The impact of substituting SFA in dairy products with MUFA or PUFA on CVD risk: evidence from human intervention studies

Katherine M. Livingstone[1]*, Julie A. Lovegrove[2] and D. Ian Givens[1]

[1]*Food Production and Quality Research Division, School of Agriculture, Policy and Development, Faculty of Life Sciences, University of Reading, Reading RG6 6AR, UK*
[2]*Department of Food and Nutritional Sciences and Institute for Cardiovascular and Metabolic Research (ICMR), School of Chemistry, Food and Pharmacy, Faculty of Life Sciences, The University of Reading, Reading RG6 6AR, UK*

Abstract

With the substantial economic and social burden of CVD, the need to modify diet and lifestyle factors to reduce risk has become increasingly important. Milk and dairy products, being one of the main contributors to SFA intake in the UK, are a potential target for dietary SFA reduction. Supplementation of the dairy cow's diet with a source of MUFA or PUFA may have beneficial effects on consumers' CVD risk by partially replacing milk SFA, thus reducing entry of SFA into the food chain. A total of nine chronic human intervention studies have used dairy products, modified through bovine feeding, to establish their effect on CVD risk markers. Of these studies, the majority utilised modified butter as their primary test product and used changes in blood cholesterol concentrations as their main risk marker. Of the eight studies that measured blood cholesterol, four reported a significant reduction in total and LDL-cholesterol (LDL-C) following chronic consumption of modified milk and dairy products. Data from one study suggested that a significant reduction in LDL-C could be achieved in both the healthy and hypercholesterolaemic population. Thus, evidence from these studies suggests that consumption of milk and dairy products with modified fatty acid composition, compared with milk and dairy products of typical milk fat composition, may be beneficial to CVD risk in healthy and hypercholesterolaemic individuals. However, current evidence is insufficient and further work is needed to investigate the complex role of milk and cheese in CVD risk and explore the use of novel markers of CVD risk.

Key words: SFA: Milk: Dairy products: CVD

Introduction

Milk and dairy products are important sources of essential micronutrients, including Ca, riboflavin and vitamin B_{12}, yet being major contributors to SFA intake, have been investigated for their role in the development of chronic diseases such as CVD, obesity and the metabolic syndrome[1]. The proportion of SFA entering the food chain from milk and dairy products is typically controlled by industrial skimming, which reduces total milk fat. Alternatively, research has shown that alteration of the diet of the dairy cow, by feeding a diet high in MUFA and/or PUFA, can lead to partial replacement of milk SFA with these unsaturated fatty acids (UFA)[2]. Critically, this strategy increases proportions of potentially cardioprotective MUFA and PUFA, while simultaneously reducing proportions of SFA. The present review will examine the limited number of human dietary intervention studies that have investigated the effects of these milk and dairy products that have been modified through alteration of the dairy cow's diet on cardiovascular risk markers.

Burden of CVD

CVD, encompassing CHD, stroke and peripheral vascular diseases, is responsible for almost half of all premature deaths in Europe (Table 1)[3]. According to the latest report by the British Heart Foundation, CVD also remains the biggest killer in the UK, responsible for one in three deaths[4]. CHD is itself the primary cause of mortality, and in 2008 accounted for one in five male and one in eight female mortalities in the UK. Evidence suggests that in Western Europe the number of mortalities from CVD is falling, whereas in Eastern Europe, mortality rates rose by as much as 7 % between 1996 and 2006[3]. Estimates suggest that a total of 7·5 million individuals in the UK are living with CVD (2·7 million with CHD, 2·1 million with angina and 1·5 million having had a myocardial infarction)[4]. As shown in Table 1, morbidity rates in the UK are also substantial, and have led to the UK having one of the highest expenditures on CVD in Europe (12 % of total healthcare costs, compared with the European average of 10 %).

Table 1. Estimated number of mortalities and cost of CHD, stroke and other vascular diseases in the UK and Europe per year*

	Mortality		Cost†			
			UK		European Union	
	UK	Europe	Direct‡	Indirect§	Direct	Indirect
CHD	88 000	1·9 M	3·2 M	5·8 M	23·5 B	25·5 B
Stroke	43 000	1·2 M	3·2 M	5·1 M	18·6 B	19·4 B
Other vascular diseases	60 000	1·2 M	8·0 M	5·4 M	105 B	
CVD total	191 000	4·3 M	30·7 M		192 B	

M, million; B, billion.
* Calculated from data provided by Allender *et al.* [3] and the British Heart Foundation[4].
† Cost in pounds sterling for the UK and euros for the European Union.
‡ Estimated direct cost of hospital care.
§ Estimated indirect cost of productivity losses as a result of CVD.

With the growing burden of CVD on the population and the economy, there is mounting pressure to reduce risk factors for the development of CVD. One of the main modifiable contributors to CVD risk, in addition to smoking, exercise, alcohol consumption and other dietary components, is a high consumption of saturated and *trans*-fat.

Saturated fat consumption

Population trends for fat consumption in the UK have changed considerably over the past century. Between 1900 and 1930, fat consumption represented 30 % total energy, after which it rose to a plateau of 40 % in the mid 1970s, following a dip during the Second World War[5]. The National Diet and Nutrition Survey from 2000/01[6] reported that the percentage of total energy from fat was 33·5 % for men and women, while the most recent results from the rolling programme for 2008/10[7] suggest that UK fat consumption is close to the target (Table 2) at 33·0 % total energy for men and 34·1 % for women. With regards to SFA intake, the National Diet and Nutrition Survey reported that the proportion of energy from SFA in 2000 was 13·4 % for men and 13·2 % for women, while between 2008 and 2010 this fell to 12·9 % total energy for men and 12·6 % for women. However, as indicated in Table 2, the proportion of energy derived from SFA is in excess of both UK and worldwide recommendations.

The potential for certain SFA to raise plasma cholesterol is well established[8,9] and more recent evidence links SFA with the production of inflammatory markers[10,11] and an impaired endothelial function[12] although any link with insulin sensitivity remains uncertain[13,14]. Given these associations between dietary SFA consumption and CVD risk, this overconsumption needs to be addressed in order to combat the burden of CVD.

Dairy products as a source of saturated fat

Winter whole milk fatty acid (FA) composition (per 100 g FA) in the UK is typically about 72 g SFA, 25 g MUFA and

3 g PUFA (KE Kliem, personal communication); however, variation in dairy management and geographical location can have considerable effects on FA composition. For example, standard winter whole milk FA composition in the USA is 65 g SFA, 28 g MUFA and 7 g PUFA[15], while in Sweden milk FA composition is 69 g SFA, 28 g MUFA and 3 g PUFA[16].

