CHAPTER 1

Dairy Food Consumption and Obesity-Related Chronic Disease

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Contents

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Abstract Dairy food comprises a range of different products with varying nutritional components. In the context of a healthy diet, dairy food may provide protection against and amelioration of chronic diseases related to obesity. These include overweight, insulin resistance/metabolic syndrome/type 2 diabetes, hypertension/stroke, and cardiovascular disease. Eliciting how dairy food may have this impact represents a challenge for modern nutritional science and requires an integration of knowledge from observational studies of population dietary patterns and disease prevalence, and experimental studies testing the effect of dairy food consumption. It also benefits from the recent identification of biomarkers of dairy fat intake and from mechanistic studies that support the plausibility of the observed effects. Future research might discriminate between types of dairy foods and focus on the synergy provided by the food matrix, rather than simply the component parts of the food.

I. INTRODUCTION

The accumulation of excess body fat has proven to be a major metabolic stress for the human body. It is known that development of insulin resistance and type 2 diabetes begins with excess intake of calories and growing adipocytes trying to store this surplus of energy (Frayn *et al.*, 2007). Further, this may lead to abnormal blood lipid profiles, and higher blood pressure levels, presenting risks to the cardiovascular system (Lebovitz, 2006). These changes all reflect systems and processes that are adapting to an environmental insult, while they serve to preserve functionality; the cascade of events represents a gradual breakdown of an otherwise healthy organism. Food lies at the heart of this scenario, for it delivers the offending excess calories, but paradoxically it also provides key nutrients and other bioactive molecules that sustain the system.

Research on the effects of food involves measurements of biomarkers of the disease entities and their end points. More recently, biomarkers of dietary intake have also been developed. Dietary assessment is critical in

this research but interview and recording methods are prone to a number of limitations, which affects both the precision and accuracy of the measurement (Hodson *et al*., 2008). Additional information from dietary biomarkers is valuable, allowing greater confidence in results. In the case of dairy foods, two saturated fatty acids, pentadecanoic acid (15:0) and heptadecanoic acid (17:0), measured either in adipose tissue, serum lipids (cholesteryl esters, phospholipids, free fatty acids, or triacylglycerol), or erythrocyte membranes, have been validated as biomarkers of milk fat intake (Baylin and Campos, 2006; Smedman *et al*., 1999; Sun *et al*., 2007b; Wolk *et al*., 1998, 2001).

Determining the exact role of the diet and of individual foods themselves represents a large scientific enterprise, but all the more necessary in an environment where food is plenty so the choices are critical. In the case of dairy food, the research is complex. Dairy food comprises a range of different products with varying nutritional components. Eliciting how dairy food may have a specific health impact represents a challenge for modern nutritional science and requires an integration of knowledge from observational studies of population dietary patterns and disease prevalence, and experimental studies testing the effect of dairy food consumption.

There is an iterative relationship between observational and experimental research. On the one hand, it is possible to advance the understanding of diet–disease relationships first observed in the laboratory in large observational studies in free-living healthy populations or disease subgroups. On the other hand, observational studies may generate hypotheses for potential causal effects between dietary patterns and disease, which can be explored in the experimental setting. The purposes and methods used in observational and experimental research differ in the sense that observational studies do not have the ability to prove causality, unlike experimental studies (Tarasuk and Brooker, 1997). The randomized controlled trial (RCT) study provides the best evidence of causality and is considered the gold standard for evaluating the efficacy of different treatments in clinical medicine and public health (Gordis, 2009). However, it may be difficult to test nutrition-related hypotheses in an RCT, due to the difficulties associated with operating a controlled intervention and the high-associated costs (Tarasuk and Brooker, 1997). There are also different observational designs including cross-sectional surveys, ecological comparisons, cohort studies, and case–control studies, all of which have their limitations. Among the observational designs, the prospective cohort study gives the highest evidence for causality (Bonita *et al*., 2006). To critically appraise the relationships between dairy food intake and obesity-related chronic diseases, it is important to evaluate both the observational and experimental evidence at hand, bearing in mind their respective strengths and shortcomings.

Finally, research that exposes the mechanisms of action of food component provides some degree of plausibility for the observed effects. However, this must be considered in the light of possible interactions within foods, known as food synergy (Jacobs *et al.*, 2009). In this sense, consideration needs to be given as to whether effects are due to single nutrients within a food, the food itself, or the whole dietary pattern in which the food is a significant part. Recent reviews have argued strongly for a greater focus on whole food research (Jacobs and Tapsell, 2007; Jacobs *et al.*, 2009) not least because it provides the evidence in a form directly related to dietary guidance.

