFA intake and CVD risk, raising doubts on the detrimental effects of high SFA intake and questioning current dietary recommendations (18-20). However, these meta-analyses have been criticised for not taking into account the importance of dietary replacement of SFA with a suitable macronutrient to assess disease risk associations. Moreover, the meta-analysis of Hooper et al reported a 17% reduction in CVD events following a reduction in SFA intake, although recognised that effects on myocardial infarction and stroke were less clear (199). Additionally, the association between high SFA intake and CVD risk is an indirect one, mediated primarily by the observed increases in LDL-C an independent CVD risk marker, with additional mechanisms such as an impact on vascular function and glycaemia (15, 53). Studies which have investigated an iso-energetic SFA replacement with unsaturated fats (UFA) have contributed towards accumulating evidence supporting a beneficial impact on CVD (23). As described in **Chapter 1**, taking into consideration the beneficial effects of certain dairy products on CVD risk and the importance of replacing high SFA intake with a suitable UFA, partial replacement of SFA in dairy fat with UFAs provides an alternative strategy to simply reducing total dairy intake or advocating for low-fat products. Supplementation of the dairy cow diet with plant oils or oil seeds leads

to the production of milk with a modified FA profile (122). Although such an approach would allow consumers to maintain the beneficial aspects of dairy consumption while limiting SFA intake into the food chain, only a small number of RCTs have investigated the impact of consuming FA-modified dairy products on human health (**Chapter 1)**. Of these studies, four observed a reduction in TC and LDL-C following chronic consumption of FA-modified dairy products in which total dairy SFA was partially replaced by MUFA. However, these studies presented heterogeneity in methodological approaches, from the supplementation strategy of the dairy cows to study populations and estimations of CVD risk. Considering the methodological variations of these studies and the apparent beneficial impact on CVD lipid risk markers (primarily TC and LDL-C), further research combining multiple risk markers would provide a more informative understanding of the effects associated with replacing SFA with UFAs in dairy products.

To address this evidence gap and to gain a better insight of the replacement of SFA with MUFA within a dairy background, a novel RCT was conducted and presented in **Chapters 2, 3** and **4**. The RESET trial investigated both the chronic and acute-within-chronic effects of SFA-reduced, MUFA-enriched dairy products (FA-modified), compared to control matched conventional products, on traditional and novel risk markers of CVD. Supplementation of the dairy cow feed with high-oleic sunflower oil led to partial replacement of total SFA and a parallel increase of total MUFA in milk fat, as described in detail in **Appendix II**. The development of the food exchange model, described in **Chapter 2**, aimed to implement specific dairy fat manipulation, without changing other components of the participants' diets. Furthermore, it underlined the importance of evaluating the efficacy and adherence of the food-exchange strategy within the dietary interventions. This was achieved by comparing post-intervention change of the two dairy diets from both the 4 day weighed diet diaries and data obtained from the analysis of the plasma phospholipid FA (PL-

FA) fraction of the participants. The weighed dietary records confirmed that overall, the FA-modified diet let to a reduction in SFA intake by 2.5% of total energy (%TE), with a parallel increase in MUFA of 3.5%TE, compared to the control diet. Comparatively, the analysis of the PL-FA data showed a modest decrease in the abundance of total SFAs and a concomitant increase in total *cis*-MUFAs. It is worth noting the potential limitation of circulating SFA as direct indicators of dietary intake, as some FA, particularly palmitic acid, can be endogenously synthesized from other FA and dietary carbohydrate (163, 274). Results from both the 4 day weighed food diaries and PL-FA analysis also highlighted the observed increase in *trans* ruminant FA (rTFA) intake following the modified diet and compared to the control diet. This is consistent with previous dairy cow supplementation studies, which have observed increased levels of ruminant TFA (rTFA) in the milk and derived dairy products, a result of ruminal biohydrogenation of UFAs (122, 203). Current evidence appears to suggest that rTFA do not detrimentally impact on human health, unlike industrial counterparts (220). The observed results from the RESET study have added to the evidence base of the impact of specifically increasing rTFA on CVD. However, further work is needed to explore the association of consuming rTFA present in FA-modified dairy products on human health.

The intervention study presented in **Chapter 3** and **4**, add further insight into the impact of three types of FA-modified dairy products (UHT milk, Cheddar cheese and butter) on both traditional (fasted circulating lipid markers, blood pressure, arterial stiffness) and novel (vascular function, inflammatory and endothelial risk markers, size distribution of LDL and HDL particles) risk markers of CVD. The nature of the chronic and acute-within-chronic design addressed important aspects of dietary intervention studies. Firstly, investigating the long-term impact of replacing habitually consumed dairy products with FA-modified study products, provided the means to evaluate potential improvements in CVD risk markers, in a population at moderate CVD risk. The observed attenuation in the rise of fasted LDL-C

concentrations from baseline values, following consumption of the FA-modified diet and compared to the control diet, confirms the importance of considering not only the macronutrient replacing dietary SFA, but also the potential impact of the dairy food matrix. Additionally, the observed increase in fasted circulating LDL-C from baseline values following intake of conventional dairy products supports the detrimental effect of total SFA on LDL-C.

One of the aims of the present intervention study was to investigate the impact of FA-modified dairy products on novel risk markers of CVD, in parallel to traditional ones. The observed increase in fasted plasma nitrite concentrations as a result of 12-week consumption of the FA-modified dairy diet is indicative of a potentially beneficial impact on endothelial function, which may further be supported by the results of the FMD response. However, it remains unanswered whether the lack of an impact on other fasted markers of CVD risk (**Chapter 3**) was due to the two dairy diets having similar amounts of protein and other micronutrients, such as calcium, which have been observed to mediate effects on blood pressure and arterial stiffness (69).

Given that many individuals consume frequent meals throughout the day, investigating the postprandial state and assessing potentially modulatory effects on both circulating lipid markers and vascular function following dietary interventions remains of great public health importance. The acute-within-chronic study (**Chapter 4)** aimed to assess whether long-term intake of FA-modified dairy products would beneficially impact on postprandial markers of CVD risk. The novel design of the acute-within-chronic study assessed postprandial risk markers both pre and post chronic consumption of the two dairy diets by implementing a two meal challenge, with study test meals representative of the dairy products of each intervention arm. The observed lower postprandial response in the incremental area under the curve (iAUC) of apolipoprotein B (apoB), following consumption of the FA-modified diet and test

meals, is indicative of an effect on triglyceride-rich lipoprotein (TRL) metabolism. This is in agreement with other studies (232, 233) which have investigated a replacement of SFA with MUFA-rich diets and suggests an overall influence on the number and size of TRLs, which may therefore lead to an increased rate of clearance in the circulation. Similarly to the chronic study in **Chapter 3**, the FA-modified diet appears to also modulate the response of plasma nitrite in the postprandial state. However, as it was a non-significant change, this finding remains to be compared with the FMD response in the fasted and postprandial state, as evidence suggests that FMD response may be in part mediated by production of nitric oxide in healthy adults and to a lesser degree in adults at CVD risk (275).

The observed increase in TFA intake in the FA-modified diet compared to the control requires further investigation. Although, as has been mentioned in this thesis, the impact of rTFA may not as detrimental as iTFA, further studies are still needed to elucidate potential mechanisms on risk markers of CVD. Limited evidence suggests that specific rTFA, such as vaccenic acid, may increase both LDL-C and HDL-C (132, 220). This mechanism may have impacted the observed results in the present intervention. However, the specific effect of the rTFA present in the FA-modified dairy products could not be adequately investigated. Future studies on FA-modified dairy products may incorporate analyses that would elucidate potential mechanisms of rTFA on risk markers.

Response to dietary manipulation has been shown to be highly heterogenous, based on a number of factors including genetic polymorphisms. In **Chapter 5**, the response to the dietary intervention according to the participants' *APOE* genotype was explored. Evidence suggests that the *APOE4* allele is associated with an observed greater LDL-C elevation in response to dietary SFA and individuals with this allele may benefit from a reduced SFA intake and a parallel replacement of a suitable macronutrient (260). However, only *E4* carriers and the *E3/E3* wild type variant were analysed retrospectively, resulting in unequal participant

numbers and gender distribution for each group and no significant interaction observed following consumption of the two dairy diets. However, the pre-intervention observed baseline differences in NEFA, HDL-C and HDL particle size are of interest, primarily as it highlights the importance of not only exploring the association between genotype and dietary manipulation in larger studies, but how these findings may be translated in a wider context for populations at risk. Dietary recommendations may in future be tailored to specific population groups, such as those at higher CVD risk based on existing non-modifiable risk factors such as genetic polymorphisms.

Overall, findings from the chronic and acute-within-chronic study suggest that consumption of FA-modified dairy products leads to a modest beneficial impact on aspects of circulating LDL-C metabolism and nitrite bioavailability. As mentioned in the research chapters, the difference in total SFA and *cis*-MUFA content between the two dairy diets, although significant, may be regarded as modest, which may explain why no further changes in the other CVD risk markers were observed. However, these are aspects that may be investigated in future studies.

It is of interest to note that although recent UK dietary guidelines have advised a further reduction in total dairy intake, there is no actual recommendation for dairy intake. In contrast, other countries such as France, advise up to 3 servings of dairy a day (for example, one serving represents 250ml milk, 40 g hard cheese, 200 g yoghurt) (276). This recommendation was recently adopted in the UK by the dairy sector (277), as a way to campaign for transparency for the role of milk and dairy, and promote their consumption as part of a healthy diet. It remains to be seen therefore, whether dietary recommendations in the UK will also change in light of the current evidence of dairy consumption on CVD risk.

6.2 Conclusions

In conclusion, the data presented in this thesis add novel insight into the impact of FA-modified dairy products on fasted and postprandial risk markers of CVD. Development and implementation of the food-exchange model was successful, as shown by data obtained from the 4 day weighed food diaries and PL-FA. Our findings show that in adults at moderate CVD risk, long-term consumption of high quantities of FA-modified dairy products (38% total energy intake) led to an attenuation in the fasted concentrations of LDL-C, compared to both baseline values and consumption of matched control dairy products. Additionally, a postprandial investigation with test meals representative of the dietary intervention products resulted in observed decreases in postprandial apoB response following the FA-modified test meals are indicative of a potential impact on TRL metabolism. A further aim was to determine whether *APOE* polymorphisms would result in an additional impact on fasted and postprandial response, following dietary fat manipulation and consumption of the dairy study products. No outcome measures were significantly modulated by the *APOE* genotype of the participants, however baseline differences in NEFA, HDL-C and HDL particle size were observed. The findings of the present PhD research have added new and valuable data on the impact of FA-modified dairy products on established and novel CVD risk markers. At the same time, several aspects of the impact of FA-modified dairy products on CVD risk require further research.

6.3 Future perspectives

The studies presented in this thesis have addressed a number of important questions, while generating new insights into potential mechanisms of the role of dairy fat on CVD risk markers, which may form the basis for future research opportunities, some of which have been proposed in previous sections of the thesis. Findings from the RCT demonstrate a modest beneficial impact on specific CVD risk markers following consumption of high

intakes of FA-modified dairy products compared with conventional dairy foods, in both the fasted and postprandial state. The observed beneficial effect of specific dairy products on fasted LDL-C concentrations and blood pressure may represent the nature of the dairy food structure and nutritional composition. Nutrient digestion and absorption are determined by the nature of the food structure and nutritional composition. In the case of dairy foods, it has been suggested that the detrimental effect of SFA on blood lipids is attenuated following consumption of specific products, such as milk, cheese or yogurt (21, 64, 77). The combination of dairy bioactive peptides, minerals and fatty acids in certain dairy products may exert beneficial effects on CVD risk marker, as suggested by increasing evidence from intervention studies. In particular, studies which have compared iso-energetic quantities of cheese and butter provide support of a food matrix effect on observed changes in circulating blood lipids (61). Previous studies on dairy fat consumption have suggested that the observed attenuation in blood lipid response may be explained by a decrease in intestinal fat absorption and bile-acid recycling, modulation of the gut microbiota or the alteration of gene expression (21). Moreover, there is a wealth of evidence to indicate that the beneficial effects of certain dairy products are due to calcium content. In particular, it has been observed that an increased intake of calcium from certain dairy products, such as cheese, leads to increased faecal fat and bile acid excretion following consumption of both low- and high-fat diets. It has been proposed that the effect of calcium on faecal fat and bile excretion is probably due to a combination of a formation of insoluble calcium-fatty acid soaps, and hydrophobic aggregations with phosphorus and bile acids (21, 25, 278). In support of this concept, a study which compared isocaloric quantities of milk, cheese and butter observed that both milk and cheese attenuated the increase in LDL-C compared to butter (279). The three diets were matched not only for energy content, but also total fat and protein; calcium content was similar for cheese and milk but not matched in butter (279). Although this was a short-term intervention study in healthy men, it provides further evidence for an impact of calcium

content in specific dairy product on circulating LDL-C. Considering the effect on fasted LDL-C and iAUC of apoB following consumption of FA-modified dairy products presented in this thesis, this is an area of research that needs further intervention-based evidence, which would include investigation into changes of the composition of gut microbiota, the role of calcium present in specific dairy products in reduced fat digestion and whether there is a dose dependent response effect which would impact on bile acid excretion.

The RCT presented in this thesis also highlights the need for mechanistic *in vitro* studies to further understand and assess the effect of the FA-modified dairy products on risk markers of disease. Specifically, the impact on TRL metabolism observed in the postprandial study may be further examined with the use of hepatocyte (HepG2) cell model uptake studies to investigate potential pathways of TRL production and clearance. Potential *in vitro* research using human aortic endothelial cells (HAEC) may also provide a platform to conduct investigations in physiological concentrations of dairy FA mixtures. A previous study which examined the effect of individual dairy FA and FA mixtures on both healthy and type II diabetic HAEC, observed that the alteration of dairy FA composition did not significantly affect endothelial markers of CVD, although there was a differential effect of individual dairy FA on production of NO and sE-selectin (280). Considering that few *in vitro* studies have investigated dairy FA mixtures in cell lines, the use of better cell models, such as co-culture of endothelial cells with vascular smooth muscle cells, remains a research area of great interest.

The genetic analysis presented in Chapter 5 does not allow definite conclusions to be drawn due to the small population size, uneven numbers of the two groups and retrospective genotype analysis. However, there is considerable interest to further explore lipid-associated SNPs in response to dietary fat manipulation and future studies could reduce the limitations mentioned above by including an increased number of participants and a prospective

genotype analysis. In addition to *APOE*, other SNPs, such as polymorphisms of lipoprotein lipase (*LPL*) are of interest. Two *LPL* SNP (rs320 and rs328), have been linked as genetic determinants which may explain the inter-individual variability of TAG and HDL-C concentrations in both the fasted and postprandial state following dietary fatty acid replacement (281-283). LPL plays a role in lipid metabolism by both hydrolysing TRL, affecting HDL-C concentrations and binding TRL to hepatic LDL receptors, mediating the clearance of these particles in the circulation (284). Considering the observed increased content of rTFA in the FA-modified dairy products and the impact of total TFA intake on HDL-C, investigation into LPL polymorphisms may add further evidence in understanding the impact of rTFA on risk markers.

Considerable research from previous studies has demonstrated that milk FA composition can be modified by supplementing the bovine feed with plant seeds or oils (200), as has been illustrated in Chapters 1 and 2 and Appendix II. The challenging aspect of partially replacing SFA with *cis*-MUFA in milk fat is the increase in rTFA. Although these FA are distinct from industrially derived TFA, further research is needed in order to fully understand their impact on health. Protection of oils/oilseeds from ruminal biohydrogenation remains an area to explore further. To date, a number of studies have looked at different methodologies, such as extrusion and saponification, as a way to minimise the increases in rTFA with varying degrees of success (122). Larger scale trials are still needed to confirm existing findings or to explore novel strategies, such as whey-gel protection of the supplement (122). However, the implementation of oil/oilseed supplementation strategies on a larger scale, aimed for a consumer market, would incur additional costs on dairy farmers. Although the financial costs of producing FA-modified dairy products was not part of the scope of the present thesis, it does represent an aspect which may affect potential future directions. According to data available from the Agriculture and Horticulture Development Board

(AHDB), purchased feed in November 2017 averaged £210 per tonne in the UK, representing a 6.4% increase from the previous year (285). With feed and forage costs representing 30-40% of the total cost of dairy farm production and farmgate prices for liquid milk declining since 2015 (285), any additional production costs would need to either be subsidised or covered by the consumer. Considering the decline in demand for dairy products and the parallel increase in market competition from plant-based alternatives, FA-modified dairy products may, at least initially, represent a niche market for dairy products valued by consumers willing to pay a premium for healthy dairy product alternatives.

References

1. World Health Organisation. Top 10 causes of death. Available from: http://www.who.int/mediacentre/factsheets/fs310/en/2017 (Accessed January 2018).

2. British Heart Foundation. Headline Statistics: Cardiovascular Disease 2017. Available from: https://www.bhf.org.uk/research/heart-statistics (Accessed January 2018).

3. Smith SC, Collins A, Ferrari R, Holmes DR, Logstrup S, McGhie DV, et al. Our time: a call to save preventable death from cardiovascular disease (heart disease and stroke). Circulation. 2012;126(23):2769-75.

4. Townsend N, Wilson L, Bhatnagar P, Wickramasinghe K, Rayner M, Nichols M. Cardiovascular disease in Europe: epidemiological update 2016. Eur Heart J. 2016;37(42):3232-45.

5. Wilkins E, Wilson L, Wickramasinghe K, Bhatnagar P, Leal J, Luengo-Fernandez R, et al. European Cardiovascular Disease Statistics 2017. European Heart Network, Brussels. 2017.

6. World Health Organisation. Chronic disease report. Available from: http://www.who.int/chp/chronic_disease_report/part2_ch1/en/index12.html (Accessed January 2018).

7. Forouzanfar MH, Afshin A, Alexander LT, Anderson HR, Bhutta ZA, Biryukov S, et al. Global, regional, and national comparative risk assessment of 79 behavioural, environmental and occupational, and metabolic risks or clusters of risks, 1990–2015: a systematic analysis for the Global Burden of Disease Study 2015. The Lancet.388(10053):1659-724.

8. Feigin VL, Roth GA, Naghavi M, Parmar P, Krishnamurthi R, Chugh S, et al. Global burden of stroke and risk factors in 188 countries, during 1990-2013: a systematic analysis for the Global Burden of Disease Study 2013. Lancet Neurol. 2016;15(9):913-24.

9. Mensink RP, Zock PL, Kester AD, Katan MB. Effects of dietary fatty acids and carbohydrates on the ratio of serum total to HDL cholesterol and on serum lipids and apolipoproteins: a meta-analysis of 60 controlled trials. Am J Clin Nutr. 2003;77(5):1146-55.

10. Bates B, Lennox B, Prentice A, Bates C, Page P, Nicholson S, et al. National Diet and Nutrition Survey. Results from Years 5–6 (combined) of the Rolling Programme (2012/13 – 2013/14). London: Public Health England; 2016.

11. Tabas I, García-Cardeña G, Owens GK. Recent insights into the cellular biology of atherosclerosis. J Cell Biol. 2015;209(1):13-22.

12. Hansson GK, Libby P, Tabas I. Inflammation and plaque vulnerability. J Intern Med. 2015;278(5):483-93.

13. Dominiczak MH, Caslake MJ. Apolipoproteins: metabolic role and clinical biochemistry applications. Ann Clin Biochem. 2011;48(Pt 6):498-515.

14. Nakajima K, Nagamine T, Fujita MQ, Ai M, Tanaka A, Schaefer E. Apolipoprotein B-48: a unique marker of chylomicron metabolism. Adv Clin Chem. 2014;64:117-77.

15. Ference BA, Ginsberg HN, Graham I, Ray KK, Packard CJ, Bruckert E, et al. Low-density lipoproteins cause atherosclerotic cardiovascular disease. 1. Evidence from genetic, epidemiologic, and clinical studies. A consensus statement from the European Atherosclerosis Society Consensus Panel. Eur Heart J. 2017.

16. Huang Y, Mahley RW. Apolipoprotein E: structure and function in lipid metabolism, neurobiology, and Alzheimer's diseases. Neurobiol Dis. 2014;72 Pt A:3-12.

17. Forouzanfar MH, Alexander L, Anderson HR, Bachman VF, Biryukov S, Brauer M, et al. Global, regional, and national comparative risk assessment of 79 behavioural, environmental and occupational, and metabolic risks or clusters of risks in 188 countries, 1990-2013: a systematic analysis for the Global Burden of Disease Study 2013. Lancet. 2015;386(10010):2287-323.

18. Siri-Tarino PW, Sun Q, Hu FB, Krauss RM. Meta-analysis of prospective cohort studies evaluating the association of saturated fat with cardiovascular disease. Am J Clin Nutr. 2010;91(3):535-46.

19. Chowdhury R, Warnakula S, Kunutsor S, Crowe F, Ward HA, Johnson L, et al. Association of dietary, circulating, and supplement fatty acids with coronary risk: a systematic review and meta-analysis. Ann Intern Med. 2014;160(6):398-406.

20. de Souza RJ, Mente A, Maroleanu A, Cozma AI, Ha V, Kishibe T, et al. Intake of saturated and trans unsaturated fatty acids and risk of all cause mortality, cardiovascular disease, and type 2 diabetes: systematic review and meta-analysis of observational studies. BMJ. 2015;351:h3978.

21. Thorning TK, Bertram HC, Bonjour JP, de Groot L, Dupont D, Feeney E, et al. Whole dairy matrix or single nutrients in assessment of health effects: current evidence and knowledge gaps. Am J Clin Nutr. 2017;105(5):1033-45.

22. Siri-Tarino PW, Chiu S, Bergeron N, Krauss RM. Saturated Fats Versus Polyunsaturated Fats Versus Carbohydrates for Cardiovascular Disease Prevention and Treatment. Annu Rev Nutr. 2015;35:517-43.

23. Briggs MA, Petersen KS, Kris-Etherton PM. Saturated Fatty Acids and Cardiovascular Disease: Replacements for Saturated Fat to Reduce Cardiovascular Risk. Healthcare (Basel). 2017;5(2).

24. Nettleton JA, Brouwer IA, Geleijnse JM, Hornstra G. Saturated Fat Consumption and Risk of Coronary Heart Disease and Ischemic Stroke: A Science Update. Ann Nutr Metab. 2017;70(1):26-33.

25. Givens D. Saturated fats, dairy foods and health: a curious paradox? Nutrition Bulletin. 2017;42:8.

26. Mozaffarian D, Micha R, Wallace S. Effects on coronary heart disease of increasing polyunsaturated fat in place of saturated fat: a systematic review and meta-analysis of randomized controlled trials. PLoS Med. 2010;7(3):e1000252.

27. Patterson E, Wall R, Fitzgerald GF, Ross RP, Stanton C. Health implications of high dietary omega-6 polyunsaturated Fatty acids. J Nutr Metab. 2012;2012:539426.