Dairy products are thus a significant dietary source of SFA, estimated to contribute up to 40 % of the UK SFA intake, and up to 60 % in other European countries[17]. Moreover, these are likely to be underestimates, given that the contribution of milk and dairy products from composite foods was not included. The most recent data compiled on milk and dairy consumption in the UK indicates that, over the past decade, milk ingestion has declined by 15 % (1766 to 1556 ml/individual per week), while cheese and yoghurt consumption has increased by 10 and 34 %, respectively (103 to 111 g/individual per week and 149 to 202 ml/individual per week, respectively)[18]. With an increased number of fat-reduced, and even SFA-reduced, products now on offer, it is no surprise that consumption of semi-skimmed milk has increased by 0·4 % year-on-year during the past decade, while the most recent data (2008–2009) for skimmed milk show a 4·3 % increase in consumption[18].

Table 2. Dietary reference values for percentage contribution of fat and fatty acids to total energy intake

	Recommendations (% energy intake)		
Fat type	SACN*	WHO[77]	AHA[78]
Total fat	<33	20–35	25–35
SFA	<10	10	<7·0
MUFA	12	15–20	7·0–17
Total PUFA	6·0–10	6·0–11	10
n-3 PUFA			
Total	>0·2	0·5–2·0	1·1–1·6
EPA + DHA (mg/d)	450	250	250
n-6 PUFA	>1·0	2·5–9·0	–
Trans	<2·0	1·0	<1·0

SACN, Scientific Advisory Committee for Nutrition; AHA, American Heart Association.
* Based on data from the Department of Health[38].

These trends highlight the response to both government and consumer demands for products believed to be healthier.

Lowering saturated fat consumption

Despite the fat in dairy products being high in SFA, epidemiological evidence suggests that these foods may not be detrimental to cardiovascular health, although most of this evidence relates to milk[19]. Milk and dairy products have a high Ca, K and Mg content and thus have been associated with reductions in blood pressure[20,21]. Additionally, milk caseins and whey proteins (predominantly hydrolysates), sources of bioactive peptides, are becoming recognised as regulators of blood pressure in a small number of human studies[22]. Research has also suggested potential cardioprotective properties associated with conjugated linoleic acid consumption, which is unique to ruminant products[23]. Simply reducing dairy product consumption is thus not the ideal strategy to lower SFA ingestion as this may limit intake of these potentially cardioprotective agents. Instead, reducing the entry of SFA into the food chain through milk and dairy products may be achieved by altering the diet of the dairy cow to attain lower concentrations of SFA. This strategy involves partially replacing milk SFA with MUFA and/or PUFA.

Replacing SFA with MUFA and/or PUFA

Studies have shown that substituting dietary SFA with cis-MUFA and/or PUFA has beneficial effects on CVD risk factors[9,24]. Mensink et al.[9] showed that a 1% iso-energetic replacement of carbohydrates with SFA increased LDL-cholesterol (LDL-C) by 0·032 mmol/l, while replacement with either cis-MUFA or PUFA reduced LDL-C by 0·009 and 0·019 mmol/l, respectively. While this meta-analysis excluded long-chain n-3 PUFA, the beneficial effects of these FA on CVD risk markers such as inflammation, blood pressure and vascular function are well established[25]. A more recent systematic review and meta-analysis suggested that replacement of 9·9% of dietary energy from SFA with PUFA resulted in an overall pooled risk reduction of 19% (relative risk 0·81; 95% CI 0·70, 0·95; $P = 0.008$), corresponding to a 10% reduction in CHD risk (relative risk 0·90; 95% CI 0·83, 0·97) for each 5% energy of increased PUFA[26]. However, the basis for this evidence is limited to a small number of studies, where the total number of cardiovascular events was only 1042. Additionally, a recent symposium suggested that a replacement of 1% energy from SFA with PUFA lowered LDL-C, with a likely 2–3% reduction in incidence of CHD[27]. Nonetheless, this conclusion is based on the previously mentioned meta-analysis[26], in addition to a study that calculated risk using a spreadsheet model that included questionable assumptions on dietary behaviour[28].

A recent systematic review by Hooper et al.[24] attempted to amalgamate evidence from randomised clinical trials and concluded that, based on 4586 events, reducing SFA and/or modifying dietary fat type lowered the risk of cardiovascular events by 14% (relative risk 0·86; 95% CI 0·77, 0·96). Specifically, subgrouping identified that this protective effect was only seen in studies of at least 2 years' duration (the review included studies of at least 6 months' duration), in studies of men only, and in studies where individuals had moderate to high risk of CVD at baseline. Furthermore, this review could not specify whether replacing SFA with MUFA or PUFA was more beneficial.

In excess of 100 studies[29] have partially replaced milk SFA with cis-MUFA and PUFA through alteration of the cow's diet. A recent study indicated that including 49 g rapeseed oil/kg DM in the dairy cow's diet can reduce SFA from 70 to 55–60 g/100 g FA by replacing them primarily with cis-MUFA, which increased from 20 to 33 g/100 g FA[2]. However, as seen in these studies, this strategy leads to the production of naturally produced ruminant trans-FA (R-TFA), which may be a concern to human health.

Production of ruminant trans-fatty acids

Comprising 65% of the total stomach capacity and playing host to a vast microbial population of ciliate protozoa, anaerobic bacteria and anaerobic fungi, the rumen is the main site of microbial fermentation and fat metabolism in the cow[30]. Rumen microbes transform dietary FA (TAG, and phospho- and galactolipids) via two processes: lipolysis and biohydrogenation. Lipolysis involves the hydrolysis of lipid ester linkages to NEFA via either plant or bacterial lipase. The latter is the process whereby UFA are first iso-merised, then hydrogenated, thereby producing a number of conjugated linoleic acid isomers and trans-MUFA, also known as R-TFA[31].

The primary ruminal biohydrogenation (RBH) intermediate of both PUFA and MUFA is trans-11−18:1, which, along with ruminant-produced conjugated linoleic acids, have been investigated for their beneficial effects on a variety of diseases, including cancer and CVD[32,33]. The pathways of RBH are complex and are dependent on the composition of the diet; for a review of RBH pathways of PUFA and MUFA, see Shingfield et al.[32].

The inclusion of UFA in the diet of the dairy cow can thus lead to increased R-TFA in the milk. Nonetheless, despite the established detrimental effects of industrially produced TFA on CVD[34], the impact of R-TFA is inconclusive. Some evidence has shown a protective role of R-TFA in CVD[35], such as trans-7-16:1[36], while a recent review and meta-analysis by Bendsen et al.[37] concluded that although studies to date have found a null relationship between R-TFA and CHD, the evidence from these limited studies is not sufficient to clearly identify the role of R-TFA in CVD. Importantly, it is generally accepted that this null relationship is due to a lower intake of R-TFA compared with industrial TFA. Moreover, current UK TFA consumption is 0·8% of food energy[7], below the national

recommended population maximum of 2%[38], and at this level is not considered detrimental.