The aim of this chapter is to review the current knowledge on dairy food consumption and obesity-related chronic illness. Recent articles published in PubMed and MedLine databases were critically appraised to describe the current knowledge and propose directions for future research.

II. OBESITY-RELATED CHRONIC DISEASE

A. Obesity and insulin resistance

Obesity occurs when energy intake exceeds energy expenditure. It is a major risk factor for the metabolic syndrome, type 2 diabetes, and cardiovascular diseases (CVDs). Overweight is defined in subjects with body mass index (BMI) >25 kg/m2 and obesity is defined in subjects with a BMI >30 kg/m2. Obesity is a major public health threat and the number of affected people is rising steadily. The World Health Organization (WHO) estimates that 700 million adults will be obese in 2015. In the United States, it is estimated that more than 30% of the adult population is obese (2005–2006), while the figure is about 25% in Australia (2008) and the United Kingdom (2007). Further in Europe, the prevalence of obesity is less than 13% in Sweden (2007) and Germany (2003) and in Italy around 10%. In Greece, 26% of the men are obese as are 18% of the women (2003). In South West Asia, 28% of the men and 43% of the women are obese in Saudi Arabia (2005) and around 24% among both sexes in Iran are obese (2005) (World Health Organization, 2009). Thus, obesity affects a great number of people throughout the world.

It is well known that obesity, especially abdominal obesity, has a number of metabolic consequences, including insulin resistance (Frayn, 2005). Insulin resistance is a state that occurs when normal concentrations of insulin produce a subnormal biological response and the decay of glucose regulation, which eventually leads to type 2 diabetes (Krentz, 1996). Insulin sensitivity varies in healthy individuals, but obese individuals are very often insulin resistant (Frayn, 2005).

B. Metabolic syndrome and type 2 diabetes

The term ''metabolic syndrome'' represents a clustering of metabolic risk factors in one individual and typically denotes disturbances in glucose and insulin metabolism, central obesity, dyslipidemia, and hypertension. Dyslipidemia typically includes high triacylglycerol levels, low high-density lipoprotein (HDL) cholesterol and high levels of small dense low-density lipoprotein (LDL) particles. Impaired fibrinolysis, increased coagulation, inflammation, and endothelial dysfunction are other disturbances associated with the metabolic syndrome (Levesque and Lamarche, 2008). The concept of risk factor clustering was first introduced by Reaven in 1988 (although described as early as in the 1920s) and was referred to as Syndrome X (Isomaa, 2003). Since the metabolic syndrome was first introduced, it has been a hot topic for researchers, but the definition and its clinical value, beyond the risk associated with its individual components, remains controversial (Alberti *et al*., 2006; Kahn *et al*., 2005a,b; Reaven, 2006).

The signs of the metabolic syndrome have been consistently observed throughout the world. The prevalence of the metabolic syndrome is increasing, increases with age, and is higher in certain ethnic groups (Isomaa, 2003; Moller and Kaufman, 2005). The prevalence of the metabolic syndrome, however, varies greatly across the globe, as a consequence of the differences in obesity prevalence between countries, as previously discussed. In a recent review by Potenza and Mechanick, the highest prevalence of the metabolic syndrome was reported in female Native Americans (57%) followed by women in India (47%) and Iran (42%), and male Native Americans (44%). In the United States, the prevalence of the metabolic syndrome among men was reported to be 27% and among women 21%, while in Australia the corresponding figures were 20% and 17% (Potenza and Mechanick, 2009).

It has been demonstrated that the metabolic syndrome predicts morbidity and mortality risk associated with CVD and type 2 diabetes, but some studies have failed to confirm this association (Isomaa *et al*., 2001; Klein *et al*., 2002; Lakka *et al*., 2002; Sundstrom *et al*., 2006; Wilson *et al*., 2005). Several factors are implicated in the etiology of the metabolic syndrome. These include both lifestyle modifiable risk factors (such as physical activity and diet), genetic factors, and perinatal influences (Isomaa, 2003). It is unlikely that the clustering of risk factors in the metabolic syndrome is caused by one single factor (Kahn *et al*., 2005b). Accumulation of adipose tissue (especially abdominal obesity) mass followed by insulin resistance are thought to be the main drivers of the syndrome (Moller and Kaufman, 2005). Thus, there is a need for effective dietary strategies to prevent the development of the metabolic syndrome and its consequences. A general reduction in calorie intake and increase in physical activity are modifiable risk factors.