28. Wang DD, Li Y, Chiuve SE, Stampfer MJ, Manson JE, Rimm EB, et al. Association of Specific Dietary Fats With Total and Cause-Specific Mortality. JAMA Intern Med. 2016;176(8):1134-45.

29. Ramsden CE, Zamora D, Leelarthaepin B, Majchrzak-Hong SF, Faurot KR, Suchindran CM, et al. Use of dietary linoleic acid for secondary prevention of coronary heart disease and death: evaluation of recovered data from the Sydney Diet Heart Study and updated meta-analysis. BMJ (Clinical research ed). 2013;346:e8707.

30. Hammad S, Pu S, Jones PJ. Current Evidence Supporting the Link Between Dietary Fatty Acids and Cardiovascular Disease. Lipids. 2016;51(5):507-17.

31. Schwingshackl L, Strasser B, Hoffmann G. Effects of monounsaturated fatty acids on cardiovascular risk factors: a systematic review and meta-analysis. Ann Nutr Metab. 2011;59(2-4):176-86.

32. Liu X, Kris-Etherton PM, West SG, Lamarche B, Jenkins DJ, Fleming JA, et al. Effects of canola and high-oleic-acid canola oils on abdominal fat mass in individuals with central obesity. Obesity (Silver Spring). 2016;24(11):2261-8.

33. Mensink R. Effects of saturated fatty acids on serum lipids and lipoproteins: a systematic review and regression analysis. World Health Organization: Geneva; 2016.

34. Astrup A, Dyerberg J, Elwood P, Hermansen K, Hu FB, Jakobsen MU, et al. The role of reducing intakes of saturated fat in the prevention of cardiovascular disease: where does the evidence stand in 2010? Am J Clin Nutr. 2011;93(4):684-8.

35. Hooper L, Summerbell CD, Thompson R, Sills D, Roberts FG, Moore HJ, et al. Reduced or modified dietary fat for preventing cardiovascular disease. Cochrane Database Syst Rev. 2012(5):CD002137.

36. Lovegrove JA, Givens DI. Dairy food products: good or bad for cardiometabolic disease? Nutr Res Rev. 2016;29(2):249-67.

37. Pimpin L, Wu JH, Haskelberg H, Del Gobbo L, Mozaffarian D. Is Butter Back? A Systematic Review and Meta-Analysis of Butter Consumption and Risk of Cardiovascular Disease, Diabetes, and Total Mortality. PLoS One. 2016;11(6):e0158118.

38. Department for Environment, Food and Rural Affairs. Milk prices and composition. Available from: https://www.gov.uk/government/collections/milk-prices-and-composition (Accessed January 2018).

39. de Oliveira Otto MC, Mozaffarian D, Kromhout D, Bertoni AG, Sibley CT, Jacobs DR, et al. Dietary intake of saturated fat by food source and incident cardiovascular disease: the Multi-Ethnic Study of Atherosclerosis. Am J Clin Nutr. 2012;96(2):397-404.

40. Food and Agriculture Organisation. Food based dietary guidelines. Available from: http://www.fao.org/nutrition/education/food-based-dietary-guidelines/regions/countries/france/en/ (Accessed January 2018).

41. Dilzer A, Park Y. Implication of conjugated linoleic acid (CLA) in human health. Crit Rev Food Sci Nutr. 2012;52(6):488-513.

42. Roe M, Pinchen H, Church S, Finglas P. McCance and Widdowson's The Composition of Foods Seventh Summary Edition and updated Composition of Foods Integrated Dataset. Nutrition Bulletin. 2015

40:3.

43. Elwood PC, Givens DI, Beswick AD, Fehily AM, Pickering JE, Gallacher J. The survival advantage of milk and dairy consumption: an overview of evidence from cohort studies of vascular diseases, diabetes and cancer. J Am Coll Nutr. 2008;27(6):723S-34S.

44. Elwood PC, Pickering JE, Givens DI, Gallacher JE. The consumption of milk and dairy foods and the incidence of vascular disease and diabetes: an overview of the evidence. Lipids. 2010;45(10):925-39.

45. Soedamah-Muthu SS, Masset G, Verberne L, Geleijnse JM, Brunner EJ. Consumption of dairy products and associations with incident diabetes, CHD and mortality in the Whitehall II study. Br J Nutr. 2013;109(4):718-26.

46. Qin LQ, Xu JY, Han SF, Zhang ZL, Zhao YY, Szeto IM. Dairy consumption and risk of cardiovascular disease: an updated meta-analysis of prospective cohort studies. Asia Pac J Clin Nutr. 2015;24(1):90-100.

47. Chen M, Li Y, Sun Q, Pan A, Manson JE, Rexrode KM, et al. Dairy fat and risk of cardiovascular disease in 3 cohorts of US adults. Am J Clin Nutr. 2016;104(5):1209-17.

48. Guo J, Astrup A, Lovegrove JA, Gijsbers L, Givens DI, Soedamah-Muthu SS. Milk and dairy consumption and risk of cardiovascular diseases and all-cause mortality: dose-response meta-analysis of prospective cohort studies. Eur J Epidemiol. 2017;32(4):269-87.

49. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem. 1972;18(6):499-502.

50. Cromwell WC, Otvos JD, Keyes MJ, Pencina MJ, Sullivan L, Vasan RS, et al. LDL Particle Number and Risk of Future Cardiovascular Disease in the Framingham Offspring Study - Implications for LDL Management. J Clin Lipidol. 2007;1(6):583-92.

51. Lamarche B, Tchernof A, Moorjani S, Cantin B, Dagenais GR, Lupien PJ, et al. Small, dense low-density lipoprotein particles as a predictor of the risk of ischemic heart disease in men. Prospective results from the Québec Cardiovascular Study. Circulation. 1997;95(1):69-75.

52. Hoogeveen RC, Gaubatz JW, Sun W, Dodge RC, Crosby JR, Jiang J, et al. Small dense low-density lipoprotein-cholesterol concentrations predict risk for coronary heart disease: the

Atherosclerosis Risk In Communities (ARIC) study. Arterioscler Thromb Vasc Biol. 2014;34(5):1069-77.

53. Griffin B. Serum low-density lipoprotein as a dietary responsive biomarker of cardiovascular disease risk: Consensus and confusion. Nutrition Bulletin. 2017;42(3).

54. Kien CL, Bunn JY, Stevens R, Bain J, Ikayeva O, Crain K, et al. Dietary intake of palmitate and oleate has broad impact on systemic and tissue lipid profiles in humans. Am J Clin Nutr. 2014;99(3):436-45.

55. Krishnan S, Cooper JA. Effect of dietary fatty acid composition on substrate utilization and body weight maintenance in humans. Eur J Nutr. 2014;53(3):691-710.

56. Fernandez ML, West KL. Mechanisms by which dietary fatty acids modulate plasma lipids. J Nutr. 2005;135(9):2075-8.

57. Dreon DM, Fernstrom HA, Campos H, Blanche P, Williams PT, Krauss RM. Change in dietary saturated fat intake is correlated with change in mass of large low-density-lipoprotein particles in men. Am J Clin Nutr. 1998;67(5):828-36.

58. Rosqvist F, Smedman A, Lindmark-Månsson H, Paulsson M, Petrus P, Straniero S, et al. Potential role of milk fat globule membrane in modulating plasma lipoproteins, gene expression, and cholesterol metabolism in humans: a randomized study. Am J Clin Nutr. 2015;102(1):20-30.

59. Thorning TK, Raziani F, Bendsen NT, Astrup A, Tholstrup T, Raben A. Diets with high-fat cheese, high-fat meat, or carbohydrate on cardiovascular risk markers in overweight postmenopausal women: a randomized crossover trial. Am J Clin Nutr. 2015;102(3):573-81.

60. Chiu S, Bergeron N, Williams PT, Bray GA, Sutherland B, Krauss RM. Comparison of the DASH (Dietary Approaches to Stop Hypertension) diet and a higher-fat DASH diet on blood pressure and lipids and lipoproteins: a randomized controlled trial. Am J Clin Nutr. 2016;103(2):341-7.

61. de Goede J, Geleijnse JM, Ding EL, Soedamah-Muthu SS. Effect of cheese consumption on blood lipids: a systematic review and meta-analysis of randomized controlled trials. Nutr Rev. 2015;73(5):259-75.

62. Micha R, Mozaffarian D. Saturated fat and cardiometabolic risk factors, coronary heart disease, stroke, and diabetes: a fresh look at the evidence. Lipids. 2010;45(10):893-905.

63. Sharrett AR, Ballantyne CM, Coady SA, Heiss G, Sorlie PD, Catellier D, et al. Coronary heart disease prediction from lipoprotein cholesterol levels, triglycerides, lipoprotein(a), apolipoproteins A-I and B, and HDL density subfractions: The Atherosclerosis Risk in Communities (ARIC) Study. Circulation. 2001;104(10):1108-13.

64. Drouin-Chartier JP, Côté JA, Labonté M, Brassard D, Tessier-Grenier M, Desroches S, et al. Comprehensive Review of the Impact of Dairy Foods and Dairy Fat on Cardiometabolic Risk. Adv Nutr. 2016;7(6):1041-51.

65. Lawes CM, Vander Hoorn S, Rodgers A, Hypertension ISo. Global burden of blood-pressure-related disease, 2001. Lancet. 2008;371(9623):1513-8.

66. James GD, Gerber LM. Measuring arterial blood pressure in humans: Auscultatory and automatic measurement techniques for human biological field studies. Am J Hum Biol. 2017.

67. Mancia G, Fagard R, Narkiewicz K, Redon J, Zanchetti A, Böhm M, et al. 2013 ESH/ESC Practice Guidelines for the Management of Arterial Hypertension. Blood Press. 2014;23(1):3-16.

68. Kris-Etherton PM, Grieger JA, Hilpert KF, West SG. Milk products, dietary patterns and blood pressure management. J Am Coll Nutr. 2009;28 Suppl 1:103S-19S.

69. Fekete Á, Givens DI, Lovegrove JA. Can milk proteins be a useful tool in the management of cardiometabolic health? An updated review of human intervention trials. Proc Nutr Soc. 2016;75(3):328-41.

70. FitzGerald RJ, Meisel H. Milk protein-derived peptide inhibitors of angiotensin-I-converting enzyme. Br J Nutr. 2000;84 Suppl 1:S33-7.

71. Maes W, Van Camp J, Vermeirssen V, Hemeryck M, Ketelslegers JM, Schrezenmeir J, et al. Influence of the lactokinin Ala-Leu-Pro-Met-His-Ile-Arg (ALPMHIR) on the release of endothelin-1 by endothelial cells. Regul Pept. 2004;118(1-2):105-9.

72. Appel LJ, Moore TJ, Obarzanek E, Vollmer WM, Svetkey LP, Sacks FM, et al. A clinical trial of the effects of dietary patterns on blood pressure. DASH Collaborative Research Group. N Engl J Med. 1997;336(16):1117-24.

73. Craddick SR, Elmer PJ, Obarzanek E, Vollmer WM, Svetkey LP, Swain MC. The DASH diet and blood pressure. Curr Atheroscler Rep. 2003;5(6):484-91.

74. Benatar JR, Sidhu K, Stewart RA. Effects of high and low fat dairy food on cardio-metabolic risk factors: a meta-analysis of randomized studies. PLoS One. 2013;8(10):e76480.

75. Soedamah-Muthu SS, Verberne LD, Ding EL, Engberink MF, Geleijnse JM. Dairy consumption and incidence of hypertension: a dose-response meta-analysis of prospective cohort studies. Hypertension. 2012;60(5):1131-7.

76. Park KM, Cifelli CJ. Dairy and blood pressure: a fresh look at the evidence. Nutr Rev. 2013;71(3):149-57.

77. Drouin-Chartier JP, Brassard D, Tessier-Grenier M, Côté JA, Labonté M, Desroches S, et al. Systematic Review of the Association between Dairy Product Consumption and Risk of Cardiovascular-Related Clinical Outcomes. Adv Nutr. 2016;7(6):1026-40.

78. Kesse-Guyot E, Vergnaud AC, Fezeu L, Zureik M, Blacher J, Péneau S, et al. Associations between dietary patterns and arterial stiffness, carotid artery intima-media thickness and atherosclerosis. Eur J Cardiovasc Prev Rehabil. 2010;17(6):718-24.

79. De Caterina R. Endothelial dysfunctions: common denominators in vascular disease. Curr Opin Clin Nutr Metab Care. 2000;3(6):453-67.

80. Versari D, Daghini E, Virdis A, Ghiadoni L, Taddei S. Endothelial Dysfunction as a Target for Prevention of Cardiovascular Disease. Diabetes Care. 2009;32:S314-S21.

81. Schächinger V, Britten MB, Zeiher AM. Prognostic impact of coronary vasodilator dysfunction on adverse long-term outcome of coronary heart disease. Circulation. 2000;101(16):1899-906.

82. Anderson TJ. Arterial stiffness or endothelial dysfunction as a surrogate marker of vascular risk. Can J Cardiol. 2006;22 Suppl B:72B-80B.

83. Corretti MC, Anderson TJ, Benjamin EJ, Celermajer D, Charbonneau F, Creager MA, et al. Guidelines for the ultrasound assessment of endothelial-dependent flow-mediated vasodilation of the brachial artery: a report of the International Brachial Artery Reactivity Task Force. J Am Coll Cardiol. 2002;39(2):257-65.

84. Lorenz MW, von Kegler S, Steinmetz H, Markus HS, Sitzer M. Carotid intima-media thickening indicates a higher vascular risk across a wide age range: prospective data from the Carotid Atherosclerosis Progression Study (CAPS). Stroke. 2006;37(1):87-92.

85. Petersen KS, Clifton PM, Keogh JB. The association between carotid intima media thickness and individual dietary components and patterns. Nutr Metab Cardiovasc Dis. 2014;24(5):495-502.

86. Ma J, Folsom AR, Lewis L, Eckfeldt JH. Relation of plasma phospholipid and cholesterol ester fatty acid composition to carotid artery intima-media thickness: the Atherosclerosis Risk in Communities (ARIC) Study. Am J Clin Nutr. 1997;65(2):551-9.

87. Ivey KL, Lewis JR, Hodgson JM, Zhu K, Dhaliwal SS, Thompson PL, et al. Association between yogurt, milk, and cheese consumption and common carotid artery intima-media thickness and cardiovascular disease risk factors in elderly women. Am J Clin Nutr. 2011;94(1):234-9.

88. Vlachopoulos C, Alexopoulos N, Stefanadis C. Aortic stiffness: prime time for integration into clinical practice? Hellenic J Cardiol. 2010;51(5):385-90.

89. Van Bortel LM, Laurent S, Boutouyrie P, Chowienczyk P, Cruickshank JK, De Backer T, et al. Expert consensus document on the measurement of aortic stiffness in daily practice using carotid-femoral pulse wave velocity. J Hypertens. 2012;30(3):445-8.

90. M R, H L, J P. Measurement of arterial stiffness: methodological aspects. Korean Circulation Journal. 2008;38:8.

91. Swampillai J DS, Fraser A. Clinical assessment of endothelial function - an update. British Journal of Diabetes and Vascular Disorders. 2005;5:4.

92. Kelly RP, Millasseau SC, Ritter JM, Chowienczyk PJ. Vasoactive drugs influence aortic augmentation index independently of pulse-wave velocity in healthy men. Hypertension. 2001;37(6):1429-33.

93. Whitworth JA, World Health Organization ItSoHWG. 2003 World Health Organization (WHO)/International Society of Hypertension (ISH) statement on management of hypertension. J Hypertens. 2003;21(11):1983-92.

94. Livingstone KM, Lovegrove JA, Cockcroft JR, Elwood PC, Pickering JE, Givens DI. Does dairy food intake predict arterial stiffness and blood pressure in men?: Evidence from the Caerphilly Prospective Study. Hypertension. 2013;61(1):42-7.

95. Crichton GE, Elias MF, Dore GA, Abhayaratna WP, Robbins MA. Relations between dairy food intake and arterial stiffness: pulse wave velocity and pulse pressure. Hypertension. 2012;59(5):1044-51.

96. Sedgwick P. Ecological studies: advantages and disadvantages. BMJ. 2014;348:g2979.

97. Packard RR, Libby P. Inflammation in atherosclerosis: from vascular biology to biomarker discovery and risk prediction. Clin Chem. 2008;54(1):24-38.

98. Minihane AM, Vinoy S, Russell WR, Baka A, Roche HM, Tuohy KM, et al. Low-grade inflammation, diet composition and health: current research evidence and its translation. Br J Nutr. 2015;114(7):999-1012.

99. Mozos I, Malainer C, Horbańczuk J, Gug C, Stoian D, Luca CT, et al. Inflammatory Markers for Arterial Stiffness in Cardiovascular Diseases. Front Immunol. 2017;8:1058.

100. Wang DD, Hu FB. Dietary Fat and Risk of Cardiovascular Disease: Recent Controversies and Advances. Annu Rev Nutr. 2017;37:423-46.

101. Labonté M, Couture P, Richard C, Desroches S, Lamarche B. Impact of dairy products on biomarkers of inflammation: a systematic review of randomized controlled nutritional intervention studies in overweight and obese adults. Am J Clin Nutr. 2013;97(4):706-17.

102. Calder PC, Ahluwalia N, Brouns F, Buetler T, Clement K, Cunningham K, et al. Dietary factors and low-grade inflammation in relation to overweight and obesity. Br J Nutr. 2011;106 Suppl 3:S5-78.

103. Calder PC, Albers R, Antoine JM, Blum S, Bourdet-Sicard R, Ferns GA, et al. Inflammatory disease processes and interactions with nutrition. Br J Nutr. 2009;101 Suppl 1:S1-45.

104. Siri-Tarino PW, Krauss RM. Diet, lipids, and cardiovascular disease. Curr Opin Lipidol. 2016;27(4):323-8.

105. Lee CH, Shih AZ, Woo YC, Fong CH, Leung OY, Janus E, et al. Optimal Cut-Offs of Homeostasis Model Assessment of Insulin Resistance (HOMA-IR) to Identify Dysglycemia and Type 2 Diabetes Mellitus: A 15-Year Prospective Study in Chinese. PLoS One. 2016;11(9):e0163424.

106. Brady LM, Gower BA, Lovegrove SS, Williams CM, Lovegrove JA. Revised QUICKI provides a strong surrogate estimate of insulin sensitivity when compared with the minimal model. Int J Obes Relat Metab Disord. 2004;28(2):222-7.

107. Jackson KG, Poppitt SD, Minihane AM. Postprandial lipemia and cardiovascular disease risk: Interrelationships between dietary, physiological and genetic determinants. Atherosclerosis. 2012;220(1):22-33.

108. Lairon D, Lopez-Miranda J, Williams C. Methodology for studying postprandial lipid metabolism. Eur J Clin Nutr. 2007;61(10):1145-61.

109. Willer CJ, Sanna S, Jackson AU, Scuteri A, Bonnycastle LL, Clarke R, et al. Newly identified loci that influence lipid concentrations and risk of coronary artery disease. Nat Genet. 2008;40(2):161-9.

110. Rankinen T, Sarzynski MA, Ghosh S, Bouchard C. Are there genetic paths common to obesity, cardiovascular disease outcomes, and cardiovascular risk factors? Circ Res. 2015;116(5):909-22.

111. Wilson PW, Schaefer EJ, Larson MG, Ordovas JM. Apolipoprotein E alleles and risk of coronary disease. A meta-analysis. Arterioscler Thromb Vasc Biol. 1996;16(10):1250-5.

112. Bennet AM, Di Angelantonio E, Ye Z, Wensley F, Dahlin A, Ahlbom A, et al. Association of apolipoprotein E genotypes with lipid levels and coronary risk. JAMA. 2007;298(11):1300-11.

113. Song Y, Stampfer MJ, Liu S. Meta-analysis: apolipoprotein E genotypes and risk for coronary heart disease. Ann Intern Med. 2004;141(2):137-47.

114. Povel CM, Boer JM, Imholz S, Dollé ME, Feskens EJ. Genetic variants in lipid metabolism are independently associated with multiple features of the metabolic syndrome. Lipids Health Dis. 2011;10:118.

115. Rasmussen KL. Plasma levels of apolipoprotein E, APOE genotype and risk of dementia and ischemic heart disease: A review. Atherosclerosis. 2016;255:145-55.

116. Minihane AM, Jofre-Monseny L, Olano-Martin E, Rimbach G. ApoE genotype, cardiovascular risk and responsiveness to dietary fat manipulation. Proc Nutr Soc. 2007;66(2):183-97.

117. Olano-Martin E, Anil E, Caslake MJ, Packard CJ, Bedford D, Stewart G, et al. Contribution of apolipoprotein E genotype and docosahexaenoic acid to the LDL-cholesterol response to fish oil. Atherosclerosis. 2010;209(1):104-10.

118. Carvalho-Wells AL, Jackson KG, Lockyer S, Lovegrove JA, Minihane AM. APOE genotype influences triglyceride and C-reactive protein responses to altered dietary fat intake in UK adults. Am J Clin Nutr. 2012;96(6):1447-53.

119. Fallaize R, Carvalho-Wells AL, Tierney AC, Marin C, Kieć-Wilk B, Dembińska-Kieć A, et al. APOE genotype influences insulin resistance, apolipoprotein CII and CIII according to plasma fatty acid profile in the Metabolic Syndrome. Sci Rep. 2017;7(1):6274.

120. Jackson KG, Lockyer S, Carvalho-Wells AL, Williams CM, Minihane AM, Lovegrove JA. Apolipoprotein E (epsilon) genotype has a greater impact on apoB-48 than apoB-100 responses to dietary fat manipulation-insights from the SATgenε study. Mol Nutr Food Res. 2017;61(4).

121. Glasser F, Ferlay A, Chilliard Y. Oilseed lipid supplements and fatty acid composition of cow milk: a meta-analysis. J Dairy Sci. 2008;91(12):4687-703.

122. Kliem KE, Shingfield KJ. Manipulation of milk fatty acid composition in lactating cows: Opportunities and challenges. European Journal of Lipid Science and Technology. 2016;118(11):1661 - 83.

123. Hulshof KF, van Erp-Baart MA, Anttolainen M, Becker W, Church SM, Couet C, et al. Intake of fatty acids in western Europe with emphasis on trans fatty acids: the TRANSFAIR Study. Eur J Clin Nutr. 1999;53(2):143-57.

124. van Poppel G. Intake of trans fatty acids in western Europe: the TRANSFAIR study. Lancet. 1998;351(9109):1099.

125. van de Vijver LP, Kardinaal AF, Couet C, Aro A, Kafatos A, Steingrimsdottir L, et al. Association between trans fatty acid intake and cardiovascular risk factors in Europe: the TRANSFAIR study. Eur J Clin Nutr. 2000;54(2):126-35.

126. Wahle K, Heys S, Rotondo D. Conjugated linoleic acids: are they beneficial or detrimental to health? Progress in lipid research. 2004;43(6):34.

127. Brouwer IA, Wanders AJ, Katan MB. Trans fatty acids and cardiovascular health: research completed? Eur J Clin Nutr. 2013;67(5):541-7.