Scope of the review

Modification of the dairy cow's diet to partially replace milk SFA with MUFA and/or PUFA has been extensively studied[29]. Critically, very few human dietary intervention studies have examined the effect of these modified dairy products on CVD risk. The question of whether replacing dairy SFA with cis-MUFA or PUFA, through alteration of bovine feeding, is beneficial to cardiovascular health, is still unclear. The present review will summarise the data from the human intervention studies (Table 3) that have used milk and dairy products with modified FA composition achieved through alteration of the diet given to the cows. Two additional studies that used processing techniques to achieve these changes will not be discussed in depth in the present review[39,40]. All the studies reviewed used a supplemental source of either MUFA or PUFA in the cow diets and based the reduction in CVD risk primarily on plasma lipid concentrations. Following a critical appraisal of these studies, potential limitations and scope for future research will be highlighted.

Evidence from intervention studies

Heterogeneity of intervention studies

Of the nine studies in the present review, three supplemented the dairy cow's diet with a high proportion of MUFA[41–43] in order to modify milk FA composition, while three fed a source of n-3 PUFA[44–46], two fed a source of n-6 PUFA[33,47] and one failed to specify whether the 'unsaturated feed' was predominately MUFA or PUFA[48]. Of the three studies that supplemented with a source of n-3 PUFA, two fed these as part of a 'livestock' approach[45,46], which entailed also modifying the FA composition of a variety of animal products in addition to milk and dairy products. As indicated in Table 3, five of the nine studies used the modified milk to produce butter, while the remaining four produced a combination of butter and other milk and dairy products. The quality of the dietary data varies considerably, with studies omitting valuable information such as FA composition of the diets[43], while the robustness of methods employed is equally diverse. Changes in blood cholesterol and TAG concentrations were used as the primary measure of CVD risk in all studies, except for one[46], although the majority of these studies also measured additional risk markers including apolipoproteins, clotting factors and blood pressure.

Supplementation with a source of MUFA

The majority of the evidence in support of a beneficial impact of modified milk and dairy products on CVD risk

markers results from studies where a source of MUFA was used as a feed supplement – notably rapeseed oil. Supplementation with a source of MUFA, instead of n-3 or n-6 PUFA, is seen as the more sustainable option, with comparably less lipid peroxidation and RBH. Poppitt et al.[48] used butter-fat that had been modified by feeding an encapsulated UFA, and measured its effects on total cholesterol (TC), LDL-C and HDL-cholesterol (HDL-C), TAG, apoA and B, NEFA, haemostatic clotting factor VII, fibrinogen and glucose. This was a double-blinded, randomised, cross-over, intervention trial where volunteers were fed either a 'control' or 'modified' butter. Through feeding alone, the SFA content of the modified butter was reduced by 16·1% (70·5–54·4 g/100 g FA) and the MUFA and PUFA content was increased by 9·9% (22·1–32·0 g/100 g FA) and 7·5% (3·0–10·5 g/100 g FA), respectively (Table 4).

As expected when feeding a highly unsaturated diet, the authors reported an increase in trans-MUFA following modification of the bovine feeding regimen (9·3% increase from 4·3 to 4·7 g/100 g FA). The bovine feeding regimen utilised in this study involved encapsulation protection technology – designed to protect from RBH – although the increases in trans-MUFA indicate incomplete protection.

Poppitt et al.[48] reported reductions in TC ($P < 0.05$) and LDL-C ($P < 0.01$) after consuming the modified butter-fat compared with the control butter. TC and LDL-C decreased by 0·36 mmol/l ($P < 0.001$) and 0·28 mmol/l ($P < 0.01$), respectively, and when calculated as percentage change from baseline, by day 22 TC and LDL-C had decreased by 7·9 and 9·5%, respectively. By using criteria set by the Cholesterol Treatment Trialists' meta-analysis[49], these reductions in LDL-C would be equivalent to an approximate reduction in absolute risk of CHD and stroke of 7 and 5%, respectively. No significant changes were reported from any of the haemostatic clotting factors, apoA and B, NEFA or serum glucose. Furthermore, no significant changes in HDL-C were observed.

Tholstrup et al.[42] produced a modified butter by feeding a basal diet (beetroots, grass silage, crushed barley and straw) supplemented with a bovine concentrate mix of 50% soyabean meal and 50% crushed rapeseed fed at 3·2 kg/cow per d. This dietary change decreased butter fat SFA concentrations from 73·7 to 56·4 g/100 g FA (Table 4).

Based on results from the eighteen subjects fed an iso-energetic saturate-replacement diet in an 8-week randomised cross-over study, Tholstrup et al.[42] concluded that feeding subjects dairy products where SFA were substituted for UFA did not lower TC or LDL-C and did not change HDL-C. A potential explanation for the lack of change in cholesterol levels may be due to the 5-fold increase in the reported trans-18:1 seen in the modified fat compared with the control (6·4 v. 1·1 g/100 g FA). However, having the smallest sample size of all the reviewed studies, the study population may also have been a limitation.

A further two intervention studies, by Noakes et al.[41] and Seidel et al.[43], investigated the effect of a variety

Table 3. Summary of randomised controlled trials investigating the impact of modified milk and dairy products in which SFA were partially replaced with MUFA and/or PUFA, by supplemental bovine feeding, on CVD markers