C. Cardiovascular diseases

More than 80,000,000 Americans (one in three) has one or more types of CVDs. These include high blood pressure, coronary heart disease (CHD), heart failure, stroke, and congenital CVDs. Healthy lifestyle characteristics that contribute to less CVDs in the population are nonsmoking, healthy weight, low cholesterol, regular physical activity, and no diabetes. A healthy diet including fruits, vegetables, whole grains, nuts, legumes, low-fat dairy food, and lean meat is also paramount to minimize the risk of CVDs, (American Heart Association, 2009). CHD is the single leading cause of death throughout the world today. During the past decades, there has, however, been a decline in CHD mortality, attributed to lifestyle changes, including diet, and new medical and surgical treatments for secondary prevention. In Sweden, for example, CHD mortality decreased more than 50% from 1986 to 2002 and it was estimated that 40% could be explained by a decrease in cholesterol levels in the population (Bjorck *et al.*, 2009).

III. DIETARY FAT AND OBESITY-RELATED CHRONIC DISEASE

The single most researched dietary element implicated in the development of obesity and CVD has been dietary fat. Early observations from the Seven Countries Study (Keys *et al.*, 1986) gave rise to the traditional diet–heart hypothesis. The diet–heart hypothesis suggests that diets high in saturated fat increase, while diets high in unsaturated fat decrease the risk of CHD in a population. In the Seven Countries Study the percentage of dietary saturated fat was strongly correlated with CHD mortality and the correlation was not as strong between total fat intake and mortality. Mortality rates were the highest in Finland and the lowest in Crete. Although informative, the Seven Countries Study was only an ecological study (making comparisons between countries), and has been much criticized on the basis of an inability to adjust for potential confounding factors (Hu *et al.*, 2001).

In a later and larger ecological study, CHD mortality rates were compared and a meta-analysis, using data from 40 different countries, was conducted (Artaud-Wild *et al.*, 1993). The main conclusion from this study was that the mortality difference was due to the different intake levels of saturated fat (and cholesterol) in the different countries. Intake levels of saturated fat were reported to be equal in Finland and France; however, mortality rates were paradoxically lower in France while higher in Finland. This difference was explained by a high consumption of dairy products in Finland, compared to a high consumption of plant foods and vegetable oils in France (Artaud-Wild *et al.*, 1993). This move to observing differences in food intake and putting nutrient intake into a dietary context and takes the focus of the question of diet–disease relationships from that

of individual nutrients to individual foods in the context of a whole diet (or cuisine). Not all reports have supported the diet–heart hypothesis (reviewed in Hu *et al.*, 2001) and it is currently being challenged, especially as it relates to weight management (Shai *et al.*, 2008).

Nonetheless, the inverse relationship between unsaturated fat and CVD is supported by the results from prospective cohort studies such as the Ireland–Boston Diet Heart Study (Kushi *et al.*, 1985) and the Nurses' Health Study (Hu *et al.*, 1997) and long-term intervention studies such as the Los Angeles Veteran Study and the Finnish Mental Hospital Study (Dayton *et al.*, 1965; Turpeinen *et al.*, 1979). In the Indo-Mediterranean Diet Heart Study (Singh *et al.*, 2002) and the Lyon Diet Heart Study (de Lorgeril *et al.*, 1999), a diet high in unsaturated fat and complex carbohydrates were proven to be potent to reduce coronary events. It has been difficult to prove a clear relationship between saturated fat and future cardiovascular events in prospective cohort studies, and this is highlighted by the recent meta-analysis described below.

This meta-analysis of prospective cohort studies evaluated the association between saturated fat and CVD and showed no significant suggestion that saturated fat is associated with an increased risk of CHD and stroke. The meta-analysis included data from 21 prospective cohort studies and included 347,747 subjects. During 5–23 years of follow-up, 11,006 individuals developed CHD or stroke. The authors concluded that prospective cohort studies only provide one category of evidence on the relation between saturated fat and CVDs and that the risk may be related to what is replacing saturated fat (Siri-Tarino *et al.*, 2010). This highlights the importance of both observational and experimental evidence in understanding diet–disease relationship and further studies are warranted.

The emphasis on the negative effects of saturated fat represents a focus of research that has a long history and substantial research investment. It may also imply that any saturated fat in the diet is problematic. Like calories, however, some saturated fat is desirable, but keeping the level down with the current food supply in Western societies is the challenge. It should also be remembered that there may be other food components of equal interest where the effects may be positive, and indeed the focus on whole food may take the issue further to focus on the best foods to deliver saturated fat in the diet.

IV. DAIRY FOODS AND OBESITY-RELATED CHRONIC DISEASE

The reported intake of dairy foods has been identified in both observational and experimental studies to be associated with improvements in several metabolic variables (Bowen *et al.*, 2005). Specific metabolic

variables hypothesized to be attenuated by dairy food intake include hypertension, body composition and fat mass, insulin resistance, and energy expenditure (Azadbakht *et al.*, 2005).