128. Jakobsen MU, O'Reilly EJ, Heitmann BL, Pereira MA, Bälter K, Fraser GE, et al. Major types of dietary fat and risk of coronary heart disease: a pooled analysis of 11 cohort studies. Am J Clin Nutr. 2009;89(5):1425-32.

129. Liu XR, Deng ZY, Hu JN, Fan YW, Liu R, Li J, et al. Erythrocyte membrane trans-fatty acid index is positively associated with a 10-year CHD risk probability. Br J Nutr. 2013;109(9):1695-703.

130. Oomen CM, Ocké MC, Feskens EJ, van Erp-Baart MA, Kok FJ, Kromhout D. Association between trans fatty acid intake and 10-year risk of coronary heart disease in the Zutphen Elderly Study: a prospective population-based study. Lancet. 2001;357(9258):746-51.

131. Bendsen NT, Christensen R, Bartels EM, Astrup A. Consumption of industrial and ruminant trans fatty acids and risk of coronary heart disease: a systematic review and meta-analysis of cohort studies. Eur J Clin Nutr. 2011;65(7):773-83.

132. Gebauer SK, Destaillats F, Dionisi F, Krauss RM, Baer DJ. Vaccenic acid and trans fatty acid isomers from partially hydrogenated oil both adversely affect LDL cholesterol: a double-blind, randomized controlled trial. The American journal of clinical nutrition. 2015;102(6):1339-46.

133. Field CJ, Blewett HH, Proctor S, Vine D. Human health benefits of vaccenic acid. Appl Physiol Nutr Metab. 2009;34(5):979-91.

134. Livingstone KM, Lovegrove JA, Givens DI. The impact of substituting SFA in dairy products with MUFA or PUFA on CVD risk: evidence from human intervention studies. Nutr Res Rev. 2012;25(2):193-206.

135. Noakes M, Nestel PJ, Clifton PM. Modifying the fatty acid profile of dairy products through feedlot technology lowers plasma cholesterol of humans consuming the products. Am J Clin Nutr. 1996;63(1):42-6.

136. Burdge GC, Tricon S, Morgan R, Kliem KE, Childs C, Jones E, et al. Incorporation of cis-9, trans-11 conjugated linoleic acid and vaccenic acid (trans-11 18 : 1) into plasma and leucocyte lipids in healthy men consuming dairy products naturally enriched in these fatty acids. Br J Nutr. 2005;94(2):237-43.

137. Tricon S, Burdge GC, Jones EL, Russell JJ, El-Khazen S, Moretti E, et al. Effects of dairy products naturally enriched with cis-9,trans-11 conjugated linoleic acid on the blood lipid profile in healthy middle-aged men. Am J Clin Nutr. 2006;83(4):744-53.

138. Townsend N, Bhatnagar P, Wilkins E, Wickramasinghe K, M R. Cardiovascular disease statistics. London: British Heart Foundation; 2015.

139. COMA. Department of Health—Committee on Medical Aspects of Food Policy. Dietary reference values for food energy and nutrients in the United Kingdom: report on the Panel of Dietary Reference Values of the Committee on Medical Aspects of Food Policy (Report of Health and Social Subjects, No. 41). London: The Stationary Office.; 1991.

140. Bates B, Cox L, Nicholson S, Page P, Prentice A, Steer T, et al. National Diet and Nutrition Survey. Results from Years 5-6 (combined) of the Rolling Programme (2012/13 – 2013/14). [cited 2016 Sept 21]. Available from: https://www.gov.uk/government/statistics/ndns-results-from-years-5-and-6-combined.

141. USDA. Scientific Report of the 2015 Dietary Guidelines Advisory Committee. Washington (DC): USDA and US Department of Health and Human Services; 2015.

142. Buttriss JL. The Eatwell Guide refreshed. Nutr Bull. 2016;41(2):135-41.

143. Soedamah-Muthu SS, Ding EL, Al-Delaimy WK, Hu FB, Engberink MF, Willett WC, et al. Milk and dairy consumption and incidence of cardiovascular diseases and all-cause mortality: dose-response meta-analysis of prospective cohort studies. The American journal of clinical nutrition. 2011;93(1):158-71.

144. Lamarche B, Givens DI, Soedamah-Muthu S, Krauss RM, Jakobsen MU, Bischoff-Ferrari HA, et al. Does milk consumption contribute to cardiometabolic health and overall diet quality? Can J Cardiol. 2016;32(8):1026-32.

145. de Goede J, Soedamah‐Muthu SS, Pan A, Gijsbers L, Geleijnse JM. Dairy Consumption and Risk of Stroke: A Systematic Review and Updated Dose‐Response Meta‐Analysis of Prospective Cohort Studies. J Am Heart Assoc. 2016;5(5):e002787.

146. Huth PJ, Park KM. Influence of Dairy Product and Milk Fat Consumption on Cardiovascular Disease Risk: A Review of the Evidence. Adv Nutr. 2012;3(3):266-85.

147. Astrup A, Rice Bradley B, Brenna J, Delplanque B, Ferry M, Torres-Gonzalez M. Regular-Fat Dairy and Human Health: A Synopsis of Symposia Presented in Europe and North America (2014–2015). Nutrients. 2016;8(8):463.

148. O'Donnell A, Spatny K, Vicini J, Bauman D. Survey of the fatty acid composition of retail milk differing in label claims based on production management practices. J Dairy Sci. 2010;93(5):1918-25.

149. Fekete ÁA, Givens DI, Lovegrove JA. The impact of milk proteins and peptides on blood pressure and vascular function: a review of evidence from human intervention studies. Nutr Res Rev. 2013;26(02):177-90.

150. Markey O, Vasilopoulou D, Givens DI, Lovegrove JA. Dairy and cardiovascular health: Friend or foe? Nutr Bull. 2014;39(2):161-71.

151. Chowdhury R, Warnakula S, Kunutsor S, Crowe F, Ward HA, Johnson L, et al. Association of Dietary, Circulating, and Supplement Fatty Acids With Coronary Risk. A Systematic Review and Meta-analysis. Ann Intern Med. 2014;160(6):398-406.

152. Siri-Tarino PW, Sun Q, Hu FB, Krauss RM. Meta-analysis of prospective cohort studies evaluating the association of saturated fat with cardiovascular disease. Am J Clin Nutr. 2010;91(3):535-46.

153. Vafeiadou K, Weech M, Altowaijri H, Todd S, Yaqoob P, Jackson KG, et al. Replacement of saturated with unsaturated fats had no impact on vascular function but beneficial effects on lipid biomarkers, E-selectin, and blood pressure: results from the randomized, controlled Dietary Intervention and VAScular function (DIVAS) study. Am J Clin Nutr. 2015;102(1):40-8.

154. Hooper L, Martin N, Abdelhamid A, Davey Smith G. Reduction in saturated fat intake for cardiovascular disease. Cochrane Database Syst Rev. 2015;6:Cd011737.

155. DeSalvo KB, Olson R, Casavale KO. Dietary guidelines for americans. JAMA. 2016;315(5):457-8.

156. World Health Organisation. Food based dietary guidelines in the WHO European Region. Copenhagen, Denmark: WHO. 2003.

157. Givens DI. Session 4: Challenges facing the food industry in innovating for health. Impact on CVD risk of modifying milk fat to decrease intake of SFA and increase intake of cis-MUFA. Proc Nutr Soc. 2008;67(4):419-27.

158. Kliem KE, Shingfield KJ. Manipulation of milk fatty acid composition in lactating cows: Opportunities and challenges. Eur J Lipid S Tech; 101002/ejlt201400543. 2016.

159. Lock AL, Givens DI, Bauman DE. Dairy Fat: Perceptions and Realities. Milk and Dairy Products as Functional Foods: John Wiley & Sons, Ltd; 2014. p. 174-97.

160. Livingstone KM, Lovegrove JA, Givens DI. The impact of substituting SFA in dairy products with MUFA or PUFA on CVD risk: evidence from human intervention studies. Nutr Res Rev. 2012;25(2):193-206.

161. Shaw DI, Tierney AC, McCarthy S, Upritchard J, Vermunt S, Gulseth HL, et al. LIPGENE food-exchange model for alteration of dietary fat quantity and quality in free-living participants from eight European countries. The British journal of nutrition. 2009;101(5):750-9.

162. Skeaff CM, Hodson L, McKenzie JE. Dietary-Induced Changes in Fatty Acid Composition of Human Plasma, Platelet, and Erythrocyte Lipids Follow a Similar Time Course. J Nutr. 2006;136(3):565-9.

163. Hodson L, Skeaff CM, Fielding BA. Fatty acid composition of adipose tissue and blood in humans and its use as a biomarker of dietary intake. Progress in lipid research. 2008;47(5):348-80.

164. Weech M, Vafeiadou K, Hasaj M, Todd S, Yaqoob P, Jackson KG, et al. Development of a food-exchange model to replace saturated fat with MUFAs and n-6 PUFAs in adults at moderate cardiovascular risk. J Nutr. 2014;144(6):846-55.

165. Wilson PW, D'Agostino RB, Levy D, Belanger AM, Silbershatz H, Kannel WB. Prediction of coronary heart disease using risk factor categories. Circulation. 1998;97(18):1837-47.

166. Kliem KE, Humphries DJ, Markey O, Vasilopoulou D, Fagan CC, Grandison AS, et al. Food chain approach to lowering saturated fat in milk and dairy products: the RESET study. Int J Dairy Technol.Submitted.

167. Givens D, Kliem K, Humphries D, Shingfield KJ, Morgan R. Effect of replacing calcium salts of palm oil distillate with rapeseed oil, milled or whole rapeseeds on milk fatty-acid composition in cows fed maize silage-based diets. Animal : an international journal of animal bioscience. 2009;3(07):1067-74.

168. Treasure T, MacRae KD. Minimisation: the platinum standard for trials?: Randomisation doesn't guarantee similarity of groups; minimisation does. BMJ (Clinical research ed). 1998;317(7155):362-3.

169. Moore C, Gitau R, Goff L, Lewis FJ, Griffin MD, Chatfield MD, et al. Successful manipulation of the quality and quantity of fat and carbohydrate consumed by free-living individuals using a food exchange model. J Nutr. 2009;139(8):1534-40.

170. Henderson L, Gregory J, Irving K, G. S. The National Diet and Nutrition Survey: adults aged 19 to 64 years. Vol. 2, Energy, protein, carbohydrate, fat and alcohol intake. London: The Stationery Office; 2003.

171. Nelson M, Atkinson M, Meyer J. A photographic atlas of food portion sizes. London: MAFF publications; 1997.

172. Kliem KE, Shingfield KJ, Livingstone KM, Givens DI. Seasonal variation in the fatty acid composition of milk available at retail in the United Kingdom and implications for dietary intake. Food Chem. 2013;141(1):274-81.

173. Glasser F, Doreau M, Ferlay A, Chilliard Y. Technical note: Estimation of milk fatty acid yield from milk fat data. J Dairy Sci. 2007;90(5):2302-4.

174. Public Health England. 2015. Scientific Advisory Committee on Nutrition Carbohydrates and Health. 2015 [cited 2016 Aug 18]. Available from: https://www.gov.uk/government/publications/sacn-carbohydrates-and-health-report

175. Henry CJ. Basal metabolic rate studies in humans: measurement and development of new equations. Public health nutrition. 2005;8(7a):1133-52.

176. Black AE. Critical evaluation of energy intake using the Goldberg cut-off for energy intake:basal metabolic rate. A practical guide to its calculation, use and limitations. International

journal of obesity and related metabolic disorders : journal of the International Association for the Study of Obesity. 2000;24(9):1119-30.

177. Wang LY, Summerhill K, Rodriguez-Canas C, Mather I, Patel P, Eiden M, et al. Development and validation of a robust automated analysis of plasma phospholipid fatty acids for metabolic phenotyping of large epidemiological studies. Genome medicine. 2013;5(4):39.

178. Burdge GC, Wright P, Jones AE, Wootton SA. A method for separation of phosphatidylcholine, triacylglycerol, non-esterified fatty acids and cholesterol esters from plasma by solid-phase extraction. The British journal of nutrition. 2000;84(5):781-7.

179. De Roos NM, Bots ML, Schouten EG, Katan MB. Within-subject variability of flow-mediated vasodilation of the brachial artery in healthy men and women: implications for experimental studies. Ultrasound Med Biol. 2003;29(3):401-6.

180. Smith LI. A tutorial on principal components analysis. Ithaca: Cornell University; 2002.

181. Xia J, Sinelnikov IV, Han B, Wishart DS. MetaboAnalyst 3.0-making metabolomics more meaningful. Nucleic acids research. 2015;43(W1):W251-7.

182. Katan MB, Deslypere JP, van Birgelen AP, Penders M, Zegwaard M. Kinetics of the incorporation of dietary fatty acids into serum cholesteryl esters, erythrocyte membranes, and adipose tissue: an 18-month controlled study. Journal of lipid research. 1997;38(10):2012-22.

183. Tholstrup T, Raff M, Basu S, Nonboe P, Sejrsen K, Straarup EM. Effects of butter high in ruminant trans and monounsaturated fatty acids on lipoproteins, incorporation of fatty acids into lipid classes, plasma C-reactive protein, oxidative stress, hemostatic variables, and insulin in healthy young men. The American journal of clinical nutrition. 2006;83(2):237-43.

184. Raatz SK, Bibus D, Thomas W, Kris-Etherton P. Total Fat Intake Modifies Plasma Fatty Acid Composition in Humans. J Nutr. 2001;131(2):231-4.

185. Chavarro JE, Kenfield SA, Stampfer MJ, Loda M, Campos H, Sesso HD, et al. Blood Levels of Saturated and Monounsaturated Fatty Acids as Markers of De Novo Lipogenesis and Risk of Prostate Cancer. Am J Epidemiol 2013;178(8):1246-55.

186. Jakobsen MU, Overvad K, Dyerberg J, Heitmann BL. Intake of ruminant trans fatty acids and risk of coronary heart disease. International journal of epidemiology. 2008;37(1):173-82.

187. Motard-Belanger A, Charest A, Grenier G, Paquin P, Chouinard Y, Lemieux S, et al. Study of the effect of trans fatty acids from ruminants on blood lipids and other risk factors for cardiovascular disease. The American journal of clinical nutrition. 2008;87(3):593-9.

188. Kliem KE, Reynolds CK, Humphries DJ, Kirkland RM, Barratt CES, Livingstone KM, et al. Incremental effect of a calcium salt of cis-monounsaturated fatty acids supplement on milk fatty acid composition in cows fed maize silage-based diets. J Dairy Sci. 2013;96(5):3211-21.

189. Bauman DE, Griinari JM. Regulation and nutritional manipulation of milk fat. Low-fat milk syndrome. Advances in experimental medicine and biology. 2000;480:209-16.

190. Lockyer S, Tzanetou M, Carvalho-Wells AL, Jackson KG, Minihane AM, Lovegrove JA. SATgenε dietary model to implement diets of differing fat composition in prospectively genotyped groups (apoE) using commercially available foods. The British journal of nutrition. 2012;108(9):1705-13.

191. Rennie KL, Siervo M, Jebb SA. Can Self-Reported Dieting and Dietary Restraint Identify Underreporters of Energy Intake in Dietary Surveys? J Am Diet Assoc. 2006;106(10):1667-72.

192. Macdiarmid J, Blundell J. Assessing dietary intake: Who, what and why of under-reporting. Nutrition research reviews. 1998;11(2):231-53.

193. Markey O, Souroullas K, Fagan CC, Kliem KE, Vasilopoulou D, Jackson KG, et al. Consumer acceptance of dairy products with a saturated fatty acid-reduced, monounsaturated fatty acid-enriched content. J Dairy Sci.In Review.

194. Kliem KE, Humphries DJ, Markey O, Vasilopoulou D, Fagan CC, Grandison AS, et al. Food chain approach to lowering saturated fat in milk and dairy products: the RESET study. J Dairy Sci.Submitted.

195. Joris PJ, Mensink RP. Role of cis-Monounsaturated Fatty Acids in the Prevention of Coronary Heart Disease. Curr Atheroscler Rep. 2016;18(7):38.

196. Van Horn L, Carson JA, Appel LJ, Burke LE, Economos C, Karmally W, et al. Recommended Dietary Pattern to Achieve Adherence to the American Heart Association/American College of Cardiology (AHA/ACC) Guidelines: A Scientific Statement From the American Heart Association. Circulation. 2016;134(22):e505-e29.

197. *Policy*. xC*DoHCoMAoF*. Dietary reference values for food energy and nutrients for the United Kingdom. Report of the Panel on Dietary Reference Values of the Committee on Medical Aspects of Food Policy. Rep Health Soc Subj (Lond). 1991;41:1-210.

198. Fekete Á, Givens DI, Lovegrove JA. The impact of milk proteins and peptides on blood pressure and vascular function: a review of evidence from human intervention studies. Nutr Res Rev. 2013;26(2):177-90.

199. Hooper L, Martin N, Abdelhamid A, Davey Smith G. Reduction in saturated fat intake for cardiovascular disease. Cochrane Database Syst Rev. 2015(6):CD011737.

200. Kliem KE, Humphries DJ, Reynolds CK, Morgan R, Givens DI. Effect of oilseed type on milk fatty acid composition of individual cows, and also bulk tank milk fatty acid composition from commercial farms. Animal. 2017;11(2):354-64.

201. Markey O, Vasilopoulou D, Kliem KE, Koulman A, Fagan CC, Summerhill K, et al. Plasma phospholipid fatty acid profile confirms compliance to a novel saturated fat-reduced, monounsaturated fat-enriched dairy product intervention in adults at moderate cardiovascular risk: a randomized controlled trial. Nutr J. 2017;16(1):33.

202. Treasure T, MacRae KD. Minimisation: the platinum standard for trials?. Randomisation doesn't guarantee similarity of groups; minimisation does. BMJ. 1998;317(7155):362-3.

203. Kliem KE, Humphries DJ, Markey O, Vasilopoulou D, Fagan CC, Grandison AS, et al. Food chain approach to lowering saturated fat in milk and dairy products: the RESET study. International Journal of Dairy Technology. 2017.

204. Moore C, Gitau R, Goff L, Lewis FJ, Griffin MD, Chatfield MD, et al. Successful manipulation of the quality and quantity of fat and carbohydrate consumed by free-living individuals using a food exchange model. J Nutr. 2009;139(8):1534-40.

205. Henderson GJ, Irving K, Swan G. The National Diet and Nutrition Survey: adults aged 19 to 64 years. Vol. 2, Energy, protein, carbohydrate, fat and alcohol intake.: London: The Stationery Office; 2003.

206. Fuller NR, Williams K, Shrestha R, Ahern AL, Holzapfel C, Hauner H, et al. Changes in physical activity during a weight loss intervention and follow-up: a randomized controlled trial. Clin Obes. 2014;4(3):127-35.

207. Flint A, Raben A, Blundell JE, Astrup A. Reproducibility, power and validity of visual analogue scales in assessment of appetite sensations in single test meal studies. Int J Obes Relat Metab Disord. 2000;24(1):38-48.

208. Bryan NS, Grisham MB. Methods to detect nitric oxide and its metabolites in biological samples. Free Radic Biol Med. 2007;43(5):645-57.

209. Vafeiadou K, Weech M, Altowaijri H, Todd S, Yaqoob P, Jackson KG, et al. Replacement of saturated with unsaturated fats had no impact on vascular function but beneficial effects on lipid biomarkers, E-selectin, and blood pressure: results from the randomized, controlled Dietary Intervention and VAScular function (DIVAS) study. Am J Clin Nutr. 2015;102(1):40-8.

210. Keys A, Anderson JT, Grande F. Serum cholesterol response to changes in the diet: IV. Particular saturated fatty acids in the diet. Metabolism. 1965;14(7):776-87.

211. Hegsted DM, McGandy RB, Myers ML, Stare FJ. Quantitative effects of dietary fat on serum cholesterol in man. Am J Clin Nutr. 1965;17(5):281-95.

212. Mensink RP, Katan MB. Effect of dietary fatty acids on serum lipids and lipoproteins. A meta-analysis of 27 trials. Arterioscler Thromb. 1992;12(8):911-9.

213. Clarke R, Frost C, Collins R, Appleby P, Peto R. Dietary lipids and blood cholesterol: quantitative meta-analysis of metabolic ward studies. BMJ. 1997;314(7074):112-7.

214. Howell WH, McNamara DJ, Tosca MA, Smith BT, Gaines JA. Plasma lipid and lipoprotein responses to dietary fat and cholesterol: a meta-analysis. Am J Clin Nutr. 1997;65(6):1747-64.

215. Soininen P, Kangas AJ, Würtz P, Suna T, Ala-Korpela M. Quantitative serum nuclear magnetic resonance metabolomics in cardiovascular epidemiology and genetics. Circ Cardiovasc Genet. 2015;8(1):192-206.

216. Touboul PJ, Hennerici MG, Meairs S, Adams H, Amarenco P, Bornstein N, et al. Mannheim carotid intima-media thickness and plaque consensus (2004-2006-2011). An update on behalf of the advisory board of the 3rd, 4th and 5th watching the risk symposia, at the 13th, 15th and 20th European

Stroke Conferences, Mannheim, Germany, 2004, Brussels, Belgium, 2006, and Hamburg, Germany, 2011. Cerebrovasc Dis. 2012;34(4):290-6.

217. Hobbs DA, Goulding MG, Nguyen A, Malaver T, Walker CF, George TW, et al. Acute ingestion of beetroot bread increases endothelium-independent vasodilation and lowers diastolic blood pressure in healthy men: a randomized controlled trial. J Nutr. 2013;143(9):1399-405.

218. Newens KJ, Thompson AK, Jackson KG, Wright J, Williams CM. DHA-rich fish oil reverses the detrimental effects of saturated fatty acids on postprandial vascular reactivity. Am J Clin Nutr. 2011;94(3):742-8.

219. Desroches S, Lamarche B. Diet and low-density lipoprotein particle size. Curr Atheroscler Rep. 2004;6(6):453-60.

220. Kuhnt K, Degen C, Jahreis G. Evaluation of the Impact of Ruminant Trans Fatty Acids on Human Health: Important Aspects to Consider. Crit Rev Food Sci Nutr. 2016;56(12):1964-80.

221. Hodges GJ, Nawaz S, Tew GA. Evidence that reduced nitric oxide signal contributes to cutaneous microvascular dysfunction in peripheral arterial disease. Clin Hemorheol Microcirc. 2015;59(1):83-95.

222. Kelm M. Nitric oxide metabolism and breakdown. Biochim Biophys Acta. 1999;1411(2-3):273-89.

223. Tholstrup T, Sandström B, Hermansen JE, Hølmer G. Effect of modified dairy fat on postprandial and fasting plasma lipids and lipoproteins in healthy young men. Lipids. 1998;33(1):11-21.

224. Stranieri S, Ricci EC, Banterle A. Convenience food with environmentally-sustainable attributes: A consumer perspective. Appetite. 2017;116:11-20.