Reference	Experimental design (n, age, study, dairy products, modified feed)	Diet	Experimental diet (% energy intake)*					Primary outcomes†
			Fat	SFA	MUFA	PUFA	UNID	
Noakes *et al.* (1996)[41]	n 33, 49 years, 8-week cross-over; 2-week low-fat, 3-week C/M; < roughage; butter, milk, cheese, ice cream; protected rapeseed/soyabean	C	36·6	18·2	9·10	2·60	6·70	TC ↓ 4·3 % (0·28 mmol/l; *P*<0·001) and LDL-C ↓ 5·3 % on M (0·24 mmol/l; *P*<0·001) relative to C. ↔ TAG or HDL-C
		M	36·9	16·0	11·9	4·20	4·80	
Tholstrup *et al.* (1998)[42]	n 18, 21–28 years, 16-week cross-over; 4-week C, 4-week M, 8-week washout; butter; 50:50 soyabean: crushed rapeseed	C	41·5	27·1	8·60	5·80	0·00	TC ↑ 5 % (0·23 mmol/l; *P*=0·006) on M relative to base-line, TAG ↑ 14 % (0·14 mmol/l; *P*<0·008) on M relative to C. ↔ TC, LDL-C or HDL-C between C and M
		M	40·5	21·2	14·60	5·00	0·00	
Poppitt *et al.* (2002)[48]	n 20, NA, 10-week double-blinded, cross-over; 3-week C, 3-week M, 4-week washout; butter; unspecified 'unsaturated' feed	C	40·0	20·0	6·00	14·0	0·00	TC ↓ 7·9 % (0·36 mmol/l) and LDL-C ↓9·5 % (0·28 mmol/l; *P* < 0·01) on M relative to C. ↔ TAG or HDL-C, apoA or B, NEFA, haemostatic clotting factor VII, fibrinogen or glucose
		M	39·0	15·0	8·00	16·0	0·00	
Seidel *et al.* (2005)[43]	n 31, 16–66 years, 13-week; 7 d habitual diet, 10 d low-fat, 18 d (C-B), 10 d low-fat, 18 d M, 10 d low-fat, 18 d (C-Ma); butter, milk, yoghurt; rapeseed cake	C-B	37·2	26·3	9·50	1·10	0·30	LDL-C ↓ 12 % (0·31 mmol/l) in NC individuals on M relative to C-B and LDL-C ↓ 8·9 % (0·36 mmol/l) in HC individuals on M and C-Ma relative to C-B. HDL-C ↑ 22 % (0·42 mmol/l; *P*<0·01) in NC individuals and ↑ 5·6 % (0·08 mmol/l) in HC individuals on M relative to C-B. LDL:HDL ↓ 30 % (0·43) in NC individuals and ↓ 17 % (0·45) in HC individuals on M relative to C-B and ↓ 6·5 % (0·19) in HC individuals on C-Ma relative to C-B. LP(a) ↓ 10·7 % (21·2 mg/l) in NC individuals on M relative to C-B. ↔ TC or TAG
		M	36·7	22·9	12·2	1·30	0·30	
Tholstrup *et al.* (2006)[33]	n 42, 19–33 years, 5-week double-blind, parallel; C or M (vaccenic acid-rich); butter; sunflower seeds	C	42·0	24·4	9·60	3·60	4·40	HDL-C ↓ 9 % (0·15 mmol/l; *P*=0·002) and TC ↓ 6 % (0·3 mmol/l; *P*=0·05) on M relative to C. ↔ LDL (*P*=0·14), TAG (*P*=0·30), Ur.8-iso-PGF$_2$ (*P*=0·95), CRP (*P*=0·67), haemostatic clotting factor VIIc (*P*=0·29), PAI-1 (*P*=0·21), insulin (*P*=0·14) or glucose (*P*=0·21)
		M	44·6	22·5	14·9	3·80	3·40	
Lacroix *et al.* (2012)[47]	n 72, 18–70 years, 8-week double-blind, randomised, cross-over, 3 d washout; butter; maize oil	C	33	9·90	14·7	5·90	2·50	HDL-C ↓ 3 % (0·05 mmol/l; *P*=0·004). ↔ TC (*P*=0·32), LDL (*P*=0·77), TAG (*P*=0·99), apoB (*P*=0·83), apoA-1 (*P*=0·09), systolic blood pressure (*P*=0·31), diastolic blood pressure (*P*=0·44)
		M	33	10·3	14·4	5·90	2·40	
Malpuech-Brugère *et al.* (2010)[44]	n 111, 18–50 years, 4-week double-blind, randomised, parallel; 72 % SFA, 2·85 % R-TFA (L0), 63·3 % SFA, 4·06 % R-TFA (L4), 56·6 % SFA, 12·16 % R-TFA (L9); butter; extruded linseed (L4) and white clover/rye grass/-linseed oil (L9)	L0	37·7	21·5	11·4	3·60	1·00	LDL-C ↓ 6 % (0·14 mmol/l; *P*=0·04) and TC ↓ 3 % (0·13 mmol/l; *P*=0·04) on L4 relative to L0. ↔ HDL
		L4	39·2	22·1	11·7	3·50	1·90	
		L9	38·8	22·6	11·9	3·60	0·70	

Nutrition Research Reviews

Table 3. Continued

Reference	Experimental design (n, age, study, dairy products, modified feed)	Diet	Experimental diet (% energy intake)*					Primary outcome†
			Fat	SFA	MUFA	PUFA	UNID	
Legrand et al. (2010)[45]	n 160, 18–65 years, 104 d double-blind randomised parallel study; C or M (n-3-rich); semi-skimmed milk, spread, cheese (plus non-dairy products); linseed	C	33.6	11.9	13.2	8.50	0.00	C relative to baseline: LDL-C ↑ 5% (0.16 mmol/l; P<0.01). ↔ TC, HDL, TAG. M relative to baseline: TAG ↑ 11% (0.16 mmol/l) and HDL-C ↓ 6% (0.09 mmol/l; P<0.01) and LDL-C ↑ 4% (0.15 mmol/l). ↔ TC ↔ between M and C
		M	33.9	15.1	14.3	4.50	0.00	
Weill et al. (2002)[46]	n 75, 25–45 years, two randomised, cross-over trials with 35 d period, 18 d washout (second used 'half dose'); butter and milk (plus non-dairy products); C or M (n-3 rich); 5% extruded linseed	C	35.1	9.40	11.9	5.60	8.20	Plasma 18:3n-3 ↑ 111% (0.49%; P<0.001), 16:0 ↓ 5% (1%), n-6:n-3 ↓ 28% (4.1%; P<0.01)
		M	35.1	8.50	12.6	6.10	7.90	

UNID, unidentified fatty acids; C, control diet; M, modified diet; TC, total cholesterol; LDL-C, LDL-cholesterol; HDL-C, HDL-cholesterol; ↑, increase; NA, not available; C-B, control butter; C-Ma, control margarine; NC, normocholesterolaemic; HC, hypercholesterolaemic; LP(a), lipoprotein lipase a; CRP, C-reactive protein; PAI-1, plasminogen activator inhibitor type 1; R-TFA, ruminant trans-fatty acid; L0, butter from animals fed on maize silage with cereal-based concentrate and soyabean meal; L4, butter from animals supplemented with extruded linseed at 4.1% of DM; L9, butter from animal grazing on white clover and perennial rye grass and supplemented with 1 kg linseed oil.
↓, significant decrease; LDL-C, LDL-cholesterol; ↔, no significant change; HDL-C, HDL-cholesterol.
* For the studies where this information was not provided[43,45,46], these were calculated based on information given.
† Results are significant at P<0.05 unless otherwise stated.

of dairy products on blood biomarkers. Noakes et al.[41] provided a low-roughage diet to reduce ruminal acetate production. Acetate, being a precursor of endogenous (de novo) short- and medium-chain FA synthesis in the mammary gland, is reduced by a low-roughage diet, which in turn is generally believed to limit SCFA synthesis (predominantly SFA). Furthermore, a protein-encapsulated lipid (rapeseed and soyabean oil) provided a source of PUFA that was partially protected from RBH.

Noakes et al.[41] provided modified milk, cheese, butter and ice cream with a SFA:MUFA:PUFA proportion of 51:39:10, compared with control products with the composition of 70:28:2 (Table 4). A detailed FA composition of diets was not provided, although R-TFA were estimated at 2·2 and 3·4 g/100 g FA for the fat-modified and conventional milk, respectively. After conducting an 8-week, randomised, cross-over study in thirty-three men and women, the modified dairy products reduced TC (−0·28 mmol/l; P<0·001) and LDL-C (−0·24 mmol/l; P<0·001) although there was no change in HDL-C. In comparison with the previous study by Tholstrup et al.[42], FA compositional data were limited and no additional markers of CVD risk were measured other than plasma cholesterol.