A. Dairy food intake, obesity, and weight management

With respect to the development of obesity itself, only one cohort study was found on adults examining the impact of dairy food per se. In this study of 19,352 postmenopausal women over a 9-year period, no association between dairy product intake and weight gain was identified (Rosell *et al.*, 2006).

In children, however, Carruth and Skinner (2001) demonstrated the potential effect of dairy product consumption in the body composition in a longitudinal study of 53 preschool children. This study reported that higher intakes of calcium and dairy products were correlated with a lower total body fat(Carruth & Skinner, 2001). Similarly, cohorts of 12,829 children between the ages of 9 and 14 were studied to determine the association between milk, calcium, dairy fat, and weight gain. Results suggested that children with a milk consumption of greater than three glasses per day were more likely to gain weight. As weight gain is the result of excess caloric intake, the authors hypothesized that this weight gain effect was the result of the additional energy associated with intake of large quantities of milk rather than the dairy product per se (Berkey *et al.*, 2005).

From an experimental perspective, current evidence to support the role of dairy in weight loss is conflicting. In a recent review of RCTs, dairy intake in the context of an energy deficit diet was found to effect weight loss in just three out of six studies. For a high dairy intake in the context of energy balance, 15 of 17 studies showed no effect on body mass, and two of these studies indicated that dairy intake was associated with weight gain (Lanou and Barnard, 2008). It should be noted, however, that within this review, several of the studies reported were designed to measure skeletal endpoints of the effect of dairy product and calcium consumption and may not have been adequately powered to detect changes in body mass or adiposity. On the other hand, in a review of 17 studies, Barr (2003) also failed to find substantial evidence to support the role of dairy foods in weight loss. The lack of consistency in results may reflect the difficulty in conducting dietary trials adequately and effectively. This is particularly the case given that the methods used to assess compliance with dietary protocols are subject to limitations (Johansson *et al.*, 1998).

While unfavorable results have been reported for dairy consumption in other RCTs (Lanou and Barnard, 2008), Zemel *et al.* (2008) have reported that individuals consuming at least three servings of dairy products per day demonstrated a higher respiratory quotient (greater

fat oxidation) and were able to consume significantly more energy without greater weight gain in comparison to individuals consuming minimal amounts ($\leq$1 serve/day) during periods of weight maintenance. Zemel *et al*. (2004) also reported a significant change in the distribution of body fat lost, following a 24-week randomized, placebo-controlled trial diet whereby 32 obese adults were prescribed a 500- kcal energy deficit per day of either a standard diet (400–500 mg dietary calcium), a high-calcium diet (800 mg calcium), or a high dairy diet (providing 1200–1300 mg calcium from dairy products). After 24 weeks, subjects consuming the lower calcium diet lost 2.5% of their body weight, while those on the supplemented and high dairy product diet lost 26% and 70% more weight, respectively. Perhaps, most importantly, in terms of ameliorating metabolic risk factors was the finding that abdominal fat loss was significantly greatest for the high dairy diet at 14.0 $\pm$ 2.3% than the high-calcium and low-calcium diets at 12.9 $\pm$ 2.2% and 5.3 $\pm$ 2.3%, respectively. Zemel *et al*. (2005) reported similar outcomes were obtained in obese African-American adults, with those consuming a diet high in dairy sources of calcium exhibiting a fat loss two times greater than those on an isocaloric diet with less than 500 mg calcium/day.

While greater loss of weight per se appears a difficult outcome to consistently prove, the trend toward greater metabolic advantage has gained momentum. In a study of 67 overweight females participating in a weight loss trial, 500 mg of calcium from either dairy origin (Lactoval) or calcium carbonate did not significantly increase weight loss compared to a placebo following a 3-week dietary intake of 4.5 MJ/day. However, a significant decline in fat-free mass (-1.46 ± 3.36 kg, $P = 0.006$) that was observed in the placebo group was not evident for either of the intervention groups, reflecting a preservation of metabolically active tissue during conditions of energy deficit (Kabrnova-Hlavata *et al*., 2008). Likewise, St-Onge *et al*. (2009) found that high milk consumption (>4 serves/day) in a group of 8–10-year-old children did not result in significant weight loss or improved body composition in comparison to children consuming a low milk intake. However, the children consuming the large quantities of milk did exhibit a reduced insulin response following an oral glucose tolerance test(St-Onge *et al*., 2009). The observed metabolic advantages might then be of greater value in clinical populations. In a randomized clinical trial of 259 diabetic patients on energy-restricted isocaloric diets, weight loss over a 6-month period was enhanced in subjects that consumed low-fat dairy products as part of their regimen (Shahar *et al*., 2007). Thus, again, there may be metabolic advantages in including dairy in weight loss programs particularly for those already under conditions of metabolic stress. Studies in obesity-prone transgenic mice support these findings. With mice consuming high-calcium diets from fortified foods or

dairy products exhibiting increased lipolysis and reduced fat gain when fed an obesity inducing diet as opposed to those consuming a low-calcium isocaloric diet (Sun and Zemel, 2004).