225. Markey O, Souroullas K, Fagan CC, Kliem KE, Vasilopoulou D, Jackson KG, et al. Consumer acceptance of dairy products with a saturated fatty acid-reduced, monounsaturated fatty acid-enriched content. J Dairy Sci. 2017.

226. Jackson KG, Armah CK, Minihane AM. Meal fatty acids and postprandial vascular reactivity. Biochem Soc Trans. 2007;35(Pt 3):451-3.

227. Hansson GK. Inflammation, atherosclerosis, and coronary artery disease. N Engl J Med. 2005;352(16):1685-95.

228. Berry SE, Tucker S, Banerji R, Jiang B, Chowienczyk PJ, Charles SM, et al. Impaired postprandial endothelial function depends on the type of fat consumed by healthy men. J Nutr. 2008;138(10):1910-4.

229. Burdge GC, Calder PC. Plasma cytokine response during the postprandial period: a potential causal process in vascular disease? Br J Nutr. 2005;93(1):3-9.

230. Ortega A, Varela LM, Bermudez B, Lopez S, Abia R, Muriana FJ. Dietary fatty acids linking postprandial metabolic response and chronic diseases. Food Funct. 2012;3(1):22-7.

231. Vafeiadou K, Weech M, Sharma V, Yaqoob P, Todd S, Williams CM, et al. A review of the evidence for the effects of total dietary fat, saturated, monounsaturated and n-6 polyunsaturated fatty acids on vascular function, endothelial progenitor cells and microparticles. Br J Nutr. 2012;107(3):303-24.

232. Roche HM, Zampelas A, Knapper JM, Webb D, Brooks C, Jackson KG, et al. Effect of long-term olive oil dietary intervention on postprandial triacylglycerol and factor VII metabolism. Am J Clin Nutr. 1998;68(3):552-60.

233. Silva KD, Wright JW, Williams CM, Lovegrove JA. Meal ingestion provokes entry of lipoproteins containing fat from the previous meal: possible metabolic implications. Eur J Nutr. 2005;44(6):377-83.

234. Jiménez-Gómez Y, Marín C, Peérez-Martínez P, Hartwich J, Malczewska-Malec M, Golabek I, et al. A low-fat, high-complex carbohydrate diet supplemented with long-chain (n-3) fatty acids alters the postprandial lipoprotein profile in patients with metabolic syndrome. J Nutr. 2010;140(9):1595-601.

235. Zampelas A, Roche H, Knapper JM, Jackson KG, Tornaritis M, Hatzis C, et al. Differences in postprandial lipaemic response between Northern and Southern Europeans. Atherosclerosis. 1998;139(1):83-93.

236. Jackson KG, Zampelas A, Knapper JM, Roche HM, Gibney MJ, Kafatos A, et al. Differences in glucose-dependent insulinotrophic polypeptide hormone and hepatic lipase in subjects of southern and northern Europe: implications for postprandial lipemia. Am J Clin Nutr. 2000;71(1):13-20.

237. Schwingshackl L, Christoph M, Hoffmann G. Effects of Olive Oil on Markers of Inflammation and Endothelial Function-A Systematic Review and Meta-Analysis. Nutrients. 2015;7(9):7651-75.

238. Vasilopoulou D, Markey O, Kliem KE, Fagan CC, Grandison AS, Humphries DJ, et al. Impact of dairy fat manipulation to replace saturated with unsaturated fatty acids in milk and dairy products on cardiovascular disease risk markers: findings from the RESET randomized controlled trial. 2017.

239. Vasilopoulou D, Markey O, Kliem KE, Fagan CC, Grandison AS, Humphries DJ, et al. Impact of two high fat dairy diets varying in fatty acid profile on cardiovascular risk factors: findings from the RESET randomized controlled trial. 2017.

240. Kliem KE, Shingfield KJ, Livingstone KM, Givens DI. Seasonal variation in the fatty acid composition of milk available at retail in the United Kingdom and implications for dietary intake. Food Chem. 2013;141(1):274-81.

241. Ulberth F, Gabernig R, Schrammel F. Flame-ionization detector response to methyl, ethyl, propyl, and butyl esters of fatty acids. Journal of the American Oil Chemist's Society. 1999;76(2):4.

242. Carstensen M, Thomsen C, Hermansen K. Incremental area under response curve more accurately describes the triglyceride response to an oral fat load in both healthy and type 2 diabetic subjects. Metabolism. 2003;52(8):1034-7.

243. Benn M. Apolipoprotein B levels, APOB alleles, and risk of ischemic cardiovascular disease in the general population, a review. Atherosclerosis. 2009;206(1):17-30.

244. Silva KD, Kelly CN, Jones AE, Smith RD, Wootton SA, Miller GJ, et al. Chylomicron particle size and number, factor VII activation and dietary monounsaturated fatty acids. Atherosclerosis. 2003;166(1):73-84.

245. Jackson KG, Maitin V, Leake DS, Yaqoob P, Williams CM. Saturated fat-induced changes in Sf 60-400 particle composition reduces uptake of LDL by HepG2 cells. J Lipid Res. 2006;47(2):393-403.

246. Marchesi S, Lupattelli G, Schillaci G, Pirro M, Siepi D, Roscini AR, et al. Impaired flow-mediated vasoactivity during post-prandial phase in young healthy men. Atherosclerosis. 2000;153(2):397-402.

247. Vogel RA, Corretti MC, Plotnick GD. Effect of a single high-fat meal on endothelial function in healthy subjects. Am J Cardiol. 1997;79(3):350-4.

248. Esser D, van Dijk SJ, Oosterink E, Müller M, Afman LA. A high-fat SFA, MUFA, or n3 PUFA challenge affects the vascular response and initiates an activated state of cellular adherence in lean and obese middle-aged men. J Nutr. 2013;143(6):843-51.

249. Nappo F, Esposito K, Cioffi M, Giugliano G, Molinari AM, Paolisso G, et al. Postprandial endothelial activation in healthy subjects and in type 2 diabetic patients: role of fat and carbohydrate meals. J Am Coll Cardiol. 2002;39(7):1145-50.

250. Jellema A, Plat J, Mensink RP. Weight reduction, but not a moderate intake of fish oil, lowers concentrations of inflammatory markers and PAI-1 antigen in obese men during the fasting and postprandial state. Eur J Clin Invest. 2004;34(11):766-73.

251. SD P. Postprandial lipemia, hemostasis, inflammatory response and other emerging risk factors for cardiovascular disease: the influence of fatty meals. Current Nutrition and Food Science. 2005;1:11.

252. Alipour A, Elte JW, van Zaanen HC, Rietveld AP, Castro Cabezas M. Novel aspects of postprandial lipemia in relation to atherosclerosis. Atheroscler Suppl. 2008;9(2):39-44.

253. Khan TA, Shah T, Prieto D, Zhang W, Price J, Fowkes GR, et al. Apolipoprotein E genotype, cardiovascular biomarkers and risk of stroke: systematic review and meta-analysis of 14,015 stroke cases and pooled analysis of primary biomarker data from up to 60,883 individuals. Int J Epidemiol. 2013;42(2):475-92.

254. Eichner JE, Dunn ST, Perveen G, Thompson DM, Stewart KE, Stroehla BC. Apolipoprotein E polymorphism and cardiovascular disease: a HuGE review. Am J Epidemiol. 2002;155(6):487-95.

255. Jofre-Monseny L, Minihane AM, Rimbach G. Impact of apoE genotype on oxidative stress, inflammation and disease risk. Mol Nutr Food Res. 2008;52(1):131-45.

256. Masson LF, McNeill G, Avenell A. Genetic variation and the lipid response to dietary intervention: a systematic review. Am J Clin Nutr. 2003;77(5):1098-111.

257. Lockyer S, Tzanetou M, Carvalho-Wells AL, Jackson KG, Minihane AM, Lovegrove JA. SATgenε dietary model to implement diets of differing fat composition in prospectively genotyped groups (apoE) using commercially available foods. Br J Nutr. 2012;108(9):1705-13.

258. Jackson KG, Lockyer S, Carvalho-Wells AL, Williams CM, Minihane AM, Lovegrove JA. Dietary fat manipulation has a greater impact on postprandial lipid metabolism than the apolipoprotein E (epsilon) genotype-insights from the SATgenε study. Mol Nutr Food Res. 2012;56(12):1761-70.

259. Chouinard-Watkins R, Conway V, Minihane AM, Jackson KG, Lovegrove JA, Plourde M. Interaction between BMI and APOE genotype is associated with changes in the plasma long-chain-PUFA response to a fish-oil supplement in healthy participants. Am J Clin Nutr. 2015;102(2):505-13.

260. Calabuig-Navarro MV, Jackson KG, Walden CM, Minihane AM, Lovegrove JA. Apolipoprotein E genotype has a modest impact on the postprandial plasma response to meals of varying fat composition in healthy men in a randomized controlled trial. J Nutr. 2014;144(11):1775-80.

261. Basu A, Devaraj S, Jialal I. Dietary factors that promote or retard inflammation. Arterioscler Thromb Vasc Biol. 2006;26(5):995-1001.

262. Koutsos A, Jackson KG, Lockyer S, Carvalho-Wells A, Minihane AM, Lovegrove JA. Greater impact of dietary fat manipulation than apolipoprotein E genotype on ex vivo cytokine production - insights from the SATgenε study. Cytokine. 2014;66(2):156-9.

263. Dietary reference values for food energy and nutrients for the United Kingdom. Report of the Panel on Dietary Reference Values of the Committee on Medical Aspects of Food Policy. Rep Health Soc Subj (Lond). 1991;41:1-210.

264. Vasilopoulou D, Markey O, Kliem K, Fagan C, Grandison A, Humphries D, et al. Impact of chronic consumption of dairy products with a differential fatty acid profile on postprandial endothelial, lipid and inflammatory responses: a randomized, cross-over, double-blinded controlled trial. 2017.

265. Carlsson M, Wessman Y, Almgren P, Groop L. High levels of nonesterified fatty acids are associated with increased familial risk of cardiovascular disease. Arterioscler Thromb Vasc Biol. 2000;20(6):1588-94.

266. Pollex RL, Hegele RA. Genetic determinants of plasma lipoproteins. Nat Clin Pract Cardiovasc Med. 2007;4(11):600-9.

267. Dallongeville J, Lussier-Cacan S, Davignon J. Modulation of plasma triglyceride levels by apoE phenotype: a meta-analysis. J Lipid Res. 1992;33(4):447-54.

268. Kim DS, Li YK, Bell GA, Burt AA, Vaisar T, Hutchins PM, et al. Concentration of Smaller High-Density Lipoprotein Particle (HDL-P) Is Inversely Correlated With Carotid Intima Media Thickening After Confounder Adjustment: The Multi Ethnic Study of Atherosclerosis (MESA). J Am Heart Assoc. 2016;5(5).

269. Martin SS, Khokhar AA, May HT, Kulkarni KR, Blaha MJ, Joshi PH, et al. HDL cholesterol subclasses, myocardial infarction, and mortality in secondary prevention: the Lipoprotein Investigators Collaborative. Eur Heart J. 2015;36(1):22-30.

270. Shaw DI, Tierney AC, McCarthy S, Upritchard J, Vermunt S, Gulseth HL, et al. LIPGENE food-exchange model for alteration of dietary fat quantity and quality in free-living participants from eight European countries. Br J Nutr. 2009;101(5):750-9.

271. Tierney AC, McMonagle J, Shaw DI, Gulseth HL, Helal O, Saris WH, et al. Effects of dietary fat modification on insulin sensitivity and on other risk factors of the metabolic syndrome--LIPGENE: a European randomized dietary intervention study. Int J Obes (Lond). 2011;35(6):800-9.

272. Thurm CW, Halsey JF. Measurement of cytokine production using whole blood. Curr Protoc Immunol. 2005;Chapter 7:Unit 7.18B.

273. Public Health England. The Eatwell Guide [Available from: https://www.gov.uk/government/publications/the-eatwell-guide.

274. Lauritzen L, Hellgren LI. Plasma phospholipid very-long-chain saturated fatty acids: a sensitive marker of metabolic dysfunction or an indicator of specific healthy dietary components? Am J Clin Nutr. 2015;101(5):901-2.

275. Green DJ, Dawson EA, Groenewoud HM, Jones H, Thijssen DH. Is flow-mediated dilation nitric oxide mediated?: A meta-analysis. Hypertension. 2014;63(2):376-82.

276. Food and Agriculture Organisation. Food based dietary guidelines 2017

277. Group DAP. Putting Dairy Back on the Daily Menu. London: APPG; 2016. p. 26.

278. Lorenzen JK, Astrup A. Dairy calcium intake modifies responsiveness of fat metabolism and blood lipids to a high-fat diet. Br J Nutr. 2011;105(12):1823-31.

279. Soerensen KV, Thorning TK, Astrup A, Kristensen M, Lorenzen JK. Effect of dairy calcium from cheese and milk on fecal fat excretion, blood lipids, and appetite in young men. Am J Clin Nutr. 2014;99(5):984-91.

280. Livingstone KM, Givens DI, Jackson KG, Lovegrove JA. Comparative effect of dairy fatty acids on cell adhesion molecules, nitric oxide and relative gene expression in healthy and diabetic human aortic endothelial cells. Atherosclerosis. 2014;234(1):65-72.

281. Tang W, Apostol G, Schreiner PJ, Jacobs DR, Boerwinkle E, Fornage M. Associations of lipoprotein lipase gene polymorphisms with longitudinal plasma lipid trends in young adults: The Coronary Artery Risk Development in Young Adults (CARDIA) study. Circ Cardiovasc Genet. 2010;3(2):179-86.

282. Ariza MJ, Sánchez-Chaparro MA, Barón FJ, Hornos AM, Calvo-Bonacho E, Rioja J, et al. Additive effects of LPL, APOA5 and APOE variant combinations on triglyceride levels and hypertriglyceridemia: results of the ICARIA genetic sub-study. BMC Med Genet. 2010;11:66.

283. Shatwan IM, Weech M, Jackson KG, Lovegrove JA, Vimaleswaran KS. Apolipoprotein E gene polymorphism modifies fasting total cholesterol concentrations in response to replacement of dietary saturated with monounsaturated fatty acids in adults at moderate cardiovascular disease risk. Lipids Health Dis. 2017;16(1):222.

284. Pirim D, Wang X, Radwan ZH, Niemsiri V, Hokanson JE, Hamman RF, et al. Lipoprotein lipase gene sequencing and plasma lipid profile. J Lipid Res. 2014;55(1):85-93.

285. AHDB UK. AHDB Dairy Market Information [18 Jan 2018]. Available from: http://dairy.ahdb.org.uk/market-information/#.Wonku6io_IU.

List of publications

Markey O, Vasilopoulou V, Givens DI, Lovegrove JA. Dairy and cardiovascular health: Friend or Foe? Nutr Bull 2014; 39:161-171.

Kliem KE, Humphries DJ, Markey O, Vasilopoulou, Fagan CC, Grandison AS, Jackson KG, Todd SC, Givens DI, Lovegrove JA. Food chain approach to lowering saturated fat in milk and dairy products: the RESET study. Int J Dairy Technol. Under review (June 2018).

Markey O, Vasilopoulou V, Kliem KE, Koulman A, Fagan CC, Summerhill K, Wang LY, et al. Plasma phospholipid fatty acid profile confirms compliance to a novel saturated fat-reduced, monounsaturated fat-enriched dairy product intervention in adults at moderate cardiovascular risk: a randomized controlled study. Nutr J 2017;16:33.

Conference abstracts

Vasilopoulou D, Markey O, Fagan CC, Kliem KE, Humphries DJ, Jackson KG, Todd S, Givens DI and Lovegrove JA. Chronic consumption of conventional and saturated-fat reduced dairy products have differential effects on low-density lipoprotein cholesterol levels in adults at moderate cardiovascular disease risk Abstract for oral presentation at the Summer Nutrition Society, July 2017

Vasilopoulou D, Markey O, Fagan CC, Kliem KE, Humphries DJ, Jackson KG, Todd S, Givens DI and Lovegrove JA. Impact of SFA-reduced and conventional dairy products on the fasting lipid profile in adults at moderate risk of cardiovascular disease: the RESET study. Abstract submitted for ISSFAL conference, South Africa, September 2016

Vasilopoulou D, Markey O, Fagan CC, Kliem KE, Humphries DJ, Jackson KG, Todd S, Givens DI and Lovegrove JA. Chronic consumption of conventional and saturated-fat reduced dairy products have differential effects on low-density lipoprotein cholesterol levels in adults at moderate cardiovascular disease risk. Proceedings of the Nutrition Society 75 (OCE3) January 2016 (oral presentation)

Vasilopoulou D, Markey O, Fagan CC, Kliem KE, Humphries DJ, Jackson KG, Todd S, Givens DI and Lovegrove JA. Associations between dairy consumption and common carotid intima media thickness in adults at risk of cardiovascular disease. Proceedings of the Nutrition Society 74 (OCE5) January 2015 (oral presentation).

Nutrition Bulletin

REVIEW

DOI: 10.1111/nbu.12086

Dairy and cardiovascular health: Friend or foe?

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Abstract

Cardiovascular disease (CVD) prevalence at a global level is predicted to increase substantially over the next decade due to the increasing ageing population and incidence of obesity. Hence, there is an urgent requirement to focus on modifiable contributors to CVD risk, including a high dietary intake of saturated fatty acids (SFA). As an important source of SFA in the UK diet, milk and dairy products are often targeted for SFA reduction. The current paper acknowledges that milk is a complex food and that simply focusing on the link between SFA and CVD risk overlooks the other beneficial nutrients of dairy foods. The body of existing prospective evidence exploring the impact of milk and dairy consumption on risk factors for CVD is reviewed. The current paper highlights that high milk consumption may be beneficial to cardiovascular health, while illustrating that the evidence is less clear for cheese and butter intake. The option of manipulating the fatty acid profile of ruminant milk is discussed as a potential dietary strategy for lowering SFA intake at a population level. The review highlights that there is a necessity to perform more well-controlled human intervention-based research that provides a more holistic evaluation of fat-reduced and fat-modified dairy consumption on CVD risk factors including vascular function, arterial stiffness, postprandial lipaemia and markers of inflammation. Additionally, further research is required to investigate the impact of different dairy products and the effect of the specific food matrix on CVD development.

Keywords: arterial stiffness, blood pressure, cardiovascular disease, dairy products, milk, saturated fatty acids

Introduction

Although mortality from cardiovascular disease (CVD) is now falling in most European countries, CVD is ranked as the leading cause of mortality in the UK and worldwide (BHF 2010; WHO 2011; Nichols *et al.* 2012). It is envisaged that the global prevalence of CVD will continue to increase and is expected to be responsible for more than 23.6 million deaths by 2030 (Smith *et al.* 2012), a figure that is largely attributable to today's dramatic demographic changes with increasing proportions of ageing and obese groups. The outcome of today's obesity and ageing trends may, if not moderated, result in unsustainable costs to global society. Currently, CVD costs the European Union (EU) economy approximately €196 billion per annum in direct and indirect charges,

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with the cost per person in the UK exceeding the EU average (Nichols *et al*. 2012). There is growing pressure to reduce risk factors for CVD at a population level. An atherogenic diet, characterised by a high intake of dietary saturated fatty acids (SFA), is a key modifiable risk factor for CVD. While the effects of the amount and type of dietary fat have been examined in relation to CVD, less focus has been placed on the role of animal-derived staple foods such as milk and dairy products, which are significant dietary sources of SFA.

Saturated fat consumption

In order to reduce the population health burden of CVD, a number of strategies can be addressed; these include increasing physical activity, improving weight profiles and reducing tobacco and alcohol intake. However, this paper will focus on strategies for combatting the overconsumption of dietary SFA.

The adverse effect of SFA on CVD risk is well established; this is primarily mediated via increases in serum lipids, particularly low-density lipoprotein (LDL)-cholesterol (Mensink *et al*. 2003). Current dietary recommendations include an intake of dietary SFA of less than 10% of total energy (DH 1991; WHO 2008). The current rolling *National Diet and Nutrition Survey* (*NDNS*), as outlined in Table 1, highlights that this dietary target is exceeded by the majority of men, women and children in the UK (DH 2012).

Substitution of dietary saturated fat with carbohydrate or unsaturated fatty acids

The question of whether replacement of dietary SFA with carbohydrate (CHO), *cis*-polyunsaturated fatty acids (*cis*-PUFA) or *cis*-monounsaturated fatty acids (*cis*-MUFA) can have beneficial effects on CVD mortality and risk has received considerable attention (Astrup *et al*. 2011; Hooper *et al*. 2012; Vafeiadou *et al*. 2012). There would seem to be no clear evidence for a benefit of substituting CHO for SFA in the prevention of CVD (Astrup *et al*. 2011; Hooper *et al*. 2012) but there is some recent evidence for the benefit of replacing SFA with unsaturated fatty acids (Jakobsen *et al*. 2009; Micha & Mozaffarian 2010; Hooper *et al*. 2012). There is good evidence that replacing SFA with *cis*-PUFA will reduce CVD mortality and risk markers, although there is less information on the effects of replacing SFA with *cis*-MUFA, mainly because few relevant randomised controlled trials have been performed (Mozaffarian *et al*. 2010). Overall, the Cochrane meta-analysis of Hooper *et al*. (2012) identified that a reduction of dietary SFA intake (where it is replaced by unsaturated fat) and/or reduction of total dietary fat lowered the risk of cardiovascular events by 14% [relative risk (RR) 0.86; 95% confidence interval (CI) 0.77–0.96] but had no effect on total mortality.

The contribution of dairy products to saturated fat intake

Milk and dairy products are one of the most significant contributors to SFA intake in the UK diet. In the context of this review, we are defining dairy as cows' milk or any food product derived from cows' milk. However, it should be noted that some dairy products (including butter and cream) do not fall into the 'milk and dairy' section of the *eatwell* plate because they are predominantly viewed as significant sources of fat (FSA 2011). Recent *NDNS* data (Table 2) suggest that milk and dairy products are responsible for 22–25% of SFA consumption in adults (DH 2011); however, it should be noted that this analysis failed to take into account the intake of composite dairy dishes (*e.g.* pizza and lasagne) as well as butter consumption, the latter contributing around 5% to daily average saturated fat intakes; thus SFA intake from milk and dairy product consumption may be underestimated.

Table 1 Average intake of saturated fatty acids in absolute terms and as a percentage of food/total energy, by age and gender (NDNS 2008/2009–2010/2011)

	Boys/Men			Girls/Women		
	4–18 years	19–64 years	≥65 years	4–18 years	19–64 years	≥65 years
SFA (g/day)	25.8 ± 8.6	28.8 ± 12.1	29.3 ± 10.7	22.7 ± 8.1	22.0 ± 9.8	23.2 ± 9.0
% food energy	12.9 ± 2.6	12.8 ± 3.3	14.4 ± 3.5	12.9 ± 2.7	12.6 ± 3.4	14.0 ± 3.7
% total energy	12.9 ± 2.6	12.0 ± 3.4	13.6 ± 3.4	12.9 ± 2.8	12.0 ± 3.3	13.7 ± 3.3

Source: DH (2012).