Seidel et al.[43] fed a diet of MUFA-rich rapeseed cake to achieve changes in milk FA concentrations, which was used to produce butter and yoghurt. As part of a 13-week, three-phase, randomised, cross-over, controlled study, subjects were fed modified milk, butter and yoghurt, non-modified milk, butter and yoghurt, as well as non-modified milk, margarine and yoghurt. As summarised in Table 3, results indicated that LDL-C was reduced (−0·32 mmol/l; P<0·05) when the modified products were consumed, while HDL-C was increased (+0·23 mmol/l; P<0·05), resulting in a reduced LDL:HDL ratio (−0·38; P<0·05). This study was the only one to compare the effects of modified dairy products between normocholesterolaemic (NC) subjects and hypercholesterolaemic (HC) subjects. Results showed that dairy products with low SFA and high MUFA/PUFA concentrations increased HDL-C (+0·42 mmol/l; P<0·01) in NC subjects. Given that HC subjects tend to have greater reductions in cholesterol than healthy individuals[50], these results were unexpected. However, the imbalance in subject group sizes (nine HC and twenty-two NC subjects) is likely to have affected the results. Nonetheless, of all the studies reviewed, that of Seidel et al.[43] was the only one to show a beneficial change in HDL-C levels (Table 3) when low-SFA-content dairy products were consumed. Moreover, this study achieved the most substantial changes in serum cholesterol fractions with the smallest difference between the percentage SFA of the modified and control milk and dairy products (Fig. 1). A potential explanation may be the minimal confounding effect from R-TFA content, although this could not be confirmed due to limited FA compositional data.

Table 4. Comparison of fatty acid (FA) profiles of test products and test diets

Reference	Diet	FA profile of test products (g/100 g FA)				FA profile of diets (g/100 g FA)*		
		SFA	MUFA	PUFA	UNID	SFA	MUFA	PUFA
Noakes *et al.* (1996)[41]	C	70·0	28·0	2·00	0·00	59·9	31·2	8·90
	M	51·0	39·0	10·0	0·00	49·8	37·1	13·1
Tholstrup *et al.* (1998)[42]	C	73·7	20·6	2·55	4·76	65·3	20·8	14·0
	M	56·4	36·8	4·00	4·78	51·9	35·8	12·3
Poppitt *et al.* (2002)[48]	C	70·5	22·1	3·00	4·40	50·0	15·0	35·0
	M	54·4	32·0	10·5	3·10	38·4	20·5	41·0
Seidel *et al.* (2005)[43]	C	70·8	25·5	3·02	0·68	70·8	25·5	3·02
	M	62·6	33·3	3·63	0·49	62·6	33·3	3·63
Tholstrup *et al.* (2006)[33]	C	60·5	12·3	1·50	25·7	64·9	25·5	9·60
	M	46·7	28·4	3·00	21·9	54·7	36·2	9·20
Lacroix *et al.* (2012)[47]	C	68·4	27·1	3·60	0·90	32·4	48·2	19·3
	M	54·2	39·6	3·80	0·90	33·7	47·1	19·3
Malpuech-Brugère *et al.* (2010)[44]	L0	72·0	22·4	5·66	0·00	58·0	32·2	9·81
	L4	63·3	29·7	6·97	0·00	52·7	37·0	10·3
	L9	56·6	33·2	10·3	0·00	48·1	38·0	13·8
Legrand *et al.* (2010)[45]	C	53·6	17·4	2·00	27·0	35·5	39·3	25·2
	M	47·4	20·4	3·20	29·0	44·6	42·1	13·2
Weill *et al.* (2002)[46]	C	43·0	20·0	3·10	33·9	34·4	43·5	21·9
	M	37·0	26·0	5·40	31·6	31·5	46·8	21·9

UNID, unidentified fatty acids; C, control diet; M, modified diet; L0, butter from animals fed on maize silage with cereal-based concentrate and soyabean meal; L4, butter from animals supplemented with extruded linseed at 4·1 % of DM; L9, butter from animal grazing on white clover and perennial rye grass and supplemented with 1 kg linseed oil.
* For the studies where this information was not provided[33,41,44,48], these were calculated based on percentage energy from fatty acids.

Supplementation with a source of n-6 PUFA

Of the studies reviewed, two were specifically designed to increase concentrations of R-TFA, predominantly 18 : 1*n*-7, in butter and investigate the effects of this modified product on CVD risk markers[33,47]. While Tholstrup *et al.*[33] introduced sunflower seeds to the cow's diet (undeclared inclusion rate) and increased total *trans*-18 : 1 from 0·4 g/100 g FA in the control butter to 5·0 g/100 g in the modified butter, Lacroix *et al.*[47] fed a mixed diet of concentrates, lucerne and maize silage, with the addition of 3·6 % maize oil and increased total *trans*-18 : 1 from 4·1 g/100 g FA in the control butter to 12·4 g/100 g in the modified butter. This was equivalent to 3 g R-TFA/d (1·2 % energy intake) in that of Lacroix *et al.*[47], and in excess of 3·6 g/d (1·4 % energy intake) in that of Tholstrup *et al.*[33]. Both are above the average UK intake of R-TFA[7].

Although both studies considerably increased concentrations of R-TFA in the modified butter products, and saw large differences in concentrations of SFA and PUFA between the modified and control butters (Table 4), they differed in diet design strategies. Critically, Tholstrup *et al.*[33] did not match percentage energy from SFA, MUFA and PUFA between the control and modified diets and subsequently showed a 5·9 % difference in percentage of energy from SFA, whereas Lacroix *et al.*[47] sought to match the percentage energy intakes between diets, with a comparable exchange of *cis*-18 : 1 for *trans*-18 : 1 in the modified diet, seeing a negligible 0·4 % difference in percentage energy from SFA between diets.

Tholstrup *et al.*[33] reported reductions in TC (-6 %; $-0·03$ mmol/l; $P = 0·05$) and HDL-C (-9 %; $-0·27$ mmol/l; $P = 0·002$), yet noted that the increase in MUFA and reduction in SFA were likely to be responsible for this rather than the R-TFA. In contrast, Lacroix *et al.*[47] saw no significant change in TC, yet did see a significant decline in HDL-C (-3 %; $-0·05$ mmol/l; $P = 0·004$). The author attributes the lack of beneficial changes in cholesterol and other markers of CVD to the study population characteristics; notably the inclusion of only healthy women, for which there is limited information.