For normal healthy overweight individuals, the benefits of including dairy foods might relate to the longer term. Gunther *et al*. (2005a) found no effect on body fat mass or weight in a study of 155 normal weight females randomized to either isocaloric control (with $<$800 mg/day of calcium from dairy products), medium (with 1000–1100 mg/day calcium from dairy products), or high (1300–1400 mg/day calcium from dairy products) dairy product intake groups, following 1 year of intervention. While these results do not support the dairy and weight loss hypothesis, a 6-month follow-up of 51 of the subjects who had participated in this trial indicated that those who had maintained a high-dairy calcium intake for a further 6 months postcompletion of the study accumulated less fat mass than their low-dairy calcium controls (Eagan *et al*., 2006). It is hypothesized thus by Eagan *et al*. (2006) and Zemel *et al*. (2008) that dairy products may prevent a small, incremental weight gain over a prolonged period of time and that studies reporting no effect of dairy product consumption on body weight may not have been designed to assess this prevention of weight regain.

B. Dairy food intake, metabolic syndrome, and type 2 diabetes

While the association between dairy food intake and obesity is less clear, observational studies have indicated that dairy food consumption may be protective against the development of metabolic syndrome and type 2 diabetes. Pereira *et al*. (2002) reported an inverse relationship between dairy product consumption and insulin resistance syndrome in a prospective study of 3157 young adults (18–30 years) with 10 years of follow-up. Further, Mennen *et al*. (2000) reported that more than four servings of dairy products per day was associated with a nonsignificant inverse association with the metabolic syndrome in women, while a significant association in men was found.

In a larger prospective study of 37,183 women with 10 years of follow-up, Liu *et al*. (2006) reported a reduction in the relative risk of type 2 diabetes in women with the highest quintile of dairy intake (relative risk: 0.79, 95% confidence interval (CI) [0.67–0.94]) after controlling for potential confounding and risk factors. A 4% reduction in risk of development of type 2 diabetes mellitus was quantified in this sample with each additional daily serving of dairy products. In a similar prospective study of 41,254 healthy males with 12 years of follow-up, Choi *et al*. (2005) report similar results, whereby highest quintiles of dairy intake were associated with a reduced relative risk of development of type 2 diabetes mellitus (relative risk: 0.77, 95% CI [0.62–0.95]) with a 9% reduction in relative risk associated with each additional serving of dairy products

per day. These findings are reasonably consistent with the analysis of metabolic effects associated with weight loss.

C. Dairy food intake and cardiovascular diseases

The relationship between dairy food intake and the risk of obesity-related chronic diseases has been the interest of much research. A high intake of dairy products has traditionally been associated with an increased risk of CVDs, attributed to the high content of saturated fatty acids (about 70%) in dairy fat (Lindmark-Månsson *et al*., 2003).

Despite these observations, several intervention studies conducted during the 1990s demonstrated that dairy products may even induce hypocholesterolemic effects in humans (Eichholzer and Stahelin, 1993); Skim milk, yogurt, and other fermented dairy products, as well as whole milk were found to induce hypocholesterolemic effects in humans (Andersson *et al*., 1995; Golay *et al*., 1990; Steinmetz *et al*., 1994). The size of these studies was small and the study duration was short. For example, Steinmetz *et al*. (1994) proposed that substitution of whole milk with skim milk may reduce the risk of CHD by 14%, following results of a 6-week crossover study in which eight healthy males were given 236 ml of whole or skim milk per day in a controlled diet setting.

This research lead to search for a ''milk factor'' that could be responsible for the observed hypocholesterolemic effects. Several components of milk were named as being the responsible component, for example, orotic acid, whey, calcium, lactose, casein, B12, and B6 (Eichholzer and Stahelin, 1993). This search for a single factor may have been too simplistic; bearing in mind that the matrix of nutrients and bioactive components contained within the different dairy foods may have been responsible for the observed effects.