Mean ± SD. NDNS, National Diet and Nutrition Survey; SFA, saturated fatty acids.

Table 2 Percentage contribution of food groups to average daily total saturated fatty acid intake, by age and gender (NDNS 2008/2009–2009/10)

	Boys/Men			Girls/Women		
Food groups (%)	4–18 years	19–64 years	≥65 years	4–18 years	19–64 years	≥65 years
Cereals and cereal products	25	18	17	24	20	18
Milk and milk products	25	22	28	27	22	24
Eggs and egg dishes	2	3	3	2	4	4
Fat spreads	9	10	13	8	10	16
Meat and meat products	22	27	22	20	23	19
Fish and fish dishes	2	3	4	1	3	5
Vegetables and potatoes	5	7	5	6	7	6
Savoury snacks	2	1	0	2	1	0
Nuts/seeds/fruits	0	1	1	0	2	0
Sugars/preserves/confectionary	7	4	2	7	5	3
Drinks	0	1	1	0	1	0
Miscellaneous	2	3	3	2	4	3

Source: DH (2011).
NDNS, National Diet and Nutrition Survey.

Effects of saturated fat in dairy products on plasma lipids and lipoproteins

A detailed review by Huth and Park (2012) revealed that diets higher in SFA from whole milk and butter increase LDL-cholesterol but may also have a beneficial impact on high-density lipoprotein (HDL)-cholesterol concentrations, resulting in a neutral or beneficial effect on the total cholesterol:HDL-cholesterol ratio. Fermented dairy products including cheese and yogurt appear to have a different impact on circulating levels of plasma lipid and lipoproteins. For example, compared to butter matched for milk fat intake, consumption of hard cheese for a 6-week period led to significantly lower concentrations of total cholesterol, LDL-cholesterol and HDL-cholesterol (Hjerpsted *et al*. 2011). However, it should be noted that butter is not necessarily a good comparator. There are a number of confounding variables that negate the drawing of firm conclusions in relation to cheese and CVD risk due to the differential effect of individual cheese varieties that differ in macronutrient content, degree of fermentation and food matrix (Huth & Park 2012). Furthermore, it highlights that the impact of dairy products on CVD risk factors may be dependent on the specific dairy food, even when supplying the same mass of dairy fat. However, further long-term studies are necessary before firmer conclusions can be drawn on the relative impact of milk and milk-derived food consumption on plasma lipids and lipoproteins.

The consumption of milk and milk-derived products and cardiovascular disease

Potential benefits of milk and dairy consumption

Dairy fats are high in SFA; however, ruminant milk is a complex food and there is much debate as to whether nutrients within dairy foods act independently or synergistically in relation to chronic disease development. The association of SFA with CVD development may be dependent on other nutrients/macronutrients in the matrix of the SFA-containing food (de Oliveira Otto *et al*. 2012). Milk is a significant source of a number of essential micronutrients including calcium, potassium and iodine. Calcium, for example, has a higher bioavailability in milk compared with that present in some other foods (Weaver *et al*. 1999). Furthermore, mineral bioavailability is enhanced by the lack of inhibitors present in milk, including phytates and oxalates, and by the presence of lactose and certain amino acids that may promote mineral absorption (FAO 2013). Moreover, lipids mediate the delivery of essential fat-soluble vitamins, including vitamins D and A, and fatty acids associated with dairy products, namely conjugated linoleic acids, may also have cardio-protective properties, although the data are inconsistent and require confirmation in further human studies (Dilzer & Park 2012). Furthermore, emerging evidence suggests that plasma phospholipid *trans*-palmitoleic acid (*trans* 16:1 *n*-7), a circulating fatty acid biomarker positively corre-

lated with self-reported intakes of dairy fat intake (whole-fat dairy products and butter), is associated with a more favourable metabolic profile and incident diabetes rate (Micha & Mozaffarian 2010; Mozaffarian *et al*. 2013). However, the aforementioned findings regarding *trans* 16:1 *n*-7 should be interpreted with caution as they do not necessarily prove cause and effect; thus, oversimplifying the relationship between dairy product intake (in terms of SFA content) and CVD risk may be misleading (Givens 2012).

Changing trends in dairy consumption

UK trends in dairy product consumption have changed markedly over recent years; this could be partly due to the negative connotations surrounding dairy products and SFA content. *Family Food* statistics published by the Department for Environment, Food and Rural Affairs (Defra) over the past 20 years show a decline in total liquid milk consumption, largely brought about by a decrease in whole milk consumption, which now accounts for around 20% of milk consumed in the UK today. Butter consumption has decreased markedly from the 1970s by about 70%, and fell around 2% lower in the decade between 2001 and 2011, while cheese and yogurt consumption increased by around 5% and 30%, respectively, over the same time period (AHDB-DairyCo 2012).

Dietary patterns associated with milk and dairy product consumption

Analysis of dietary patterns is recognised as an alternate and complementary strategy for investigating the association between diet and disease risk. As dietary patterns are more representative of overall food consumption, they may facilitate a more valid prediction of CVD risk compared with assessment of an individual nutrient or food (Hu 2002). Prospective data from 88 517 middle-aged women indicated that adherence to the Dietary Approaches to Stop Hypertension (DASH) diet, characterised by a moderate intake of low-fat dairy, legumes and nut products, high intake of fruit, vegetables and wholegrains, and low intake of red meat, was inversely associated with risk of coronary heart disease (CHD) and stroke during a 24-year follow-up period (Fung *et al*. 2008). This finding was in agreement with the *Atherosclerosis Risk in Communities Study* which illustrated that a dietary pattern rich in dairy and nut products, but less meat, is associated with a lower risk of incident hypertension in middle-aged adults (Weng *et al*. 2013). However, many international and national dietary guidelines recommend the reduced intake of full-fat dairy products as one aspect of a dietary pattern linked to reducing risk of CHD (Erlinger & Appel 2005).

Dairy consumption and cardiovascular disease risk: Prospective evidence

A number of studies have investigated the effect of milk and dairy products on different CVD events. As previously mentioned, there is a lack of robust evidence on the potential differential effects of individual dairy foods on CVD risk as most observational studies combine dairy products as a single food group, although milk is better studied. A meta-analysis of prospective cohort studies reported that, overall, high milk consumption does not increase the RR of CHD (Elwood *et al*. 2008). A second meta-analysis, which combined prospective cohort and clinical studies, revealed that there was insufficient evidence for an association between milk consumption and CHD (RR 0.94; 95% CI 0.75–1.13) (Mente *et al*. 2009). A later more extensive meta-analysis on milk and dairy consumption and CVD events concluded that high consumption of milk was related to a significant reduction in risk of stroke development (Elwood *et al*. 2010). Table 3 is largely based on Elwood *et al*. (2010) but has been updated by the addition of data from six recently published studies (Bonthuis *et al*. 2010; Goldbohm *et al*. 2011; Sonestedt *et al*. 2011; Soedamah-Muthu *et al*. 2012; Avalos *et al*. 2013; van Aerde *et al*. 2013). A recent study showed that compared with the lowest quintile of dairy consumption, total dairy intake was inversely related to myocardial infarction (MI) risk following an 11.6-year follow-up period (HR 0.77; 95% CI 0.63–0.95). Further analysis revealed that butter used on bread was positively correlated with MI risk, while total cheese intake had an inverse risk association (Patterson *et al*. 2013). This evidence supports the hypothesis that dairy products, excluding butter, are associated with no detrimental effect, and in some cases a significant reduced CVD risk.

Hypertension is one of the key risk factors for CVD development and is influenced by gene polymorphisms, nutrition, the environment and interactions between these factors. Milk and milk-derived products provide essential micronutrients (such as calcium, potassium and iodine) and protein (whey, casein and specific bioactive peptides), some of which have been associated with beneficial hypotensive effects, either independently or synergistically (Kris-Etherton *et al*. 2009). There are a number of proposed mechanisms by which milk and its components could reduce blood pressure (BP; for a

Table 3 Summary of the relative risk for milk and dairy consumption and CVD events

Disease outcome	Number of cohort studies (number used in analyses)	Adjusted RR (95% CI) for milk/dairy consumption*	Significance of heterogeneity between studies
Ischaemic heart disease	22 (17)	0.92 (0.86, 0.99)	$P = 0.765$
All strokes	12	0.79 (0.68, 0.91)	$P = 0.001$
Haemorrhagic stroke	5	0.75 (0.60, 0.94)	$P = 0.014$
Subarachnoid bleed	3	0.93 (0.84, 1.02)	$P = 0.004$
All-cause mortality	12 (9)	0.91 (0.78, 1.05)	$P = 0.070$

Source: Adapted from Givens *et al*. (2014).

*Estimate of the risk of each disease in individuals with the highest consumption of milk/dairy products compared to the risk in individuals with the lowest consumption.

CI, confidence interval; CVD, cardiovascular disease; RR, relative risk.

detailed review, see Fekete *et al*. 2013). Bioactive peptides present in casein and whey proteins have been observed to play a role in controlling BP by inhibiting the action of angiotensin-I-converting enzyme, resulting in vasodilation (FitzGerald & Meisel 2000), by modulating the release of endothelin-1 by endothelial cells (Maes *et al*. 2004) and acting as opioid receptor ligands increasing nitric oxide production which mediates arterial tone (Kris-Etherton *et al*. 2009). An important consideration is the potential impact of a threshold dependency mechanism whereby benefit is conferred in those at low nutrient status, such as calcium, whereas in individuals with adequate baseline status, little effect is observed (McCarron *et al*. 1991; Wennersberg *et al*. 2009; Park & Cifelli 2013). This has important considerations in respect of public health advice on dairy consumption within population groups with different nutritional status. Although the *Rotterdam Study* found an inverse association between low-fat dairy intake and hypertension risk in older adults (Engberink *et al*. 2009), limited evidence exists as to the potential additional benefit of low-fat dairy foods and the type of dairy products in relation to BP reduction. Low-fat dairy is the product of choice in most trials, yet both low- and high-fat alternatives appear to have an overall beneficial effect in relation to BP (Ralston *et al*. 2012). Fumeron *et al*. (2011) reported that consumption of either a variety of dairy products excluding cheese, or cheese alone, and the calcium density of the diet were associated with a lower 9-year diastolic BP after analysing data from the *Epidemiological Study on the Insulin Resistance Syndrome*. Moreover, data from the *Caerphilly Prospective Study* illustrated that when compared to non-milk consumers, men who consumed >586 ml/day had a 10.4 mmHg lower systolic BP after a 22.8-year follow-up (Livingstone *et al*. 2013). Unsurprisingly, greater hypotensive effects of dairy consumption are observed in those with hypertension or who present with calcium sensitivity. In normotensive subjects, dairy consumption is often related to retaining BP homeostasis rather than hypotensive effects (Park & Cifelli 2013).

Elasticity of the blood vessels can be influenced by chronic dietary patterns (Kesse-Guyot *et al*. 2010). Cardiovascular events and all-cause mortality are independently predicted by carotid-femoral pulse wave velocity (PWV), the gold standard measurement of arterial stiffness (Vlachopoulos *et al*. 2010; Van Bortel *et al*. 2012). Further evidence from the *Caerphilly Prospective Study* highlighted that, with the exception of butter consumption, dairy product intake does not impact negatively on PWV (Livingstone *et al*. 2013). Furthermore, augmentation index, another indicator of arterial stiffness, was 1.8% lower in men with the highest quartiles of dairy food consumption (Livingstone *et al*. 2013). Similarly, cross-sectional study findings illustrated that dairy food intake was inversely correlated with PWV (Crichton *et al*. 2012a).

A low-grade systemic inflammation is recognised as a major factor contributing to the development and progression of a number of disorders related to CVD (Labonte *et al*. 2013). Cross-sectional studies that have investigated the relationship between dairy intake and low-grade systemic inflammation have found an inverse association (Salas-Salvado *et al*. 2008; Esmaillzadeh & Azadbakht 2010). However, a review that grouped several studies involving overweight or obese subjects found a degree of heterogeneity which hinders any definite conclusions (Labonte *et al*. 2013). Although there is evidence supporting a beneficial association between dairy consumption and inflammation, the mechanisms are still unclear and studies are either underpowered or use more than one type of dairy product as an intervention, making it difficult to distinguish between dairy products (Labonte *et al*. 2013).

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Dietary strategies for lowering consumption of saturated fat and the implication for cardiovascular disease

Low-fat milk and dairy products

When compared with low-fat alternatives, there is no established nutritional benefit of whole-fat dairy consumption, except in young children; therefore, the intake of low-fat milk and milk-related products may be considered an effective strategy to lower SFA intake. However, there is currently no consensus on whether fat-reduced dairy foods are associated with a reduced risk of CVD (Benatar *et al*. 2013). Observational studies have indicated that low-fat dairy consumption is an effective strategy to promote lower BP levels (Engberink *et al*. 2009; Toledo *et al*. 2009; van Meijl & Mensink 2011), circulating markers of inflammation (Esmaillzadeh & Azadbakht 2010), the ratio of total cholesterol:HDL-cholesterol (Mensink *et al*. 2003) and LDL-cholesterol concentration (Kai *et al*. 2013), as well as aid in weight maintenance or reduction (Abargouei *et al*. 2012). The *Nurses' Health Study* cohort illustrated that the associated RR of CHD varied according to consumption of high-fat (RR 1.12; 95% CI 1.05–1.20) or low-fat dairy consumption (RR 0.80; 95% CI 0.73–0.87) (Hu *et al*. 1999). On the contrary, findings from a prospective population-based cohort of 33 636 women suggested there were no significant differences between consumption of specific low-fat and high-fat dairy products and MI risk (Patterson *et al*. 2013). Furthermore, findings from a 12-month randomised crossover trial concluded that inclusion of reduced-fat dairy products in the diets of overweight adults had no impact on cardio-metabolic outcomes, including blood lipids, BP and arterial compliance (Crichton *et al*. 2012b). However, before it can be clearly established whether or not removal of milk fat is beneficial to overall cardio-metabolic health, further evidence from well-controlled human intervention studies is required.

Altering the dairy cow diet to manipulate the fatty acid profile of milk

As an alternative to promoting low-fat dairy product consumption, modification of the fatty acid profile of bovine milk offers a strategy for lowering the population's intake of SFA, by removing SFA from the food chain, while preserving the beneficial contributions that dairy products make to the protein and micronutrient content of the human diet (Shingfield *et al*. 2008). Over 100 studies have explored the potential of partially replacing milk SFA with *cis*-MUFA or *cis*-PUFA through supplementation of the bovine diet with plant oils or oilseeds (Givens & Shingfield 2006; Glasser *et al*. 2008). Through a reduced synthesis of short- and medium-chain SFA by the mammary gland, this feeding strategy enhances the long-chain (>C18) unsaturated fatty acid concentration in the milk (Doreau *et al*. 1999). Inclusion of 49 g/kg of dry matter of rapeseed oil in the ruminant diet for a 28-day period increased *cis*-MUFA from 20 to 33 g/100 g FA, while reducing SFA from 70 to 55–60 g/day FA (Givens *et al*. 2009). Although a more substantial decrease in SFA (~20 g/100 g of FA) has been documented, the alteration to milk FA composition was adversely linked to voluntary bovine nutrient intake and milk yield compared to the control diet (Givens *et al*. 2003). In order for modification of the composition of ruminant-derived foods to be recognised as a sustainable strategy for reducing SFA intake at a population level, it is essential to find an optimal balance between maximising the unsaturated FA profile of the milk and minimising the impact of the supplementation on animal performance (Givens 2008). Furthermore, it should be considered that ruminal biohydrogenation of PUFA results in the formation of intermediates including *trans* 18:1 and leads to small increases in PUFA relative to MUFA concentrations and therefore, it might be more feasible to supplement the bovine diet with MUFA (Shingfield *et al*. 2013).

In addition to the reductions in SFA and increases in *cis*-MUFA, inclusion of unsaturated fatty acids into the bovine-feeding regimen can lead to increased concentrations of naturally produced ruminant *trans* fatty acid (rTFA), namely linoleic acid isomers and *trans* MUFA, in the milk. The intake of *trans* fatty acids (TFA) from industrially hydrogenated vegetable oils is known to have a negative impact on cardiovascular health (Mozaffarian *et al*. 2006; Brouwer *et al*. 2010) and, accordingly, there has been a significant reduction in the level of 'industrial' TFA (iTFA) in the food chain (Hulshof *et al*. 1999). Conversely, the association between rTFA and CVD remains inconclusive (Gebauer *et al*. 2011; Brouwer *et al*. 2013) with some studies showing a cardio-protective effect of ruminant sources of *trans* fats (Mozaffarian *et al*. 2006; Jakobsen *et al*. 2008). In an attempt to resolve the conflicting reports, a systematic review and meta-analysis was undertaken by Bendsen *et al*. (2011). They reported that the RR for high vs. low quintiles of total TFA intake (2.8 to approximately 10 g/day) was 1.22 (95% CI 1.08–1.38; $P = 0.002$) for CHD events and 1.24 (95% CI 1.07–1.43; $P = 0.003$) for fatal CHD. rTFA intake (0.5–1.9 g/day) was not significantly associated with CHD risk (RR 0.92; 95% CI 0.76–1.11; $P = 0.36$) although neither

was iTFA. There was, however, a trend towards a positive association (RR 1.21; 95% CI 0.97–1.50; $P = 0.09$) for iTFA intake. The authors concluded that while iTFA may be positively related to CHD, rTFA is not, but the limited number of studies available prevented a firm conclusion concerning whether the source of TFA is important. The lack of an association of rTFA with CHD risk may be due to lower intake levels (Bendsen *et al*. 2011). However, at levels currently consumed in the UK diet, there is no evidence of risk from rTFA.

Over the past 10–15 years, the total TFA intake in the UK diet has decreased substantially as a result of voluntary action by the UK food industry; this has led to a greater proportion of the total dietary *trans* fats originating from rTFA (SACN 2007). However, while the proportion of dietary rTFA has increased, the absolute intake of ruminant fat is unchanged. The current dietary intake of TFA in the UK (0.7% of food energy in adults) (DH 2012) is below the recommended population maximum (2% of food energy intake) (DH 1991), with milk and milk products contributing to around 25% of this intake (DH 2011). Consequently, TFA intake from ruminant sources is not seen as a major cause of concern to cardiovascular health at a population level (Tardy *et al*. 2011; Brouwer *et al*. 2013). However, it has yet to be determined whether increasing rTFA intake, through manipulation of the fatty acid profile of milk and dairy products to decrease SFA content, impacts on cardiovascular health (Livingstone *et al*. 2012).

Impact of modified dairy products on cardiovascular disease risk factors

A review of the current evidence suggests that consumption of modified feed-reduced SFA milk and milk products may be beneficial to CVD risk in healthy and hypercholesterolaemic populations when compared to commercially available whole milk dairy products (Livingstone *et al*. 2012). However, it should be noted that there is a distinct lack of human intervention-based research (Givens 2012; Livingstone *et al*. 2012) and the studies that have been performed have relied on plasma lipid levels as a predictor of CVD risk and on butter as the main test food. Some selected data illustrate that, in comparison to conventional milk, cheese, butter and ice cream (70 g/100 g SFA, 28 g/100 g *cis*-MUFA), total cholesterol and LDL-cholesterol were significantly lowered following a 3-week period of consuming matched fat-modified dairy products (51 g/100 g SFA, 39 g/100 g *cis*-MUFA) (Noakes *et al*. 1996). Dairy products are complex, nutrient-dense foods and focusing on a single outcome measure could lead to misleading conclusions by failing to establish the impact on other CVD risk factors.

Evidence suggests that some of the effects of SFA on CVD risk are mediated by impairment in endothelial function and subsequent establishment of atherosclerosis (Nicholls *et al*. 2006; Blumenthal *et al*. 2010; Vafeiadou *et al*. 2012) and by influencing postprandial lipaemia (Berry & Sanders 2005). Endothelial dysfunction, an early modifiable event in the coronary atherosclerotic process, is positively associated with increased risk of CVD (De Caterina 2000; Schachinger *et al*. 2000). Flow-mediated dilation, which measures the vasodilatory response of the brachial artery to an increase in blood flow-associated shear stress and carotid intima-media thickness, can be used to non-invasively assess endothelial function and arterial structural changes, respectively (Anderson 2006). As previously discussed, it is recommended that arterial stiffness, a surrogate marker of central arterial function, should also be evaluated when exploring the impact of modified dairy consumption on cardiovascular risk factors (Givens 2012). Postprandial lipaemia, characterised by elevated and prolonged triacylglycerol concentrations in the fed state, is influenced by the type and quantity of the meal fat (Chong *et al*. 2010; Jackson & Lovegrove 2012; Jackson *et al*. 2012). Postprandial lipaemia is a significant independent risk marker of CVD that requires attention in future modified fat studies (Nordestgaard *et al*. 2007). Further research is warranted to examine the impact of fat-modified or any total low-fat or full-fat dairy consumption on holistic measures of CVD including vascular function, arterial stiffness, postprandial lipaemia and inflammation.

This is currently being addressed at the University of Reading in the *RESET* (*REplacement of SaturatEd fat in dairy on Total cholesterol*) *Study* (ClinicalTrials.gov NCT02089035), a 3-year Medical Research Council funded project that is investigating the impact of reducing SFA intake by using modified milk and dairy products on vascular function and CVD risk biomarkers, without limiting dairy product consumption. This will be achieved by producing milk and dairy products that have a substantial proportion of the SFA replaced with *cis*-MUFA. In a randomised, crossover, double-blind, controlled study, it will be determined whether modified dairy product consumption improves vascular function and other CVD risk biomarkers relative to typical commercially available products in both acute and chronic settings. The project, which started in late 2013, will provide unique evidence to inform public health policy on food-based dietary recommendations for CVD risk reduction.

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Conclusion

Much of the UK population currently exceeds the dietary SFA recommendation of <11% of food energy intake, with milk and dairy products as a group making a considerable contribution to SFA intake in the average UK diet. While it is intuitive to consider further reducing dairy consumption as a means of decreasing SFA intake, epidemiological data suggest that this strategy may be counterproductive, given the array of cardio-metabolic benefits that milk products appear to offer to human health. Fat-reduced or fat-modified dairy product consumption may offer a more feasible option for reducing intake of SFA with minimal change to habitual eating patterns and, hence, CVD risk at a population level. Nevertheless, before the impact of both fat-reduced and modified dairy consumption on CVD risk reduction can be evaluated, it is essential to conduct more robust, controlled human intervention-based research using both traditional and novel assessments of cardiovascular risk. Additionally, more research is required to differentiate between dairy food matrices.

Acknowledgements

O. Markey and D. Vasilopoulou are supported by the Medical Research Council (MR/K020218/1).

Conflict of interest

The authors declare no conflict of interest.

References

Abargouei AS, Janghorbani M, Salehi-Marzijarani M *et al.* (2012) Effect of dairy consumption on weight and body composition in adults: a systematic review and meta-analysis of randomized controlled clinical trials. *International Journal of Obesity* **36**: 1485–93.