Supplementation with a source of n-3 PUFA

While the majority of evidence for the beneficial effect of modified milk and dairy products originates from studies where bovine diets were fed with a high proportion of MUFA, a small number of studies have looked specifically at *n*-3 PUFA products and will be reviewed subsequently. However, it is worthy of note that two of these studies adopted a 'livestock' approach, thereby modifying not only milk and dairy product FA composition but also that of other animal products, such as eggs, pork and chicken[44,45]. Subsequently, it was not possible to differentiate the effect of consumption of modified milk and dairy products from that of other modified animal products. The source of *n*-3 PUFA in the following studies was plant oils. Although supplementation with marine oils is an option, it can adversely affect rumen function[51]. An important plant source of *n*-3 FA, linseed, is a more economical
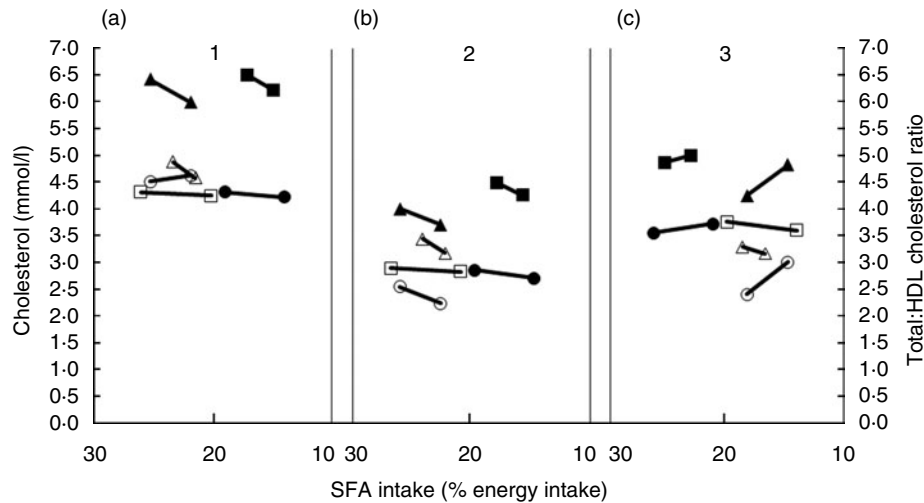
Fig. 1. Effect of decreasing the percentage energy from milk fat SFA concentrations on total cholesterol concentration (a), LDL-cholesterol concentration (b) and total:HDL-cholesterol ratio (c). (■), Noakes et al.[41]; (□), Tholstrup et al.[42]; (●), Poppitt et al.[48]; (○), Seidel et al.[43] normocholesterolaemic subjects; (▲), Seidel et al.[43] hypercholesterolaemic subjects; (△), Tholstrup et al.[33]. Cholesterol was measured in plasma with the exception of two studies[43,48], where this was measured in serum.

and sustainable option and has been investigated in these studies for any beneficial effects on milk FA composition and CVD risk markers.

One of the first studies to look at the link between linseed-enriched bovine diets and human serum FA concentrations was Weill et al.[46]. They conducted a 'livestock' approach by introducing varying amounts of extruded linseed supplement to modify the animal products from dairy cows (5 %), laying hens (10 %), pigs (2·5 %) and broiler chickens (3·5 %). For the duration of this study volunteers were not permitted to consume any fish or any products high in linseed or n-3. This study used human serum FA composition as a marker of CVD risk, although this can prove inconclusive due to further metabolism of FA after leaving the plasma[52]. Nonetheless, this study showed a substantial increase in 18:3n-3 (+119 %) in the modified dairy products and a corresponding decrease in 16:0 (−24 %). These modified dairy products successfully increased serum 18:3n-3 by 111 % (P<0·001), and resulted in a 5 % decrease in 16:0 (P<0·05) and a 28 % reduction in the n-6:n-3 ratio (P<0·01). These differences proved surprising, as estimations of SFA, MUFA and PUFA intake as percentage of total energy intake suggested very little difference between the test and control periods (Table 3).

Weill et al.[46] failed to show an increase in the long-chain n-3 PUFA 20:5n-3 and 22:6n-3 (P>0·05) in the modified dairy products. Furthermore, it is questionable whether the 111 % increase in serum 18:3n-3 (0·44 to 0·93 g/100 g) seen in the subjects that consumed the modified milk and dairy products would afford health benefits. Although 18:3n-3 is a precursor for endogenous synthesis of 20:5 and 22:6n-3, in human tissue the conversion is very inefficient[53]. Therefore, the significant increase in serum concentrations of 20:5n-3 (P<0·05) seen in the study subjects is likely to be attributable mainly to the

consumption of the n-3-enriched animal products (egg, pork and chicken), which doubled in 20:5n-3 (1·2 to 2·4 g/100 g FA) following linseed feeding.

In a randomised, double-blinded trial, Legrand et al.[45] provided a variety of modified animal products to 160 overweight volunteers over a 90 d period. In addition to a number of anthropometric measurements, plasma lipids (FA composition, cholesterol and TAG) were measured. The experimental animal products were of a similar nature to the previously mentioned study by Weill et al.[46] in that the bovine diet was supplemented with extruded linseed. While there was no significant change in erythrocyte SFA or n-3 PUFA concentrations between the control and the experimental group, there was an increase in erythrocyte MUFA (5 %; P>0·01) and a decrease in erythrocyte n-6 PUFA concentration (−10 %; P>0·001) in the experimental group compared with the control group (inter-group). Although there were no inter-group differences in total n-3 plasma FA content (18:3n-3, 20:5n-3 and 22:6n-3), there was a lower total n-3 content in the control group (−13 %; P>0·001) relative to their baseline measurements and an increase in the experimental group (+13 %; P>0·01) relative to their group baseline measures (intra-group). As fish consumption was prohibited during this study, it was suggested that the animal products provided EPA and DHA, despite a low dietary intake, due to synthesis from 18:3n-3 precursors.

Despite the favourable MUFA and PUFA plasma concentrations of the subjects, Legrand et al.[45] observed no significant differences in plasma cholesterol between the experimental and the control groups (see Table 3 for significant intra-group changes in cholesterol from baseline). Nonetheless, there was a numerical increase in TC, HDL-C, LDL-C and TAG reported in the experimental group. As previously mentioned, no significant changes

in erythrocyte SFA concentrations in the experimental group were observed, despite a numerical difference, and a 9% greater SFA content in the experimental diet compared with the control (2·6% difference in energy from SFA), which may account for the lack of change in plasma lipids. Moreover, the low n-6 PUFA in the experimental diet, subsequent erythrocyte n-6 PUFA levels and impact of potentially atherogenic R-TFA[37] may also be responsible for these non-significant results (although R-TFA were not reported and so their impact on CVD risk markers cannot be ascertained).

Of the three linseed studies reviewed, the study by Malpuech-Brugère et al.[44] reported the most beneficial effect on CVD risk factors. This was a single-centre, randomised, double-blind, parallel-intervention, 4-week controlled study where cows were fed either no linseed (L0), extruded linseed (L4; 4·1% of DM), or linseed oil (L9; 1 kg). Based on consumption of modified milk, double cream and cookies, LDL-C decreased by 6% ($-0·14$ mmol/l; $P=0·04$) and TC by 3% ($-0·13$ mmol/l; $P=0·04$) on the products made from the extruded linseed diet relative to the control. The high inclusion level of linseed oil in L9 resulted in no changes in plasma lipids despite having the lowest SFA content compared with L0 and L4 (56·6 v. 63·3 and 72%, respectively). This lack of beneficial effect was possibly due to the high R-TFA concentrations (12·16 g/100 g FA). Furthermore, a 1 kg inclusion level of linseed oil may have negative effects on DM intake and milk yield due to disruption of rumen function[54].