The evidence from intervention studies gives us important information regarding diet–disease relationships and it may be possible to delineate mechanisms at play for the observed effects in an intervention study. However, based on the results from shorter term interventions it is difficult to draw firm conclusions regarding longer term effects on intermediate endpoints such as BMI, waist circumference, and blood pressure, and even more so on cardiovascular endpoints such as heart disease. The evidence gathered in prospective cohort (longitudinal) studies investigating associations between dairy products and/or milk fat biomarkers and intermediate and cardiovascular endpoints address the question of longer term effects. Longitudinal studies are advantageous in that, unlike clinical interventions, large samples can be studied over a long period of time, but the disadvantage is that these studies cannot infer direct causality as they are not conducted under controlled experimental conditions.

In the Caerphilly cohort (UK) of elderly men, for example, an inverse, but not statistically significant, relationship was reported between a higher milk intake (0.5 l vs. no milk intake) and the risk of both ischemic stroke and ischemic heart disease. This study included 2512 men of whom 493 had an event of ischemic heart disease and 185 had an ischemic stroke during 20–24 years of follow-up. In this study, information on milk drinking was obtained in a semiquantitive food frequency questionnaire (Elwood *et al.*, 2004a) which may have been a limitation.

Likewise, in a meta-analysis of prospective cohort studies inclusive of over 600,000 subjects, Elwood *et al.* (2004b) reported a 10–15% reduction in the incidence of CVD in individuals that reported drinking the largest quantities of milk compared to those drinking the least amount. Similarly, an approximate 20% risk reduction of stroke events was reported in high milk drinkers relative to individuals with limited intake (Elwood *et al.*, 2004b). Another meta-analysis of four prospective cohort studies revealed a close to 10% reduction in the relative risk of type 2 diabetes in those who reported high intakes of milk in comparison to those with low reported intakes (relative risk: 0.92, 95% CI [0.86–0.97]) (Elwood *et al.*, 2008). Finally, in a prospective Scottish study, milk consumption was inversely related to age-adjusted, all-cause, cardiovascular and CHD mortality. The risk of death from stroke was also inversely but nonsignificantly related to regular consumption of milk (Ness *et al.*, 2001).

In a recent review by Gibson *et al.* (2009), the effect of dairy foods on CHD in prospective cohort studies was evaluated. The review was based on data from 15 studies originating from 12 well-known cohort studies such as the Health Professionals Follow-up Study, the Iowa Women's Health Study, the Nurses' Health Study, the Oxford Vegetarian Study, and the British Regional Heart Study. Follow-up periods ranged from 8 to 20 years and included more than 288,000 adults of both men and women. Four of the studies did not find any association between dairy intake and CHD, while the majority of studies produced mixed results. In these studies the mixed results were hypothesized to be related to intake of different dairy foods (Gibson *et al.*, 2009). This raises an important point on the impact of the individual food matrix. The lack of consistency in results may well represent too much variation in delivery of bioactive component is the diet. It has implications for the interpretation of results and the design of future research. A trend toward protection across the spectrum of obesity-related diseases appears to be emerging, but the detail on the actual food type may be confounding results.

Thus, there is evidence that dairy products may influence the development of obesity-related chronic diseases in a beneficial way as a significant component of a healthy diet. This perspective lies in contradistinction to the delivery of saturated fat. It is quite possible to include the saturated fat content of dairy food if the diet is balanced with

other low fat foods such as cereals, fruits, and vegetables. There may also be other mechanisms at play that better reflect the combinations of nutrients and bioactive compounds found in dairy foods. The evidence from the research discussed thus far, however, gives little information on possible mechanisms. The next part of this chapter examines the components of milk and dairy products and discusses possible mechanisms that may account for the positive effects observed with dairy food consumption.

V. COMPONENTS OF DAIRY FOOD

Cow's milk is a complex and dynamic fluid that contains all nutrients needed for the development and growth of the calf. Milk contains lipids (dairy fat), high-quality protein, vitamins, minerals, and other bioactive components. The nutritional composition in milk varies depending on factors such as breed and age of the cow and the forage composition (Haug *et al.*, 2007). Table 1.1 presents the different components of milk and their respective concentration per liter. Also presented in the table is an approximation of the daily contribution (%) of the different components in milk to the diet for adults, as well as the main health effects.

The many diverse components of milk have demonstrable effects on human health. Perhaps, the most commonly associated component of dairy food is that of dietary calcium. Dairy products provide the most significant contribution to dietary calcium intake in the modern Western diet. It has been estimated that dairy products contribute to >72% of dietary calcium in the United States (Huth *et al.*, 2006). Calcium is an important mineral for maintenance of optimal bone health (Bonjour *et al.*, 2009) and is an integral component of key metabolic pathways relating to, for example, muscle contraction both in skeletal and smooth muscle (Cheng and Lederer, 2008). Further, dairy products contribute other essential nutrients in the diet, such as proteins, phosphorus, potassium, zinc, magnesium, selenium, folate, riboflavin, vitamin B12, and vitamin A (Haug *et al.*, 2007; Huth *et al.*, 2006). Low-fat milk alternatives are fortified with vitamin A and vitamin D which is added to milk and fermented milk in many countries making it an important source for vitamin D (Huth *et al.*, 2006).