AHDB-DairyCo (Agriculture and Horticulture Development Board) (2012) Purchases of Milk and Dairy. Available at: http://www.dairyco.org.uk/market-information/dairy-sales-consumption/#.Uz6-X61g6Fc (accessed 7 March 2014).

Anderson TJ (2006) Arterial stiffness or endothelial dysfunction as a surrogate marker of vascular risk. *Canadian Journal of Cardiology* **22**: 72B–80B.

Astrup A, Dyerberg J, Elwood P *et al.* (2011) The role of reducing intakes of saturated fat in the prevention of cardiovascular disease: where does the evidence stand in 2010? *American Journal of Clinical Nutrition* **93**: 684–8.

Avalos EE, Barrett-Connor E, Kritz-Silverstein D *et al.* (2013) Is dairy product consumption associated with the incidence of CHD? *Public Health Nutrition* **16**: 2055–63.

Benatar JR, Sidhu K & Stewart RA (2013) Effects of high and low fat dairy food on cardio-metabolic risk factors: a meta-analysis of randomized studies. *PLoS ONE* **8**: 0076480.

Bendsen NT, Christensen R, Bartels EM *et al.* (2011) Consumption of industrial and ruminant trans fatty acids and risk of coronary heart disease: a systematic review and meta-analysis of cohort studies. *European Journal of Clinical Nutrition* **65**: 773–83.

Berry SE & Sanders TA (2005) Influence of triacylglycerol structure of stearic acid-rich fats on postprandial lipaemia. *Proceedings of the Nutrition Society* **64**: 205–12.

BHF (British Heart Foundation) (2010) Coronary heart disease statistics 2010. Available at: http://www.bhf.org.uk/publications/view-publication.aspx?ps=1001546 (accessed 5 December 2013).

Blumenthal JA, Babyak MA, Hinderliter A *et al.* (2010) Effects of the DASH diet alone and in combination with exercise and weight loss on blood pressure and cardiovascular biomarkers in men and women with high blood pressure: the ENCORE study. *Archives of Internal Medicine* **170**: 126–35.

Bonthuis M, Hughes MC, Ibiebele TI *et al.* (2010) Dairy consumption and patterns of mortality of Australian adults. *European Journal of Clinical Nutrition* **64**: 569–77.

Brouwer IA, Wanders AJ & Katan MB (2010) Effect of animal and industrial trans fatty acids on HDL and LDL cholesterol levels in humans-a quantitative review. *PLoS ONE* **5**: 0009434.

Brouwer IA, Wanders AJ & Katan MB (2013) Trans fatty acids and cardiovascular health: research completed? *European Journal of Clinical Nutrition* **67**: 541–7.

Chong MF, Lockyer S, Saunders CJ *et al.* (2010) Long chain n-3 PUFA-rich meal reduced postprandial measures of arterial stiffness. *Clinical Nutrition* **29**: 678–81.

Crichton GE, Elias MF, Dore GA *et al.* (2012a) Relations between dairy food intake and arterial stiffness: pulse wave velocity and pulse pressure. *Hypertension* **59**: 1044–51.

Crichton GE, Howe PR, Buckley JD *et al.* (2012b) Dairy consumption and cardiometabolic health: outcomes of a 12-month crossover trial. *Nutrition & Metabolism* **9**: 19.

de Oliveira Otto MC, Mozaffarian D, Kromhout D *et al.* (2012) Dietary intake of saturated fat by food source and incident cardiovascular disease: the Multi-Ethnic Study of Atherosclerosis. *American Journal of Clinical Nutrition* **96**: 397–404.

De Caterina R (2000) Endothelial dysfunctions: common denominators in vascular disease. *Current Opinion in Lipidology* **11**: 9–23.

DH (Department of Health) (1991), Dietary Reference Values for Food Energy and Nutrients for the United Kingdom. Vol. 41: Report on Health and Social Subjects. London: Her Majesty's Stationery Office.

DH (Department of Health) (2011) National Diet and Nutrition Survey: Headline results from Years 1 and 2 (combined) of the rolling programme 2008/9–2009/10. Available at: https://www.gov.uk/government/publications/national-diet-and-nutrition-survey-headline-results-from-years-1-and-2-combined-of-the-rolling-programme-2008-9-2009-10 (accessed 6 December 2013).

DH (Department of Health) (2012) National Diet and Nutrition Survey: Headline results from Years 1, 2 and 3 (combined) of the rolling programme 2008/9–2010/11. Available at: http://webarchive.nationalarchives.gov.uk/20130402145952/http://transparency.dh.gov.uk/2012/07/25/ndns-3-years-report/ (accessed February 2014).

Dilzer A & Park Y (2012) Implication of conjugated linoleic acid (CLA) in human health. *Critical Reviews in Food Science and Nutrition* **52**: 488–513.

Doreau M, Chillard Y, Rulquin H *et al.* (1999) Manipulation of milk fat in dairy cows. In: *Recent Advances in Animal Nutrition*, pp. 81–109. Nottingham University Press: Nottingham.

Elwood PC, Givens DI, Beswick AD *et al.* (2008) The survival advantage of milk and dairy consumption: an overview of evidence from cohort studies of vascular diseases, diabetes and cancer. *Journal of the American College of Nutrition* **27**: 723S–34S.

Elwood PC, Pickering JE, Givens DI *et al.* (2010) The consumption of milk and dairy foods and the incidence of vascular disease and diabetes: an overview of the evidence. *Lipids* **45**: 925–39.

Engberink MF, Geleijnse JM, de Jong N *et al.* (2009) Dairy intake, blood pressure, and incident hypertension in a general Dutch population. *Journal of Nutrition* **139**: 582–7.

Erlinger T & Appel LJ (2005) Dietary patterns and coronary heart disease risk. In: *Coronary Heart Disease Epidemiology*, 2nd edn. Available at: http://dx.doi.org/10.1093/acprof:oso/9780198525738.003.0014 (accessed 4 April 2014).

Esmaillzadeh A & Azadbakht L (2010) Dairy consumption and circulating levels of inflammatory markers among Iranian women. *Public Health Nutrition* **13**: 1395–402.

FAO (Food and Agriculture Organization) (2013) Milk and dairy products in human nutrition. Available at: http://www.fao.org/publications (accessed December 2013).

Fekete AA, Givens DI & Lovegrove JA (2013) The impact of milk proteins and peptides on blood pressure and vascular function: a review of evidence from human intervention studies. *Nutrition Research Reviews* **26**: 177–90.

FitzGerald RJ & Meisel H (2000) Milk protein-derived peptide inhibitors of angiotensin-I-converting enzyme. *British Journal of Nutrition* **84**: S33–7.

FSA (Food Standards Agency) (2011) The Eatwell Plate. Available at: http://www.Eatwell.gov.uk/healthydiet/Eatwellplate/ (accessed 2 February 2014).

Fumeron F, Lamri A, Abi Khalil C *et al.* (2011) Dairy consumption and the incidence of hyperglycemia and the metabolic syndrome: results from a French prospective study, Data from the Epidemiological Study on the Insulin Resistance Syndrome (DESIR). *Diabetes Care* **34**: 813–17.

Fung TT, Chiuve SE, McCullough ML *et al.* (2008) Adherence to a DASH-style diet and risk of coronary heart disease and stroke in women. *Archives of Internal Medicine* **168**: 713–20.

Gebauer SK, Chardigny JM, Jakobsen MU *et al.* (2011) Effects of ruminant trans fatty acids on cardiovascular disease and cancer: a comprehensive review of epidemiological, clinical, and mechanistic studies. *Advances in Nutrition* **2**: 332–54.

Givens DI (2008) Session 4: Challenges facing the food industry in innovating for health. Impact on CVD risk of modifying milk fat to decrease intake of SFA and increase intake of cis-MUFA. *Proceedings of the Nutrition Society* **67**: 419–27.

Givens DI (2012) Milk in the diet: good or bad for vascular disease? *Proceedings of the Nutrition Society* **71**: 98–104.

Givens DI & Shingfield KJ (2006) Optimising dairy milk fatty acid composition. In: *Improving the Fat Content of Foods*, pp. 252–80. Woodhead Publishing Ltd: Cambridge.

Givens DI, Allison R & Blake JS (2003) Enhancement of oleic acid and vitamin E concentrations of bovine milk using dietary supplements of whole rapeseed and vitamin E. *Animal Research* **52**: 531–42.

Givens DI, Kliem KE, Humphries DJ *et al.* (2009) Effect of replacing calcium salts of palm oil distillate with rapeseed oil, milled or whole rapeseeds on milk fatty-acid composition in cows fed maize silage-based diets. *Animal* **3**: 1067–74.

Givens DI, Livingstone KM, Pickering JE *et al.* (2014) Milk: White elixir or white poison? An examination of the associations between dairy consumption and disease in human subjects. *Animal Frontiers* **4**: 8–15.

Glasser F, Ferlay A & Chilliard Y (2008) Oilseed lipid supplements and fatty acid composition of cow milk: a meta-analysis. *Journal of Dairy Science* **91**: 4687–703.

Goldbohm RA, Chorus AM, Galindo Garre F *et al.* (2011) Dairy consumption and 10-y total and cardiovascular mortality: a prospective cohort study in the Netherlands. *The American Journal of Clinical Nutrition* **93**: 615–27.

Hjerpsted J, Leedo E & Tholstrup T (2011) Cheese intake in large amounts lowers LDL-cholesterol concentrations compared with butter intake of equal fat content. *American Journal of Clinical Nutrition* **94**: 1479–84.

Hooper L, Summerbell CD, Thompson R *et al.* (2012) Reduced or modified dietary fat for preventing cardiovascular disease. *Cochrane Database of Systematic Reviews* **5**: CD002137.

Hu FB (2002) Dietary pattern analysis: a new direction in nutritional epidemiology. *Current Opinion in Lipidology* **13**: 3–9.

Hu FB, Stampfer MJ, Manson JE *et al.* (1999) Dietary saturated fats and their food sources in relation to the risk of coronary heart disease in women. *American Journal of Clinical Nutrition* **70**: 1001–8.

Hulshof KF, van Erp-Baart MA, Anttolainen M *et al.* (1999) Intake of fatty acids in western Europe with emphasis on trans fatty acids: the TRANSFAIR Study. *European Journal of Clinical Nutrition* **53**: 143–57.

Huth PJ & Park KM (2012) Influence of dairy product and milk fat consumption on cardiovascular disease risk: a review of the evidence. *Advances in Nutrition* **3**: 266–85.

Jackson KG & Lovegrove JA (2012) Impact of probiotics, prebiotics and synbiotics on lipid metabolism in humans. *Nutrition and Aging* **1**: 181–200.

Jackson KG, Walden CM, Murray P *et al.* (2012) A sequential two meal challenge reveals abnormalities in postprandial TAG but not glucose in men with increasing numbers of metabolic syndrome components. *Atherosclerosis* **220**: 237–43.

Jakobsen MU, Overvad K, Dyerberg J *et al.* (2008) Intake of ruminant trans fatty acids and risk of coronary heart disease. *International Journal of Epidemiology* **37**: 173–82.

Jakobsen MU, O'Reilly EJ, Heitmann BL *et al.* (2009) Major types of dietary fat and risk of coronary heart disease: a pooled analysis of 11 cohort studies. *American Journal of Clinical Nutrition* **89**: 1425–32.

Kai SH, Bongard V, Simon C *et al.* (2013) Low-fat and high-fat dairy products are differently related to blood lipids and cardiovascular risk score. *European Journal of Preventive Cardiology* **3**: 3.

Kesse-Guyot E, Vergnaud A-C, Fezeu L *et al.* (2010) Associations between dietary patterns and arterial stiffness, carotid artery intima-media thickness and atherosclerosis. *European Journal of Cardiovascular Prevention & Rehabilitation* **17**: 718–24.

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Kris-Etherton PM, Grieger JA, Hilpert KF *et al.* (2009) Milk products, dietary patterns and blood pressure management. *American College of Nutrition* **28**: 103S–19S.

Labonte ME, Couture P, Richard C *et al.* (2013) Impact of dairy products on biomarkers of inflammation: a systematic review of randomized controlled nutritional intervention studies in overweight and obese adults. *American Journal of Clinical Nutrition* **97**: 706–17.

Livingstone KM, Lovegrove JA & Givens DI (2012) The impact of substituting SFA in dairy products with MUFA or PUFA on CVD risk: evidence from human intervention studies. *Nutrition Research Reviews* **25**: 193–206.

Livingstone KM, Lovegrove JA, Cockcroft JR *et al.* (2013) Does dairy food intake predict arterial stiffness and blood pressure in men?: Evidence from the Caerphilly Prospective Study. *Hypertension* **61**: 42–7.

McCarron DA, Morris CD, Young E *et al.* (1991) Dietary calcium and blood pressure: modifying factors in specific populations. *American Journal of Clinical Nutrition* **54**: 215S–219S.

Maes W, Van Camp J, Vermeirssen V *et al.* (2004) Influence of the lactokinin Ala-Leu-Pro-Met-His-Ile-Arg (ALPMHIR) on the release of endothelin-1 by endothelial cells. *Regulatory Peptides* **118**: 105–9.

Mensink RP, Zock PL, Kester AD *et al.* (2003) Effects of dietary fatty acids and carbohydrates on the ratio of serum total to HDL cholesterol and on serum lipids and apolipoproteins: a meta-analysis of 60 controlled trials. *American Journal of Clinical Nutrition* **77**: 1146–55.

Mente A, de Koning L, Shannon HS *et al.* (2009) A systematic review of the evidence supporting a causal link between dietary factors and coronary heart disease. *Archives of Internal Medicine* **169**: 659–69.

Micha R & Mozaffarian D (2010) Saturated fat and cardiometabolic risk factors, coronary heart disease, stroke, and diabetes: a fresh look at the evidence. *Lipids* **45**: 893–905.

Mozaffarian D, Katan MB, Ascherio A *et al.* (2006) Trans fatty acids and cardiovascular disease. *New England Journal of Medicine* **354**: 1601–13.

Mozaffarian D, Micha R & Wallace S (2010) Effects on coronary heart disease of increasing polyunsaturated fat in place of saturated fat: a systematic review and meta-analysis of randomized controlled trials. *PLoS Medicine* **7**: 1000252.

Mozaffarian D, de Oliveira Otto MC, Lemaitre RN *et al.* (2013) Trans-palmitoleic acid, other dairy fat biomarkers, and incident diabetes: the Multi-Ethnic Study of Atherosclerosis (MESA). *American Journal of Clinical Nutrition* **97**: 854–61.

Nicholls SJ, Lundman P, Harmer JA *et al.* (2006) Consumption of saturated fat impairs the anti-inflammatory properties of high-density lipoproteins and endothelial function. *Journal of the American College of Cardiology* **48**: 715–20.

Nichols M, Townsend N, Scarborough P *et al.* (2012) European Cardiovascular Disease Statistics. European Heart Network, Brussels, European Society of Cardiology, Sophia Antipolis.

Noakes M, Nestel PJ & Clifton PM (1996) Modifying the fatty acid profile of dairy products through feedlot technology lowers plasma cholesterol of humans consuming the products. *American Journal of Clinical Nutrition* **63**: 42–6.

Nordestgaard BG, Benn M, Schnohr P *et al.* (2007) Nonfasting triglycerides and risk of myocardial infarction, ischemic heart disease, and death in men and women. *Journal of the American Medical Association* **298**: 299–308.

Park KM & Cifelli CJ (2013) Dairy and blood pressure: a fresh look at the evidence. *Nutrition Reviews* **71**: 149–57.

Patterson E, Larsson SC, Wolk A *et al.* (2013) Association between dairy food consumption and risk of myocardial infarction in women differs by type of dairy food. *Journal of Nutrition* **143**: 74–9.

Ralston RA, Lee JH, Truby H *et al.* (2012) A systematic review and meta-analysis of elevated blood pressure and consumption of dairy foods. *Journal of Human Hypertension* **26**: 3–13.

Salas-Salvado J, Garcia-Arellano A, Estruch R *et al.* (2008) Components of the Mediterranean-type food pattern and serum inflammatory markers among patients at high risk for cardiovascular disease. *European Journal of Clinical Nutrition* **62**: 651–9.

SACN (Scientific Advisory Committee on Nutrition) (2007) Update on trans fatty acids and health. Position statement. Available at: http://www.sacn.gov.uk/pdfs/sacn_trans_fatty_acids_report.pdf (accessed 2 March 2014).

Schachinger V, Britten MB & Zeiher AM (2000) Prognostic impact of coronary vasodilator dysfunction on adverse long-term outcome of coronary heart disease. *Circulation* **101**: 1899–906.

Shingfield KJ, Chilliard Y, Toivonen V *et al.* (2008) Trans fatty acids and bioactive lipids in ruminant milk. *Advances in Experimental Medicine and Biology* **606**: 3–65.

Shingfield KJ, Bonnet M & Scollan ND (2013) Recent developments in altering the fatty acid composition of ruminant-derived foods. *Animal* **1**: 132–62.

Smith SC, Collins A, Ferrari R *et al.* (2012) Our time: a call to save preventable death from cardiovascular disease (heart disease and stroke). *Circulation* **126**: 2769–75.

Soedamah-Muthu SS, Masset G, Verberne L *et al.* (2012) Consumption of dairy products and associations with incident diabetes, CHD and mortality in the Whitehall II study. *British Journal of Nutrition* **7**: 1–9.

Sonestedt E, Wirfalt E, Wallstrom P *et al.* (2011) Dairy products and its association with incidence of cardiovascular disease: the Malmo diet and cancer cohort. *European Journal of Epidemiology* **26**: 609–18.

Tardy AL, Morio B, Chardigny JM *et al.* (2011) Ruminant and industrial sources of trans-fat and cardiovascular and diabetic diseases. *Nutrition Research Reviews* **24**: 111.

Toledo E, Delgado-Rodriguez M, Estruch R *et al.* (2009) Low-fat dairy products and blood pressure: follow-up of 2290 older persons at high cardiovascular risk participating in the PREDIMED study. *British Journal of Nutrition* **101**: 59–67.

van Aerde MA, Soedamah-Muthu SS, Geleijnse JM *et al.* (2013) Dairy intake in relation to cardiovascular disease mortality and all-cause mortality: the Hoorn Study. *European Journal of Nutrition* **52**: 609–16.

van Meijl LE & Mensink RP (2011) Low-fat dairy consumption reduces systolic blood pressure, but does not improve other metabolic risk parameters in overweight and obese subjects. *Nutrition, Metabolism & Cardiovascular Diseases* **21**: 355–61.

Vafeiadou K, Weech M, Sharma V *et al.* (2012) A review of the evidence for the effects of total dietary fat, saturated, monounsaturated and n-6 polyunsaturated fatty acids on vascular function, endothelial progenitor cells and microparticles. *British Journal of Nutrition* **107**: 303–24.

Van Bortel LM, Laurent S, Boutouyrie P *et al*. (2012) Expert consensus document on the measurement of aortic stiffness in daily practice using carotid-femoral pulse wave velocity. *Journal of Hypertension* **30**: 445–8.

Vlachopoulos C, Aznaouridis K & Stefanadis C (2010) Prediction of cardiovascular events and all-cause mortality with arterial stiffness: a systematic review and meta-analysis. *Journal of American College of Cardiology* **55**: 1318–27.

Weaver CM, Proulx WR & Heaney R (1999) Choices for achieving adequate dietary calcium with a vegetarian diet. *American Journal of Clinical Nutrition* **70**: 543s–548s.

Weng L-C, Steffen L, Szklo M *et al*. (2013) A diet pattern with more dairy and nuts, but less meat is related to lower risk of developing hypertension in middle-aged adults: the Atherosclerosis Risk in Communities (ARIC) Study. *Nutrients* **5**: 1719–33.

Wennersberg MH, Smedman A, Turpeinen AM *et al*. (2009) Dairy products and metabolic effects in overweight men and women: results from a 6-mo intervention study. *American Journal of Clinical Nutrition* **90**: 960–8.

WHO (World Health Organization) (2008) Interim summary of conclusions and dietary recommendations on total fat and fatty acids. Available at: http://www.who.int/nmh/publications/ncd_report2010/en/ (accessed 5 December 2013).

WHO (World Health Organization) (2011) Global status report on noncommunicable diseases 2010. Available at: http://www.who.int/nmh/publications/ncd_report_full_en.pdf (accessed 5 December 2013).

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Interpretive summary:

Food chain approach to lowering saturated fat in milk and dairy products: the RESET study

Kliem

Supplementing dairy cow diets with high-oleic sunflower oil produces milk lower in saturated fatty acids/higher in monounsaturated fatty acids compared with the fatty acid profile of typical UK retail milk. This composition was maintained when this dietary strategy was used on a number of occasions over two years, with no difference in profile between UHT milk, butter and Cheddar cheese made from this milk. This dietary strategy could be used to manufacture modified dairy products which may have beneficial effects on human health.

Food chain approach to lowering saturated fat in milk and dairy products: the RESET study

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ABSTRACT

Despite a decline in overall consumption of saturated fatty acids (**SFA**) by UK adults over the past decade, intakes still exceed recommended levels, which may contribute to increased risk of cardiovascular disease. Milk and dairy products are a major source of SFA in the UK adult diet, and one strategy to minimise SFA in the food chain is to supplement lactating cow diets with unsaturated fatty acid-rich plant oils, thereby replacing some milk SFA with *cis*-monounsaturated fatty acids (**MUFA**). Human intervention studies are required to determine whether consuming dairy products with SFA partially replaced with *cis*-MUFA reduces cardiovascular disease risk. However, a major hurdle to the implementation of such studies is the requirement for large volumes of SFA-reduced, MUFA-enriched dairy products with a consistent composition. Therefore, in this study a diet containing high oleic acid sunflower oil was fed to lactating cows over two production periods spanning almost two years to partially replace milk fat SFA with *cis*-MUFA. Resulting milk (modified) was used for UHT milk, butter and Cheddar cheese production, and compared with products with fatty acid (**FA**) profiles typical of retail products (conventional). The SFA concentration of lipid from modified products was lower than that of conventional products (70 vs 53 g/100 g FA, respectively), and was replaced by *cis*-MUFA (mainly *cis*-9 18:1) and *trans*-MUFA. FA profile of modified products was consistent over the production periods with no effect of processing. This food chain approach was successful in producing modified dairy products of consistent FA profile, suitable for use in a human intervention study.

Keywords: Milk, dairy product, saturated fatty acid, monounsaturated fatty acid

INTRODUCTION

Milk and dairy products are a major source of fat and fatty acids (**FA**) in the UK adult diet, contributing to 18 % total fat, 28 % saturated fatty acid (**SFA**), 38 % *trans* fatty acid (**TFA**) intake (Bates et al., 2016) and 12 % *cis*-monounsaturated fatty acid (**MUFA**) intake (Hobbs, personal communication). The contribution of dairy products to UK SFA intake has declined slightly since 2003 (Henderson et al., 2003), probably due to a decrease in whole fat milk consumption between 2003-2013 (DEFRA, 2013), but at the population level total SFA intake still exceeds current recommendations (12.1 % total energy intake (**EI**); Bates et al., 2016) vs 11 % total EI; Department of Health, 1994, or 10 % total EI; WHO, 2010). A reduction in dietary TFA from industrially hydrogenated food sources has led to an increase in contribution of these fatty acids from dairy products but overall TFA consumption has declined (Henderson et al., 2003; Bates et al., 2016) and is below the maximum recommended intake of 2 % total energy (SACN, 2007).