Discussion

The studies included in the present review were specifically those that tested the effects of milk and dairy products that had been modified through alteration of the cow's diet. However, as summarised in Table 3, the methodologies utilised differed considerably, notably the choice of fat supplement in the dairy cow diet, choice of milk or dairy product tested, number of subjects and study design. For example, while Tholstrup et al.[33] conducted a 16-week cross-over study where a total of eighteen men (aged 21–28 years) were fed a butter that had been produced from a bovine diet of soyabean and crushed rapeseed, Legrand et al.[45] ran a 15-week parallel study in 106 overweight men and postmenopausal women (aged 18–65 years) where butter was produced from a bovine diet high in linseed. Such vast shifts in focus provide insight into a range of cause-and-effect scenarios, and it is understandable that not all studies can be directly comparable. However, there is a distinct lack of quality information provided in these papers.

This lack of information is clearly illustrated by Poppitt et al.[48], where there was a failure to specify the feed given to the dairy cows, and by Seidel et al.[43], who neglected to detail the FA profile of the products and diets.

However, having said this, Seidel et al.[43] had one of the most robust study designs, and showed some of the most interesting changes in blood cholesterol, in both NC and HC individuals. Furthermore, of the four studies that included milk as a test product, only Legrand et al.[45] indicated whether the milk was full fat or not.

In addition to this lack of detailed study information, there was an evident, yet unexplainable, discrepancy between the FA profile of the modified products and the modified diets in many of these studies (see Table 4). The most notable example of such a discrepancy was Poppitt et al.[48], where the PUFA content of the control and modified products was 3 and 10·5 g/100 g, respectively, whereas the PUFA content of the control and modified diets was 35 and 41 g/100 g, respectively. The study arms were matched for energy intake and all meals were provided, yet such a discrepancy would suggest that not all the dietary fat was substituted with the modified fat. Despite stating that the only dairy fat given to the volunteers was the control and modified butter, this only made up 50% of total dietary fat and thus the remaining 50% remains unaccounted for. It is thus apparent that a non-dairy fat source contributed a significant proportion of PUFA to the diets, which could have significantly made an impact on blood cholesterol changes.

Likewise, Noakes et al.[41] and Lacroix et al.[47] reported that the PUFA content of the control and modified products was 2 and 10 g/100 g FA and 3·6 and 3·8 g/100 g, respectively, while that of the diet was 8·9 and 13·1 g/100 g FA and 19·3 and 19·3 g/100 g, respectively (Table 4). Noakes et al.[41] restricted volunteers to a low-fat background diet of 15% energy from fat (with the remaining 20% energy from fat coming from the control and modified dairy products) and suggested consumption of foods of known fat content, in addition to provision of low-fat frozen meals. In contrast, Lacroix et al.[47] focused on ensuring that the experimental butters were the sole source of R-TFA in the diets; the remaining fat in the diets was made up with various vegetable and animal oils (to maintain equal percentages of SFA, MUFA and PUFA in both diets). Thus while Noakes et al.[41] sought to control for other fat sources, the background diet in the control group appeared to substantially increase their intake of PUFA during the study. Moreover, Lacroix et al.[47] designed the study to focus on the effect of R-TFA, eliminating any effect of a low-SFA, high-MUFA/PUFA diet, thus rendering the difference in profiles between the product and the diet irrelevant.

Tholstrup et al.[33,42], Legrand et al.[45] and Weill et al.[46] also showed large discrepancies between the FA profile of the products and the diets (Table 4). Yet contrary to the before-mentioned studies[41,47], the FA information provided by these particular studies is incomplete and in some cases up to 33·9 g/100 g FA are unidentified. Subsequently, drawing parallels between the FA profiles of the products and the diets becomes arbitrary.

Nevertheless, despite considerable disparities between studies, there are some very similar trends in changes in blood cholesterol concentrations. As illustrated in Fig. 1, there is a noticeable downward trend in TC concentrations, as the percentage of energy from SFA decreases across all studies, except for NC individuals in the study of Seidel *et al.*[43]. Critically, the increase in TC in these NC individuals is due to a substantial increase in HDL-C, rather than an increase in LDL-C (Table 3). In effect, Seidel *et al.*[43] (both NC and HC subjects) was the only study to show a significant increase in HDL-C. Reassuringly, there is a consistent downward trend across all studies for LDL-C, as percentage energy from SFA decreases (Fig. 1). In turn, Seidel *et al.*[43] (both NC and HC subjects) was the only study to report a significant reduction in both the total:HDL and LDL:HDL ratios with a reduction of percentage energy from SFA. Interestingly, the slopes of these reductions in both NC and HC individuals are remarkably similar (Fig. 1). In contrast, both studies by Tholstrup *et al.*[33,42] show a detrimental increase in both total:HDL-C and LDL-C:HDL-C ratios, due to Tholstrup *et al.*[42] failing to show any significance changes in plasma cholesterol concentrations and Tholstrup *et al.*[33] significantly reducing HDL-C.

Changes in cholesterol from four studies[44–47] were not included in Fig. 1 due in part to one study having used only serum FA levels as markers of CVD risk[46], but also due to the difference in study designs. Malpuech-Brugère *et al.*[44] and Legrand *et al.*[45] both used parallel designs and, thus, using baseline cholesterol concentrations, these were reported as changes from baseline. However, while Legrand *et al.*[45] provided both the significance of change between baseline and day 90 in the control and modified groups as well as between day 90 of the control and modified groups, Malpuech-Brugère *et al.*[44] only reported the significance of 'estimate mean effects' (change between baseline and day 90) between the control and the modified group. Additionally, Lacroix *et al.*[47] employed a study design that aimed to focus solely on the effect of R-TFA on CVD risk markers. By matching the percentage of energy from SFA across both diets, it was not appropriate to include this study to look at the relationship between change in percentage energy from SFA and cholesterol.

The studies included in the present review were those that specifically tested the effects of milk and dairy products that had been modified through alteration of the cow's diet. As previously mentioned, an alternative approach for reducing SFA intake from milk and dairy products is the production of low-fat products by the skimming of milk fat. However, although studies testing the effects of these foods on CVD markers have shown some promise – as reviewed by Hooper *et al.*[24] – altering the FA composition to increase proportions of potentially beneficial MUFA and PUFA would appear to offer the potential for greater health benefits to the consumer. Moreover,

the palatability and texture of milk and dairy products rely heavily on their fat content, with consumer research indicating a preference for the mouthfeel of higher-fat dairy products compared with their low-fat alternatives[55].

A further strategy for modifying milk fat content that was not covered in the present review is the addition of oils directly to the milk or dairy products to increase proportions of MUFA and PUFA without intervention at the farm level. Wood *et al.*[40] demonstrated that by blending equal parts of butter with olive oil or sunflower-seed oil the ratio of SFA:MUFA:PUFA can be changed (butter, 57:32:11; butter−olive oil, 39:46:15; butter−sunflower-seed oil, 37:31:32); however, when fed to thirty-eight healthy men, these modified butters did not reduce serum lipid levels below baseline values. Although this approach is advantageous in its simplicity, manipulation of the dairy cow's diet not only leads to an altered milk FA composition, but may also beneficially affect ruminant health, milk yield[56] and reduce methane emissions[57].