Full fat milk contains about 3% fat and triacylglycerols account for about 95% of the lipid fraction. Other components of the lipid fraction are diacylglycerols, cholesterol, phospholipids, and free fatty acids. The lipid structures contain many fatty acids from all major classes, that is, saturated, monounsaturated, and polyunsaturated fatty acids (Haug *et al.*, 2007). More than 60% of the fatty acids in cow's milk and consequently in dairy products are saturated, including shorter and medium

TABLE 1.1 Milk composition and percent contribution to the daily dietary reference intake of some nutrients in 0.5 l whole milk and their main health effects

Milk component	Concentration in 1 l whole milk[a]	Percent contribution of 0.5 l whole milk to reference intake (%)[b]	Health effects
Fat	33 g/l		Energy rich
Saturated fatty acids	19 g/l		Increase HDL, small dense LDL, and total cholesterol. Inhibition of bacteria, virus
Oleic acid	8 g/l		Prevent CHD, gives stable membranes
Lauric acid	0.8 g/l		Antiviral and antibacterial
Myrisitc acid	3.0 g/l		Increase LDL and HDL
Palmitic acid	8 g/l		Increase LDL and HDL
Linoleic acid	1.2 g/l		Omega-6 fatty acid
Alpha linolenic acid[a]	0.75 g/l		Omega-3 fatty acid
Protein	32 g/l	30–40	Essential amino acids, bioactive proteins, peptides. Enhanced bioavailability
Lactose	53 g/l		Lactosylation products
Calcium	1.1 g/l	40–50	Bones, teeth, blood pressure, weight control
Magnesium	100 mg/l	12–16	For elderly, asthma treatment
Zinc	4 mg/l	18–25	Immune function, gene expression

TABLE 1.1 (*continued*)

Milk component	Concentration in 1 l whole milk[a]	Percent contribution of 0.5 l whole milk to reference intake (%)[b]	Health effects
Selenium	37 ug/l	30	Cancer, allergy, CHD
Vitamin E	0.6 mg/l	2	Antoixidant
Vitamin A	280 ug/l	15–20	Vision, cell differentiation
Folate	50 ug/l	6	DNA synthesis, cell division, amino acid metabolism
Riboflavin	1.83 mg/l	60–80	Prevent ariboflavinosis
Vitamin B12	4.4 ug/l	90	Key role in folate metabolism

From Haug *et al.* (2007); doi:10.1186/1476-511X-6-25 (Original publisher: Biomed Central).
[a] Data from USDA Food Composition Data in Haug *et al.* (2007).
[b] Dietary reference intake (DRI) for men and women in Haug *et al.* (2007).

chain fatty acids (2:0–10:0) and longer chain fatty acids (12:0–18:0). The wide range of fatty acids contained in dairy fat is unique in the food supply (Gibson *et al.*, 2009) and it is today possible to identify over 400 different fatty acids with modern chromatography techniques (Lecerf, 2009).

VI. EFFECTS OF DAIRY FOOD COMPONENTS

A. Micronutrients

Even though the effects of dairy products may be a result of the synergy between individual components, each nutrient and compound has a biological function of its own.

Phosphorus, vitamin D, and calcium are all needed for bone health (Huth *et al.*, 2006) and selenium has a role in the immune and antioxidant systems and in DNA synthesis and repair. Zinc is necessary for DNA repair, cell growth, gene expression, and is an essential part of some enzymes and metalloproteins. Magnesium has been implicated in the prevention of CVDs (Haug *et al.*, 2007). For example, it is known that calcium, phosphorus, and magnesium may mediate beneficial effects on

several risk factors for stroke, such as blood pressure, insulin resistance, platelet aggregation, and atherosclerotic processes (Massey, 2001).

Calcium is often identified as one of the key components that may explain observed effects of dairy products on health. Heaney (2003) suggests that ensuring adequate dietary calcium intakes at a population level may decrease the prevalence of obesity or weight gain in 60–80% of women. Nevertheless, an RCT where overweight individuals were provided 1500 mg daily of elemental calcium found no significant affect on the prevention of weight or fat gain (Yanovski *et al.*, 2009). The effect of a single nutrient could not prove so potent.