It has been shown that replacing dietary SFA with *cis*-PUFA reduces CVD risk factors, including fasting serum total, LDL and total:HDL-cholesterol concentrations (Vafeiadou et al., 2015). The most effective means of replacing SFA with UFA in milk fat is by altering the dairy cow diet (Kliem & Shingfield, 2016). Due to the more extensive rumen biohydrogenation of dietary PUFA compared with *cis*-MUFA (Shingfield et al., 2008), and the greater proportion of *cis*-MUFA compared with PUFA in milk fat (Kliem et al., 2013), replacement with *cis*-MUFA offers a greater potential for SFA reduction in milk fat. To date only nine studies have assessed the impact of consuming dairy products modified using this dietary strategy (replacing milk SFA with either MUFA or PUFA) on cardiovascular health outcomes in humans (Livingstone et al., 2012). Five of these studies used only butter as the test dairy product, thus not representing the nutritional composition of a range of dairy products and food matrices. Two studies (Noakes

et al., 1996; Seidel et al., 2005) included a range of dairy products modified by changing the cow diet in their interventions, but these only lasted for relatively short periods of time (up to three weeks) and involved small numbers of participants. It is not known whether the FA composition of milk and dairy products produced this way would be consistent over longer periods of time, especially as milk FA response to dietary oilseeds appears to vary according to differences in other dietary nutrients (Lerch et al., 2012a).

The main objectives of this study were twofold; firstly to identify whether UHT milk, butter and Cheddar cheese could be produced with a consistent FA profile over a two-year period, from milk modified using an oleic-acid rich supplementation strategy. A consistent composition is essential if this technique is to be applied on a larger scale. The current study utilised a high oleic acid (*cis*-9 18:1) sunflower oil to maximise the replacement of SFA with MUFA, after previous research highlighted the potential of these oils (Loor et al., 2002; Kliem et al., 2011). The second objective was to compare the FA composition of the modified dairy products with products containing FA profiles typical of UK retail milk during winter months (average SFA 71.5 g/100 g total FA, *cis*-MUFA 21.2 g/100 g FA; Kliem et al., 2013), and for this to be consistent across products.

MATERIALS AND METHODS

This study was part of the larger, Medical Research Council-funded RESET (**RE**placement of **S**atura**E**d fat in dairy on **T**otal cholesterol, Clinicaltrials Gov ID: NCT02089035) study which aimed to investigate the impact of SFA-reduced, MUFA-enriched dairy product consumption on traditional and novel markers of CVD risk.

Production of Modified Milk

Between December 2013 and May 2015, groups of multiparous Holstein-Friesian cows were recruited to produce milk with a modified FA profile. For the purposes of the RESET human intervention study, production was divided into two periods; Production period 1 (**P1**) took place between December 2013 and September 2014, involving a total of 58 cows (mean ± standard error parity 4.0 ± 0.12; milk yield at start 35.0 ± 0.77 litres/day, and days in lactation 181 ± 7.7), producing a total of approximately 12,500 litres of milk which was used to produce modified UHT milk, butter and cheese for the human intervention. Production period 2 (**P2**) took place between November 2014 and May 2015, involving a total of 41 cows (mean +/- standard error parity 4.0 ± 0.12; milk yield at start 33.4 ± 0.91 litres/day, and days in lactation 205 ± 7.2) producing a total of 16,350 litres of milk. Cows recruited to produce modified milk were adapted to a TMR diet an example of which is presented in Table 1. Attempts were made to replicate this diet for each production batch. The diet had a forage:concentrate ratio of 50:50 on a DM basis, with the forage consisting of maize silage, grass silage, grass hay and wheat straw. The diet was supplemented with 43 g/kg DM of high oleic acid sunflower oil (AAK Ltd., Hull, East Yorkshire, HU9 5PX, UK) so that it would supply a cow consuming 23 kg DM per day with 1 kg oil. Cows were adapted to this diet for a period of four weeks before any milk collection was made. Subsamples of modified milk were taken and preserved with potassium dichromate (1 mg/ml; Lactabs; Thompson and Capper, Runcorn, UK) following the adaptation period for milk compositional analysis. A further subsample was frozen to measure the FA profile prior to product manufacture.

Manufacture of Dairy Products

UHT Milk Processing. UHT milk, i.e. both modified and conventionally-produced milk, was produced three times, twice during P1 and once during P2. The first P1 production run was carried out at Reaseheath College (Nantwich, UK) with the remaining production runs

carried out at Frampton's Ltd (Shepton Mallet Somerset, UK). Raw conventional milk was provided by Arla Foods UK (Taw Valley Creamery, North Tawton, UK), and represented retail milk with a typical UK winter dairy FA profile. Conventional milk was standardised to match the fat content of the modified milk using skimmed milk provided by A.E Rodda & Son Ltd., (Redruth, Cornwall, UK), for the purposes of the human intervention study. Raw conventional milk was pumped to a tank and skimmed milk, at the level required to match the modified milk fat content, was added. The milk was agitated in the tank for 5 minutes and a sample removed and analysed to ensure the fat content was correct. UHT processing was carried out in a pilot scale UHT plant. The milk was preheated to 85ºC using a plate heat exchanger and homogenised. The milk was then heated to 142ºC for 5 seconds using direct steam infusion and cooled to 15ºC and aseptically packaged into 5 kg aseptic bag (Reaseheath) or 330 ml aseptic cartons (Framptons). All UHT milk was stored at 4ºC until required.

Cheddar cheese processing. Conventional Cheddar cheese was provided by Arla Foods UK (Taw Valley Creamery), to represent retail mild Cheddar cheese with a typical UK winter dairy FA profile. Modified Cheddar cheese was manufactured at the University of Reading Pilot Plant (Reading, UK). Both cheese types were produced once during P1 and P2. The processing parameters for the modified cheese were selected to mimic the commercial process used at Arla. Modified raw milk was pasteurised at 73ºC for 15 seconds in a high-temperature short-time pasteuriser (flow rate 300 L/h) using a plate heat exchanger. The milk was cooled to 32ºC and transferred to 100 L cheese vats. Starter culture **(**R 604, Chr. Hansen) was added (0.15 g/L) to the vat and allowed to ripen under stirring for 50 minutes. Enzyme (CHY-MAX, Chr. Hansen) was added (0.24 ml/L) and stirred for a further 3 minutes, before the stirrers were removed. Cutting time was 90 minutes after starter addition and was visually confirmed by the cheesemaker. The coagulum was cut by hand using a coagulum cutting knives. The temperature

was increased slowly until it reached 42ºC and this scalding process continued for 1h. Stirring then commenced and continued for 1 h. Whey was subsequently drawn off the cheese vat. The resulting curd underwent a cheddaring process by piling and turning the curd 4 times. The curd was then milled using a cheese mill and dry salted (0.02 kg/kg) and placed into a stainless steel cheese mould. The mould was placed in a horizontal cheese press and pressed at 7 kPa overnight. The next day the cheese was vacuum packed and placed in an 8ºC ripening room for three months. After ripening, cheese was apportioned into 350 g, vacuum packed and stored at 2ºC.

Butter processing. Conventional butter was provided by Arla Foods UK (Taw Valley Creamery) from winter butter stocks during P1 and P2. Modified P1 butter was manufactured at Reaseheath College (Nantwich, UK) and modified P2 butter was manufactured at Ty Tanglwyst Dairy (Bridgend, South Wales). In both cases cream was separated from the milk using a disc bowl separator, pasteurised, and aged at 4ºC overnight. The cream was transferred to a churn and churned until butter grains were formed. The buttermilk was drained off and the butter grains were further worked to create a continuous emulsion. Salt was then added (1.7 g/100 g) and the butter was further worked to ensure even distribution of the salt. Butter was apportioned into 250 g, packaged in butter wrap and stored at -20°C until required.

Chemical Analysis of Milk and Dairy Products

A sample of high oleic acid sunflower oil used during P1 and P2 and subsample of the TMR diet was analysed for FA profile using a modified version of the one step transesterification of Sukhija & Palmquist, (1988). Briefly, 50 mg oil or 300 mg TMR was incubated with an internal standard (methyl heneicosanoate, 51535, Sigma Aldrich Company Ltd., Dorset, UK) at 60°C in the presence of 0.4 M sulphuric acid in methanol and toluene as an extraction solvent, for 2

h (oil) or 3 h (TMR). Following neutralisation, resulting fatty acid methyl esters (**FAME**) in toluene were allowed to stand over sodium sulphate for 30 min to remove methanol residues before being quantified by gas chromatography (**GC**; Bruker 350, Bruker, Germany). The GC was equipped with a flame ionisation detector and 100 m fused silica capillary column (CP-SIL 88, Agilent Technologies, Cheshire, UK), and GC conditions were as published previously (Kliem et al., 2013). Carbon deficiency in the flame ionization detector response for FAME containing 4- to 10-carbon atoms was accounted for using a combined correction factor which also converted FAME to FA (Ulberth et al., 1999). FA were quantified using internal standard peak area, and results were also expressed as g/100 g FA.

Samples of milk taken just prior to dairy product production and UHT milk (modified and conventional) was analysed for FA profile according to the method of Kliem et al. (2013). Briefly, lipid in 1 ml thawed, warmed (to 40°C) milk was extracted in duplicate using a mixture of diethyl ether and hexane (IDF 1: 2010(E), International Dairy Federation, 2010, Brussels, Belgium) and extracts were transesterified to FAME according to previously described procedures (Kliem et al., 2013). GC conditions and FAME identification were as described above. Methyl esters not available as authentic standards were identified by gas chromatography-mass spectrometry (**GC-MS**; Thermo Trace GC coupled to ITQ 1100 mass spectrometer using helium as a carrier gas) analysis of 4, 4-dimethyloxazoline (**DMOX**) derivatives prepared from FAME. Preparation of DMOX derivatives and parameters used for GC-MS analysis were largely in accordance with earlier reports (Shingfield et al., 2006), however a split ratio of 1:14 was used for injection and the online reference library of DMOX electron impact ionisation spectra was http://lipidlibrary.aocs.org. Results were expressed as g/100 g FA. Lipid in 50 mg conventional and modified butter was first warmed gently before 1 ml distilled water added, and mixed vigorously to emulsify butter fat. Extraction and

methylation continued as with milk and results were expressed as g/100 g total FA. Lipid in 3 g conventional and modified cheese was firstly hydrolysed using 100 ml 3M HCl, and the resulting residue filtered through Whatman 1 filter paper prior to drying at 60°C for 18 h. Lipid was extracted from the residue using petroleum ether (Brown & Mueller-Harvey, 1999), and the amount of lipid calculated gravimetrically. Lipid was then gently warmed before 50 mg was transferred to clean glass tubes, and methylation of extracted FA was conducted as for milk and butter.

Nutritional analysis (energy, protein, fat, carbohydrate, ash and moisture) of the dairy products from each cohort was conducted in duplicate by SGS United Kingdom Ltd. (ISO 17025 accredited laboratory; Ellesmere Port, Cheshire, UK). To calculate protein content the obtained nitrogen result was multiplied by the standard dairy nitrogen conversion factor (6.38) to account for the fraction of non-protein nitrogen in each sample (Maubois & Lorient, 2016). Micronutrient content analysis (calcium, magnesium, sodium and phosphorus) was conducted in duplicate by inductively coupled plasma-optical emission spectrometry (Quaternary Scientific (QUEST), University of Reading, Berkshire, UK). Results were expressed on a dry and fresh weight basis.

Data Analysis

For each product separately, nutrient composition data were analysed using an ANOVA (Minitab17) which included effects of production period and treatment. FA composition of all products were analysed using an ANOVA (Minitab17) which initially included effects of product, production period, treatment, and period by treatment interactions. When there was no effect of product this was removed from the model. Least squares means ± s.e.m. are reported, and differences were considered significant at $P < 0.01$ to account for multiplicity.

RESULTS

The FA profile of the high oleic acid sunflower oil used during production P1 and P2 is presented in Table 2. There was a slight change in FA profile during P2, when it contained a lower proportion of *cis*-9 18:1 and higher proportion of both 16:0 and 18:2 n-6.

The macronutrient composition of the dairy products is presented in Table 3. There was no significant effect of production period or treatment on macronutrient content.

There was no effect of product for each individual FA identified and hence the term was removed from the model, and the FA data presented are means across milk, cheese and butter. Lipid from modified dairy products had a lower (P<0.001) total SFA content than lipid from conventional dairy products, which was mainly due to a 12 g/100 g FA lower concentration of 16:0, but also all SFA ≤ 14:0 including branched chain SFA such as 13:0 anteiso, 14:0 iso and 15:0 anteiso (Table 4). In contrast, concentration of 18:0 was higher (P<0.001) in the modified dairy products. There was no effect of production period on SFA content apart from 17:0 iso and 20:0 which were more abundant in P1 products (P<0.01).

Treatment also had an effect on both *cis*- and *trans*- MUFA (Table 4), with lipid from modified dairy products having a higher (P<0.001) concentration of both. Of the *cis*-MUFA, *cis*-9 18:1 was the most abundant (Table 5), and concentration was at least 50 % greater in modified dairy compared with conventional products (Table 5). There were also notable differences in most of the other 18:1 isomers identified, modified products containing higher (P<0.01) lipid concentrations of *cis*-11, *cis*-13, *trans*-6-8, *trans*-9, *trans*-10, *trans*-12, *trans*-15 and *trans*-16 18:1 (the predominant isomer switching from *trans*-11 18:1 in control products to *trans*-10 18:1

in modified products). Aside from the 18:1 isomers, there were treatment differences in *cis*-9 10:1, *cis*-9 12:1, *trans*-9 14:1, *cis*-13 16:1 and *cis*-9 17:1, all of which were lower ($P<0.01$) in modified dairy products.

Total n-6 and n-3 PUFA concentrations were not different between lipid from conventional and modified products (Table 4). In contrast, the concentration of total conjugated linoleic acid (**CLA**) isomers was greater ($P=0.001$) in modified products (Table 4). Of the non-methylene interrupted 18:2 isomers, *cis*-9, *trans*-13, *cis*-9, *trans*-14 and *cis*-9, *trans*-12 18:2 were higher ($P<0.01$) in concentration in modified products (Table 6). There was an effect of period for *cis*-9, *trans*-13 which was more abundant ($P<0.05$) in P1 products (Table 6).

DISCUSSION

One of the main challenges of food chain interventions is to maintain a supply of the test food that is consistently different to the control product over the period of the study. This study produced greater volumes of modified dairy products over a longer period of time than other published studies (Livingstone et al., 2012). It has been reported previously that the milk FA profile resulting from supplementation with oilseeds can be affected by the chemical composition of other dietary constituents such as starch, or changes in DM intake (Lerch et al., 2012a). Therefore, it is important to demonstrate consistency for the purposes of both controlled human dietary intervention studies, and also for future commercial application and consumer consumption.

Overall there was little difference in macronutrient composition of both the control and modified milk and dairy products, and this was consistent over the two production periods. Feeding unsaturated oils to dairy cows often suppresses milk fat concentration (Halmemies-

Beauchet-Filleau et al., 2011), mainly due to the inhibitory effect of intermediates of rumen biohydrogenation on mammary FA synthesis (Bauman et al., 2011). In the current study, modified cheese was numerically lower (- 5.6 g/100 g) in fat content than the control cheese due to the raw modified milk being lower in total fat. It was necessary to standardise the raw conventional milk prior to UHT so that the fat content matched that of the modified milk for the purposes of the human intervention study. A study reporting the fat content of Mozzarella cheese made from milk where cows were fed incremental amounts of a linseed supplement reported no linear effect on cheese lipid content (Oeffner et al., 2013). However this study fed an extruded linseed supplement, which may have afforded the constituent oil some degree of protection from rumen biohydrogenation. This suggests that a more suitable approach for production of modified milk on a commercial scale may be to use oilseed supplements which are protected from rumen biohydrogenation.

Despite being produced across two production period over the course of two years, there was no effect of production period on differences between the main FA and groups in modified and control products, even with minor differences in FA profile of the supplemental oil fed to cows during the two production periods. Decreases in milk fat concentrations of de novo-synthesised SFA and increases in 18:0 and *cis*-9 18:1 following oilseed supplementation were found to be comparable over two consecutive lactations, but changes in most *trans*-18:1 and 18:2 isomers varied depending on year (Lerch et al., 2012b). This was probably due to differences in starch contents of the experimental diets across the two years (Lerch et al., 2012a). Transient changes in concentration of certain CLA and *trans*-18:1 isomers have been observed in response to oilseed supplementation over shorter periods of time (Roy et al., 2006). However the current study employed a dietary adaptation period of 4 weeks prior to milk collection, which should

have minimised any variation and successfully produced consistent modified milk essential for effective utilisation in the RESET human intervention study.

There were differences in most individual FA concentrations between conventional and modified products. All short (4:0-10:0) and medium (12:0-16:0) chain SFA were lower in modified products, which was balanced by higher 18:0, *cis*-9 18:1 and intermediates of biohydrogenation concentrations which supports previous studies (Loor et al., 2002; Kliem et al., 2011). A meta-analysis of recent data reported that the majority of the milk SFA responses to plant oil supplements in dairy cow diets is due to decreases in 12:0, 14:0 and 16:0 concentrations (Kliem & Shingfield, 2016). This can mainly be attributed to increases in the supply of ≥ 16 carbon FA leaving the rumen and inhibiting acetyl CoA carboxylase transcription and activity in the mammary gland (Barber et al., 1997).

SFA in modified milk were mostly replaced with *cis*-9 18:1, which was the predominant FA in the oil supplement. The current study aimed to feed 1 kg oil/cow/day, which equated to around 800 g additional *cis*-9 18:1. In this unprotected oil form it would be expected that some rumen biohydrogenation of *cis*-9 18:1 would occur as has been reported in detailed *in vivo* studies (e.g. Loor et al., 2002), however there was a 10 g/100 g FA difference in *cis*-9 18:1 concentration between conventional and modified products in the current study, in line with previous studies (Kliem et al., 2011). Milk fat *cis*-9 18:1 is derived from two sources – diet and endogenous desaturation of 18:0 by mammary epithelial cell Δ^9-desaturase. One *in vivo* study estimated that this enzyme was responsible for almost 60 % of *cis*-9 18:1 present in milk fat (Mosley & McGuire, 2007). Dairy products from the current study contained a higher concentration of 18:0 than control products, which may have contributed to the increase in milk fat *cis*-9 18:1.

A proportion of dairy SFA was also replaced by TFA, which are intermediates of rumen biohydrogenation of dietary unsaturated FA (Harfoot & Hazlewood, 1997). The majority of TFA identified in the current study were *trans* 18:1 isomers. *In vitro* studies have established that *cis*-9 18:1 is converted to a range of *trans* 18:1 isomers during incubation with rumen-derived microorganisms (McKain et al., 2010). Furthermore, *trans*-9 18:1 has been shown to further isomerise during *in vitro* incubation with rumen bacteria to a range of positional *trans* 18:1 isomers (Proell et al., 2002). The predominant isomer in the conventional dairy products was *trans*-11 18:1, but in the modified products, *trans*-10 18:1 predominated. *Trans*-10 18:1 is thought to arise as an intermediate of 18:2 n-6 biohydrogenation in response to changed rumen conditions on certain diets (Bauman et al., 2011), such as those which lower rumen pH (Palmquist et al., 2005). A recent review concluded that the predominance of *trans*-10 18:1 in ruminant products is more common than previously thought (Aldai et al., 2013), however very few milk-based studies have resulted in concentrations of *trans*-10 18:1 of the level observed in the current study. Roy et al. (2006) and Shingfield et al. (2005) both reported relatively high concentrations of trans-10 18:1 in milk fat after feeding oilseed supplements (18 and 13 g/100 g total FA, respectively), with suggested possible reasons being increased supply of 18:2 n-6 and low rumen pH. In the current study, 18:2 n-6 content of the oil was low, so the high *trans*-10 18:1 content of modified milk would have been primarily due to isomerisation of *cis*-9 18:1 within the rumen. This was suggested after a similar effect was observed when olive oil (high in *cis*-9 18:1) was fed to dairy sheep (Gomez-Cortes et al., 2008). *Trans*-10 18:1 has often been thought partially responsible for milk fat depression, but studies involving abomasally-infused *trans*-10 18:1 have reported inconsistent results (Lock et al., 2006; Shingfield et al., 2009)

Enriched concentrations of CLA in milk are usually due to an increased supply of 18:2 n-6 or 18:3 n-3 to the rumen, biohydrogenation of which results in increased *trans*-11 18:1 available

to mammary Δ^9 desaturase and therefore increased *cis*-9, *trans*-11 CLA, the predominant isomer (Palmquist et al., 2005). In the current study modified products had a higher overall CLA concentration than conventional products, but there was no difference in *trans*-11 18:1. Either desaturation of *trans*-11 18:1 was extremely efficient, or the increase in CLA concentration observed was due to increases in other CLA isomers.

The observed difference in SFA and MUFA content between control and modified products is comparable to that of previous studies (Livingstone et al., 2012). However few previous studies reported a detailed FA profile. The studies of Tholstrup et al. (2006) and Lacroix et al. (2012) were specifically designed to observe the effects of increased ruminant-derived TFA, and as such the modified products contained enriched concentrations of *trans*-11 18:1 in particular. The current study reported a greater concentration of *trans*-10 18:1 in modified products and the potential for these TFA to impact on CVD risk factors remains to be investigated.

CONCLUSIONS

In conclusion, feeding a specially formulated diet containing a high oleic acid sunflower oil to dairy cows over two production periods resulted in dairy products with a consistent FA profile, lower in SFA (including 12:0, 14:0 and 16:0), and higher in *cis*- and *trans*-MUFA (particularly *cis*-9 18:1 and *trans*-10 18:1) than conventionally-produced dairy products. Processing the modified milk into UHT milk, butter and Cheddar cheese had minor effects on FA profile. This technique is therefore suitable for the production of modified dairy products, with a consistent FA profile, which are suitable for use in large-scale intervention studies, where products with consistent composition are required over a longer period of time. This technique is also suitable for production of modified dairy foods on a commercial scale.

ACKNOWLEDGEMENTS

This study was funded by the UK Medical Research Council (MRC project number MR/K020218/1), in collaboration with Arla Foods UK. The authors gratefully acknowledge AAK (UK) Ltd. for providing the high-oleic sunflower oil in-kind. The authors would like to acknowledge the assistance of Miss J. Guo and Mrs R. Morgan during preparation of samples for analysis, staff at the Centre for Dairy Research for care of the animals, Mrs Val Bines for her expert input into the cheese manufacturing and Mr Chris Bussey for milk processing. The authors declare no conflicts of interest.