Summary of the evidence

The present review has highlighted the heterogeneity of the limited number of studies investigating the association between the consumption of modified milk and dairy products and CVD risk. Of the eight studies that measured blood cholesterol, four reported a significant reduction in TC and LDL-C following chronic consumption of modified milk and dairy products for a period of 4–13 weeks. Thus, the over-arching conclusion from these studies is that consumption of milk and dairy products, where SFA have been partially replaced with MUFA and PUFA, has a beneficial impact on CVD lipid risk markers, but further evidence is required before a clear conclusion can be drawn.

Gaps in the evidence

As discussed, the heterogeneity between these chronic intervention studies makes forming well-powered estimations of CVD risk difficult. The variations in study designs, test products and estimations of CVD risk between studies highlight a number of areas that require further investigation, notably the potentially contrasting effects of different milk and dairy products on CVD risk, as well as the strength of cardiovascular risk markers utilised.

The milk and cheese paradox

The role of milk in cardiovascular health has been the centre of much debate. Despite milk fat having a SFA content of 70–75 %, epidemiological evidence indicates that milk is potentially cardioprotective[58]. Elwood *et al.*[58] conducted a meta-analysis of ten studies looking specifically at the effect of milk on CVD and calculated a pooled estimate of relative odds, relative to the risk in subjects with the lowest consumption, of 0·87 (95 % CI

0·74, 1·03) for IHD, 0·83 (95 % CI 0·77, 0·90) for ischaemic stroke and 0·84 (95 % CI 0·78, 0·90) for any vascular event. Although the information was not available for most studies, the authors suggest that the milk drunk was predominantly whole milk, with semi-skimmed milk only becoming available during the latter years of these studies.

Furthermore, the role of cheese consumption in CVD risk is of interest. There is limited information on epidemiological links between cheese consumption and CVD risk due to cheese often being grouped collectively with butter, which is thought to increase LDL-C and TC[59]. Nonetheless, two large studies identified no association between cheese consumption and CVD, while positively linking consumption of other dairy products[60,61]. Additional evidence from epidemiological and dietary intervention studies suggests that cheese has a neutral effect on plasma cholesterol[62], yet, importantly, highlights the type of cheese as an important variable for risk[63]. Moreover, a recent study by Hjerpsted et al.[64] demonstrated that cheese lowers LDL-C when compared with butter intake of equal fat content.

The most plausible explanation proposed for the potentially protective role of milk and cheese is the presence of the cardioprotective agents such as Ca and bioactive milk peptides[65], which are low in other dairy products such as butter. While Ca is thought to reduce blood pressure by acting on parathyroid hormone[66], milk peptides may be cardioprotective by inhibiting angiotensin-converting enzyme associated with the production of angiotensin II, a potent vasoconstrictor[67].

This epidemiological and intervention-based evidence in support of milk and cheese as potential protective agents for cardiovascular health does not adequately distinguish between individual dairy products, and studies investigating specific dairy products are limited. Milk and cheese were incorporated into four of the nine studies reviewed in the present article[41,43,45,46]; however, other dairy products, notably butter, were included and thus do not provide a clear picture of the role of milk and cheese compared with other dairy products. The complex nature of milk and cheese, coupled with their widespread consumption, warrants further investigation.

Measure of CVD risk

In addition to the gap in evidence surrounding the role of milk and cheese, a greater diversity of CVD risk markers is required to help elucidate the impact of modified dairy products on CVD. For over 60 years research has considered the use of plasma lipids – HDL-C, LDL-C, TC – as indicators of CVD risk[68,69]. However, including holistic measures of CVD such as blood pressure, inflammation and vascular function is especially important when evaluating the effect of milk and dairy products due to their counterbalancing effects on CVD risk.

Furthermore, the presence of inflammatory markers such as IL-6, C-reactive protein and TNF-α are additional markers of CVD risk due to their role in atherosclerotic lesion progression[70]. Although all studies in the present review, except for Noakes et al.[41], investigated a number of these markers, no significant results were found. More research is needed to elucidate this area.

In addition to the use of blood pressure and inflammation as markers of CVD risk, the use of vascular reactivity techniques in research settings has been steadily increasing; such techniques include pulse wave velocity/analysis, laser Doppler imaging, digital volume pulse and the 'gold standard' flow-mediated dilatation. Evidence suggests a strong link between endothelial dysfunction and CVD[71]. One such study, by Halcox et al.[72], highlighted endothelial dysfunction as a predictor of CVD based on a longitudinal prospective study in 308 patients, where coronary vascular resistance and epicardial diameter were measured.

These techniques base their estimation of CVD risk on the responsiveness of the vascular endothelial cell wall to stimuli. When healthy, the endothelium is elastic and actively produces mediators, such as NO, that inhibit leucocyte adhesion, modulate smooth muscle proliferation and inhibit platelet aggregation[73]. However, when damaged by mediators of vascular dysfunction such as smoking[74], diet[10] and obesity[75], the endothelium becomes stiffer and less responsive to vasodilation stimuli, increasing the chance of cardiovascular-related diseases.

Importantly, a recent study has supported the use of measures of endothelial function (pulse wave velocity and pulse pressure) in assessing the role of dairy intake on CVD risk by showing a linear decrease in pulse wave velocity (P for trend $=0·018$) and pulse pressure (P for trend $=0·013$) with increasing frequencies of dairy product intake[76]. Therefore, based on emerging evidence, measurement of the responsiveness of the vascular endothelial cells, blood pressure measures and markers of inflammation may offer a more appropriate indicator of CVD risk than blood lipids alone.

Conclusions

Over 100 studies have investigated the impact of modifying the bovine diet to alter milk FA composition, notably to lower concentrations of SFA by substituting the diet with a source of MUFA or PUFA. However, few studies have assessed the effects of these modified products on CVD risk markers in a human intervention study. The studies presented in this review provided a high-UFA (MUFA, PUFA or mixed) feed supplement to the bovine diet to modify milk FA concentrations. However, the provision of a high-UFA diet to the cow leads to increased R-TFA in these test foods. While the atherogenic potential of these R-TFA is yet to be clarified, with many studies inadequately reporting R-TFA concentrations, the question of whether lowering SFA concentrations, with the corresponding

increase in R-TFA concentrations, is beneficial to cardiovascular health is a poignant question.

While the majority of the studies evaluated in the present review showed a beneficial effect on at least one biomarker of CVD risk, the need for more convincing evidence is abundantly apparent. Specifically, insufficient evidence exists for dairy products other than butter and their relationship with CVD risk, most notably milk and cheese, which may be cardioprotective. Moreover, all studies to date have measured plasma lipid markers as their primary measure of CVD risk. In order to evaluate a more complete picture of CVD risk, determination of other risk factors such as blood pressure and inflammatory markers, as well as novel, vascular endothelium-based measures of risk, would seem prudent.

Acknowledgements

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