REFERENCES

Aldai, N., M. de Renobales, L. J. R. Barron and J. K. G. Kramer. 2013. Highlight Article summary: What are the trans fatty acids issues in foods after discontinuation of industrially produced trans fats? Ruminant products, vegetable oils, and synthetic supplements. Eur. J. Lipid Sci. Technol. 115:1378-1401.

Barber, M. C., R. A. Clegg, M. T. Travers and R. G. Vernon. 1997. Lipid metabolism in the lactating mammary gland. Biochim. Biophys. Acta 1347:101 - 126.

Bates, B., L. Cox, S. Nicholson, P. Page, A. Prentice, T. Steer and G. Swan. 2016. National Diet and Nutrition Survey' Results from years 5 and 6 (combined) of the rolling programme (2012/2013 - 2013/2014). Public Health England, London, UK.

Bauman, D. E., K. J. Harvatine and A. L. Lock. 2011. Nutrigenomics, rumen-derived bioactive fatty acids, and the regulation of milk fat synthesis. Annu. Rev. Nutr. 31:299-319.

Brown, R. H. and I. Mueller-Harvey. 1999. Evaluation of the novel Soxflo technique for rapid extraction of crude fat in foods and animal feeds. J. AOAC Int. 82:1369-1374.

DEFRA 2013. Family Food Survey. https://www.gov.uk/government/statistical datasets/family-food-datasets. Accessed September 2016.

Department of Health 1994. Nutritional Aspects of Cardiovascular Disease. Report on Health and Social Subjects no. 46. H. M. Stationery Office, London, UK.

Gomez-Cortes, P., P. Frutos, A. R. Mantecon, M. Juarez, M. A. de la Fuente and G. Hervas. 2008. Addition of olive oil to dairy ewe diets: effect on milk fatty acid profile and animal performance. J. Dairy Sci. 91:3119-3127.

Halmemies-Beauchet-Filleau, A., T. Kokkonen, A. M. Lampi, V. Toivonen, K. J. Shingfield and A. Vanhatalo. 2011. Effect of plant oils and camelina expeller on milk fatty acid composition in lactating cows fed diets based on red clover silage. J. Dairy Sci. 94:4413-4430.

Harfoot, C. G. and G. P. Hazlewood. 1997. Lipid metabolism in the rumen. Pages 382-426 in The Rumen Microbial Ecosystem. P. N. Hobson and C. S. Stewart, ed. Blackie Academic & Professional, London, UK.

Henderson, L., J. Gregory, K. Irving and G. Swan. 2003. The National Diet and Nutrition Survey: Adults aged 19-64 years vol 2. Energy, Protein, Carbohydrate, Fat and Alcohol Intake. London (UK): TSO.

Kliem, K. E., K. J. Shingfield, D. J. Humphries and D. I. Givens. 2011. Effect of replacing calcium salts of palm oil distillate with incremental amounts of conventional or high oleic acid milled rapeseed on milk fatty acid composition in cows fed maize silage-based diets. Animal, 5:1311-1321.

Kliem, K. E., K. J. Shingfield, K. M. Livingstone and D. I. Givens. 2013. Seasonal variation in the fatty acid composition of milk available at retail in the United Kingdom and implications for dietary intake. Food Chem. 141:274-281.

Kliem, K. E. and K. J. Shingfield. 2016. Manipulation of milk fatty acid composition in lactating cows: Opportunities and challenges. Eur. J. Lipid Sci. Technol. doi:10.1002/ejlt.201400543.

Lacroix, E., A. Charest, A. Cyr, L. Baril-Gravel, Y. Lebeuf, P. Paquin, P. Y. Chouinard, P. Couture and B. Lamarche. 2012. Randomized controlled study of the effect of a butter naturally enriched in *trans* fatty acids on blood lipids in healthy women. Am. J. Clin. Nutr. 95:318-325.

Lerch, S., A. Ferlay, D. Pomiès, B. Martin, J. A. Pires and Y. Chilliard. 2012a. Rapeseed or linseed supplements in grass-based diets: effects on dairy performance of Holstein cows over 2 consecutive lactations. J. Dairy Sci. 95:1956-1970.

Lerch, S., A. Ferlay, K. J. Shingfield, B. Martin, D. Pomiès and Y. Chilliard, Y. 2012b. Rapeseed or linseed supplements in grass-based diets: Effects on milk fatty acid composition of Holstein cows over two consecutive lactations. J. Dairy Sci. 95:5221-5241.

Livingstone, K. M., J. A. Lovegrove and D. I. Givens. 2012. The impact of substituting SFA in dairy products with MUFA or PUFA on CVD risk: evidence from human intervention studies. Nutr. Res. Rev. 25:193-206.

Lock, A. L., C. Tyburczy, D. A. Dwyer, K. J. Harvatine, F. Destaillats, Z. Mouloungui and D. E. Bauman. 2006. *Trans*-10 octadecenoic acid does not reduce milk fat synthesis in dairy cows. J. Nutr. 137:71-76.

Loor, J. J., J. H. Herbein and T. C. Jenkins. 2002. Nutrient digestion, biohydrogenation and fatty acid profiles in blood plasma and milk fat from lactating holstein cows fed canola oil or canolamide. Anim. Feed Sci. Technol. 97:65 - 82.

Maubois, J. L. and D. Lorient. 2016. Dairy proteins and soy proteins in infant foods nitrogen-to-protein conversion factors. Dairy Sci. Technol. 96:15-25.

McKain, N., K. J. Shingfield and R. J. Wallace. 2010. Metabolism of conjugated linoleic acids and 18:1 fatty acids by ruminal bacteria: products and mechanisms. Microbiology 156:579-588.

Mosley, E. E., and M. A. McGuire. 2007. Methodology for the in vivo measurement of the Δ^9-desaturation of myristic, palmitic and stearic acids in lactating dairy cattle. Lipids 42: 939-945.

Noakes, M., P. J. Nestel and P. M. Clifton. 1996. Modifying the fatty acid profile of dairy products through feedlot technology lowers the plasma cholesterol of humans consuming the prodicts. Am. J. Clin. Nutr. 63:42-46.

Oeffner, S. P., Y. Qu, J. Just, N. Quezada, E. Ramsing, M. Keller, G. Cherian, L. Goddick and G. Bobe. 2013. Effect of flaxseed supplementation rate and processing on the production, fatty acid profile, and texture of milk, butter, and cheese. J. Dairy Sci. 96:1177-1188.

Palmquist, D. L., A. L. Lock, K. J. Shingfield and D. E. Bauman. 2005. Biosynthesis of conjugated linoleic acid in ruminants and humans. Adv. Food Nutr. Res. 50:179-217.

Proell, J. M., E. E. Mosley, G. Powell and T. C. Jenkins. 2002. Isomerization of stable isotopically labelled elaidic acid to *cis* and *trans* monoenes by ruminal microbes. J. Lipid Res. 43:2072-2076.

Roy, A., A. Ferlay, K. J. Shingfield and Y. Chilliard. 2006. Examination of the persistency of milk fatty acid composition responses to plant oils in cows given different basal diets, with particular emphasis on *trans*-$C_{18:1}$ fatty acids and isomers of conjugated linoleic acid. Anim. Sci. 82:479-492.

SACN 2007. Scientific Advisory Committee on Nutrition. Update on *trans* fatty acids and health. London (UK): TSO; 2007. 49.

Shingfield, K. J., C. K. Reynolds, B. Lupoli, V. Toivonen, P. Delmonte, J. M. Griinari, A. S. Grandison and D. E. Beever. 2005. Effect of forage type and proportion of concentrate in the diet on milk fatty acid composition in cows given sunflower oil and fish oil. Anim. Sci. 80:225-238.

Shingfield, K. J., C. K. Reynolds, G. Hervas, J. M. Griinari, A. S. Grandison and D. E. Beever. 2006. Examination of the persistency of milk fatty acid composition responses to fish oil and sunflower oil in the diet of dairy cows. J. Dairy Sci. 89:714-732.

Shingfield, K. J., S. Ahvenjärvi, V. Toivonen, A. Vanhatalo, P. Huhtanen and J. M. Griinari. 2008. Effect of incremental levels of sunflower-seed oil in the diet on ruminal lipid metabolism in lactating cows. Br. J. Nutr. 99:971-983.

Shingfield, K. J., A. Saebo, P. C. Saebo, V. Toivonen and J. M. Griinari. 2009. Effect of abomasal infusions of a mixture of octadecenoic acids on milk fat synthesis in lactating cows. J. Dairy Sci. 92:4317-4329.

Seidel C. T. Deufel and G. Jahreis. 2005. Effects of fat-modified dairy products on blood lipids in humans in comparison with other fats. Ann. Nutr. Metab. 49:42-48.

Sukhija, P. S. and D. L. Palmquist. 1988. Rapid determination of total fatty acid content and composition of feedstuffs and faeces. J. Agric. Food Chem. 36:1202-1206.

Tholstrup, T., M. Raff. S. Basu, P. Nonboe, K. Sejrsen and E. M. Straarup. 2006. Effects of butter high in ruminant *trans* and monounsaturated fatty acids on lipoproteins, incorporation of fatty acids into lipid classes, plasma C-reactive protein, oxidative stress, hemostatic variables, and insulin in healthy young men. Am. J. Clin. Nutr. 83:237-243.

Ulberth, F., R. G. Gabernig and F. Schrammel. 1999. Flame-ionization detector response to methyl, ethyl, propyl and butyl esters of fatty acids. J. Am. Oil Chem. Soc. 76:263-266.

Vafeiadou, K., M. Weech, H. Altowaijri, S. Todd, P. Yaqoob, K. G. Jackson and J. A. Lovegrove. 2015. Replacement of saturated with unsaturated fats had no impact on vascular function but beneficial effects on lipid biomarkers, E-selectin, and blood pressure: results from the randomized, controlled Dietary Intervention and VAScular function (DIVAS) study. Am. J. Clin. Nutr. 102:40-48.

WHO. (2010). FAO, Fats and Fatty Acids in Human Nutrition. Report of an Expert Consultation. Food and Agriculture Organisation of the United Nations. Rome, Italy.

Table 1. Ingredients and analysed chemical composition of the cow diet used for each production batch (g/kg dry matter (DM) or as stated)

	g/kg DM
Ingredients	
Maize silage	350
Grass silage	52
Grass hay	33
Straw	33
Wheat by-product[1]	86
Concentrate mix[2]	372
Calcium salts of palm oil distillate[3]	11
Salt[4]	4
Limestone	4
Minerals and vitamins	11
High oleic acid sunflower oil[5]	43
Chemical composition	
DM (g/kg fresh)	515
Organic matter	932
Crude protein	144
Neutral detergent fibre	351
Acid detergent fibre	199
Starch	212
ME (MJ/kg DM)[6]	12.6
Fatty acids	
16:0	2.9
18:0	0.9
18:1 *cis*-9	14.6
18:2 n-6	6.1
18:3 n-3	0.05
Total fatty acids	30.0

[1]CTraffordgold®; KW Alternative Feeds Ltd., Barrow Hill Barns, Andover, SP11 7RG, UK

[2] Containing (g/kg DM): Cracked wheat, 180; Soyabean meal 160; Rapeseed meal, 175; Sugar beet feed, 140; Wheat distillers, 140; Soya hulls, 120; Molasses, 33; Megalac®, 17; Urea, 11; Minerals (KW Alternative Feeds Ltd., Barrow Hill Barns, Andover, SP11 7RG, UK), 22).

[3] Megalac®; Volac International Ltd., Royston, Hertfordshire, SG8 5QX, UK

[4] Dairy Direct, Church Farm, Bury St Edmunds, IP28 6PX, UK.

[5]AAK (UK) Ltd., Hull, East Yorkshire, HU9 5PX, UK.

Table 2. Fatty acid composition of the high oleic acid sunflower oil used during the two production periods (g/100 g total fatty acids)

Fatty acid	*Production period 1*	*Production period 2*
16:0	3.6	4.1
18:0	3.1	2.7
18:1 *cis*-9	82.1	80.4
18:2 n-6	7.5	10.1
18:3 n-3	0.26	0.27

Table 3. Macronutrient composition of the modified and conventional dairy products (average across production periods 1 and 2; units as stated, least square means ± s.e.m.)

	UHT milk			Butter			Cheddar cheese		
	Conventional	Modified	s.e.m.	Conventional	Modified	s.e.m.	Conventional	Modified	s.e.m.
Energy (kJ/100 g)	220	232	1.4	3037	3024	22.1	1688	1527	10.1
Energy (kcal/100 g)	52.5	55.4	0.33	739	735	5.4	407	368	2.4
Total carbohydrate (g/100 g)	4.4	4.7	0.02	1.93	0.98	0.108	3.1	2.6	0.24
Ash (g/100 g)	0.72	0.77	0.010	1.8	1.6	0.08	4.0	3.9	0.50
Moisture (g/100 g)	88.8	88.3	0.01	14.9	16.0	0.46	36.2	39.2	0.50
Nitrogen (g/100 g)	0.47	0.51	0.02	0.04	0.07	0.007	3.7	4.2	0.07
Protein (g/100 g)[1]	3.0	3.3	0.03	0.23	0.44	0.041	23.1	26.4	0.42
Fat (g/100 g)	2.5	2.6	0.14	81.1	81.1	0.63	33.6	28.0	0.19

Calcium (mg/100 g dry)	1147	1090	28.8	19.1	20.6	2.78	1242	1428	105.0
(mg/100 g fresh)	126	119	7.1	17.0	17.9	2.32	801	911	58.4
Magnesium (mg/100 g dry)	105	101	0.1	2.1	2.1	0.24	46.6	47.4	1.65
(mg/100 g fresh)	11.5	11.1	0.35	1.9	1.8	0.20	30.1	30.3	0.80
Sodium (mg/100 g dry)	378	417	22.7	795	577	87.7	1123	1187	82.2
(mg/100 g fresh)	41.4	45.5	1.19	707	502	73.8	726	760	46.4
Phosphorus (mg/100 g dry)	896	819	30.0	25.4	27.0	3.68	836	939	57.0
(mg/100 g fresh)	98.1	89.2	6.31	22.6	23.5	3.11	539	599	30.7

[1]Calculated by nitrogen conversion factor 6.38 (Maubois & Lorient, 2016)

Table 4. Mean fatty acid composition of the lipid from all modified and conventional dairy products over two production periods (g/100 g total fatty acids, least squares means for milk, butter and cheese ± s.e.m)

	Period 1		Period 2		s.e.m.[1]	P[2]		
	Conventional	Modified	Conventional	Modified		Production period	Treatment	Interaction
4:0	2.9	2.2	2.9	2.1	0.06	0.668	<0.001	0.124
6:0	1.8	1.1	1.9	1.0	0.04	0.732	<0.001	0.057
8:0	1.11	0.62	1.14	0.54	0.030	0.420	<0.001	0.088
10:0	2.7	1.5	2.8	1.2	0.07	0.056	<0.001	0.033
10:1 *cis*-9	0.28	0.13	0.29	0.13	0.011	0.727	<0.001	0.547
11:0	0.053	0.004	0.025	0.002	0.0138	0.329	0.031	0.375
12:0	3.5	2.0	3.5	1.6	0.13	0.194	<0.001	0.133
12:1 *cis*-9	0.10	0.05	0.10	0.05	0.005	0.893	<0.001	0.453
13:0	0.10	0.05	0.09	0.05	0.010	0.601	0.003	0.859
13:0 anteiso	0.09	0.06	0.09	0.05	0.004	0.618	<0.001	0.418
14:0	11.3	8.5	11.4	7.4	0.30	0.188	<0.001	0.077
14:0 iso	0.08	0.07	0.09	0.07	0.005	0.669	0.008	0.842

14:1 *cis*-9	1.02	0.95	1.04	1.01	0.041	0.366	0.231	0.663
14:1 *trans*-9	0.22	0.16	0.24	0.17	0.010	0.210	<0.001	0.686
15:0	1.10	0.71	1.14	0.71	0.038	0.613	<0.001	0.585
15:0 anteiso	0.43	0.38	0.42	0.35	0.008	0.021	<0.001	0.406
16:0	33.3	20.9	33.8	22.2	0.69	0.220	<0.001	0.602
16:0 iso	0.20	0.19	0.20	0.20	0.010	0.650	0.721	0.629
16:1 *cis*-9 + 17:0 anteiso	1.7	1.6	1.5	1.5	0.07	0.050	0.596	0.340
16:1 *cis*-11	0.21	0.13	0.42	0.40	0.065	0.007	0.516	0.651
16:1 *cis*-13	0.15	0.05	0.17	0.05	0.011	0.412	<0.001	0.417
16:1 *trans*-9	0.03	0.10	0.10	0.05	0.101	0.377	0.001	0.640
17:0 iso	0.33	0.36	0.29	0.31	0.012	0.008	0.073	0.588
17:0	0.48	0.36	0.49	0.33	0.005	0.090	<0.001	0.013
17:1 *cis*-9	0.20	0.16	0.20	0.15	0.003	0.333	<0.001	0.089
18:0	9.6	14.0	9.4	13.0	0.32	0.089	<0.001	0.217
18:0 iso	0.03	0.02	0.06	0.03	0.008	0.026	0.019	0.026
∑ 18:1 *trans*	2.8	9.4	2.6	10.2	0.30	0.348	<0.001	0.130
∑ 18:1 *cis*	20.0	29.6	19.3	30.3	0.74	0.994	<0.001	0.407

∑ CLA[3]	0.57	0.71	0.59	0.98	0.065	0.195	0.001	0.025
∑ NMI[4] 18:2	2.1	2.3	2.1	2.5	0.17	0.605	0.084	0.857
18:3 n-3	0.32	0.23	0.40	0.30	0.032	0.060	0.020	0.872
19:0[5]	0.10	0.10	0.08	0.08	0.016	0.326	0.970	0.966
20:0	0.15	0.15	0.14	0.13	0.003	0.009	0.063	0.118
20:1 *cis*-9	0.10	0.10	0.09	0.08	0.009	0.130	0.604	0.394
20:1 *cis*-11	0.09	0.09	0.02	0.01	0.045	0.108	0.802	0.883
20:2 n-6	0.005	0.008	0.012	0.005	0.0066	0.771	0.719	0.447
20:3 n-6	0.06	0.07	0.07	0.06	0.008	0.634	0.255	0.918
20:4 n-6	0.10	0.07	0.10	0.06	0.007	0.625	0.001	0.300
20:5 n-3	0.05	0.01	0.05	0.02	0.008	0.488	0.002	0.321
22:0	0.05	0.08	0.05	0.08	0.007	0.851	0.003	0.370
22:2 n-6	0.020	0.006	0.034	0.003	0.0119	0.727	0.082	0.554
22:5 n-3	0.08	0.04	0.08	0.04	0.009	0.582	0.002	0.717
24:0	0.02	0.03	0.04	0.04	0.008	0.189	0.478	0.531
∑ SFA[6]	70.3	54.3	70.9	52.2	0.97	0.479	<0.001	0.204
∑ *cis*-MUFA[7]	23.0	32.2	22.5	32.9	0.67	0.932	<0.001	0.372

∑ *trans*-MUFA[7]	3.3	10.0	3.2	10.8	0.29	0.268	<0.001	0.125
∑ *trans* fatty acids	4.0	10.7	3.8	11.8	0.31	0.208	<0.001	0.082
∑ n-3 PUFA[8]	0.63	0.48	0.72	0.59	0.054	0.096	0.033	0.791
∑ n-6 PUFA[8]	1.7	1.9	1.9	2.0	0.18	0.547	0.578	0.865

[1] Standard error of the mean for n=12 measurements

[2] Refers to the significance of overall effect of period, treatment and their interaction

[3] CLA – conjugated linoleic acid

[4] NMI - non methylene-interrupted

[5] Co-elutes with 18:1 *cis*-15

[6] SFA – saturated fatty acids

[7] MUFA – monounsaturated fatty acids

[8] PUFA – polyunsaturated fatty acids

Table 5. Mean 18:1 isomer composition of the lipid from all modified and conventional dairy products over two production periods (g/100 g total fatty acids, least squares means for milk, butter and cheese ± s.e.m)

	Period 1		Period 2		s.e.m.[1]	P[2]		
	Conventional	Modified	Conventional	Modified		Production period	Treatment	Interaction
cis-9 18:1[3]	18.8	28.2	18.4	28.9	0.72	0.859	<0.001	0.439
cis-11 18:1	0.61	0.81	0.44	0.70	0.048	0.023	0.001	0.522
cis-12 18:1	0.22	0.16	0.21	0.19	0.016	0.467	0.042	0.318
cis-13 18:1	0.09	0.16	0.08	0.11	0.017	0.103	0.017	0.364
cis-16 18:1	0.06	0.06	0.05	0.04	0.005	0.091	0.454	0.294
trans-6, -7, -8 18:1	0.24	1.28	0.24	1.25	0.290	0.955	0.008	0.961
trans-9 18:1	0.20	0.94	0.18	1.39	0.119	0.102	<0.001	0.082
trans-10 18:1	0.36	4.01	0.41	2.49	0.413	0.113	<0.001	0.095
trans-11 18:1	1.10	0.94	0.85	1.81	0.428	0.490	0.375	0.227
trans-12 18:1	0.38	1.01	0.32	1.09	0.065	0.816	<0.001	0.310
trans-15 18:1	0.40	0.94	0.47	1.82	0.231	0.074	0.004	0.119
trans-16 18:1[4]	0.31	0.35	0.31	0.48	0.024	0.023	0.003	0.026

[1] Standard error of the mean for n=12 measurements
[2] Refers to the significance of overall effect of period, treatment and their interaction
[3] Co-elutes with 18:1 *trans*-13/14
[4] Co-elutes with 18:1 *cis*-14.

Table 6. Mean non-methylene-interrupted 18:2 isomer composition of the lipid from all modified and conventional dairy products over two production periods (all values as mg/100 g total fatty acids, least square means for milk, butter and cheese ± s.e.m.)

	Period 1		Period 2		s.e.m.[1]	P[2]		
	Conventional	Modified	Conventional	Modified		Production period	Treatment	Interaction
cis-9, *trans*-13 18:2	182	238	177	334	11.4	0.004	<0.001	0.002
cis-10, *trans*-14 18:2	122.6	122.6	100.0	92.1	9.12	0.020	0.679	0.680
cis-9, *trans*-14 18:2	80.4	100.8	75.4	107.9	5.83	0.861	0.002	0.330
cis-9, *trans*-12 18:2	33.7	50.4	26.9	54.0	3.78	0.697	<0.001	0.206
trans-9, *cis*-12 18:2	7.5	8.2	46.0	15.5	10.80	0.067	0.205	0.187
trans-11, *cis*-15 18:2	122.5	105.5	66.7	134.4	23.56	0.585	0.312	0.110
cis-9, *cis*-12 18:2	1449	1629	1563	1686	141.3	0.561	0.316	0.845

[1] Standard error of the mean for n=12 measurements

[2] Refers to the significance of overall effect of period, treatment and their interaction