In contrast, intervention studies by the Zemel team have demonstrated greater weight loss in obese adults when consuming diets high in dairy products in comparison to calcium supplementation alone. This suggests that the observed dairy product-mediated effects are the likely result of a complex matrix of nutrients and bioactive components contained within the whole dairy food in addition to calcium (Zemel, 2004). The Honolulu Heart Program study further illustrated that the effects of dairy foods and calcium intakes are not the same. In this study 3150 older middle-aged men (55–68 years) were followed up for 22 years. They had a habitual low calcium intake; 95% of the population had a calcium intake that was lower than 1000 mg/day. The results showed that total calcium intake was unrelated, but calcium from dairy sources was inversely associated, with having a stroke (Abbott *et al.*, 1996).

There is a great deal of evidence that calcium and potassium have beneficial effects on blood pressure. The first evidence regarding an inverse association between intake of calcium and blood pressure came from the first National Health and Nutrition Examination Survey in the United States from 1984, as reviewed by Huth *et al.* (2006). In the Dietary Approaches to Stop Hypertension (DASH) study, it was shown that a diet that was lower in total and saturated fat and higher in fruits and vegetables compared to a ''typical American'' diet and including three servings of low-fat dairy produced significant reductions of both systolic and diastolic blood pressure (Appel *et al.*, 1997). The risk of hypertension was also inversely related to dairy product intake in the Rotterdam study (Engberink *et al.*, 2009).

In the Women's Health Initiative Calcium/Vitamin D Trial, a large number of postmenopausal women ($n = 36{,}282$) were supplemented with 1000 mg of elemental calcium and 400 IU vitamin D daily or placebo for 7 years. The study found no significant decrease in either systolic or diastolic blood pressure (Margolis *et al.*, 2008). The results from the Women's Health Initiative Trial further emphasized the importance of dairy per se rather than calcium for the effect on blood pressure.

Dairy foods provide an array of important micronutrients each of which has an identified role. In terms of outcomes associated with

obesity-related disease, however, these effects appear more likely to reflect the whole food rather than any individual component nutrients on their own.

B. Milk-derived peptides

Milk-protein-derived peptides including those derived from casein have demonstrated effects in reducing hypertension, arterial stiffness, and LDL cholesterol in both human and animal studies (Turpeinen *et al.*, 2009). Boelsma and Kloek (2008) recently reviewed the evidence of the effects of lactotripeptides on blood pressure in humans. These milk-derived peptides contain angiotensin-converting enzyme lactotripeptides including IPP (isoleucine-proline-proline) and VPP (valine-proline-proline). In several countries such as USA, Spain, Finland, Switzerland, Iceland, UK, and Italy, blood pressure-lowering products enriched with lactotripeptides are available in the market. The studies reviewed showed that there in general was a blood pressure-lowering effect of lactotripeptides ingested as tablets, fermented milk, or in a fruit and vegetable drink versus placebo (Boelsma and Kloek, 2008). There may be a difference between concentrated effects from supplements and the small doses delivered through the regular consumption of foods, but either way the effects appear to positively support health.

C. Trans-fatty acids

It is known that the intake of trans-fatty acids is strongly related to the development of CVDs. It is also known that trans-fatty acids increase LDL-cholesterol, triglyceride concentrations, and Lp(a) and affect prostaglandin balance and thereby thromogenesis, all with an impact on the development of CVDs (Hu *et al.*, 2001). Trans-fatty acids naturally occur in dairy products, with vaccenic acid (18:1 trans-11) being the most abundant fatty acid. The content of trans-fatty acids in milk fat is normally between 3% and 6%. Natural trans-fatty acids may be less atherogenic than industrially produced trans-fatty acids; however, this is debatable. The food matrix may have an impact and there may be a threshold level for the effect of trans-fatty acids to be seen. Considering the low content of trans-fatty acids in dairy products, it will be difficult for most people to consume a dangerous amount of trans-fatty acids within the context of a normal diet (Bryngelsson, 2008).

The TRANSFACT study was a randomized, controlled, crossover study ($n = 46$), investigating the effects of industrially compared to natural trans-fatty acids (11–12 g/day, representing about 5% of daily energy). The study showed that trans-fatty acids from industrially produced sources resulted in lower plasma HDL-cholesterol concentrations

compared to trans-fatty acids from natural sources. Also, the effect occurred only in women (Chardigny *et al.*, 2008).

Given their biological origins in the food chain, all foods are likely to contain compounds that are less beneficial to human health than others. In some cases (and notably in plant foods), these compounds may be detrimental as they form part of the organisms natural defense systems. In the case of milk products the trans-fats have a yet undiscovered role for humans, but like saturated fats their contribution to the human diet must be viewed in the context of the total diet.

D. Conjugated linoleic acid

Conjugated linoleic acid (CLA) is a fatty acid, naturally occurring following isomerization of linoleic acid by bacteria in the digestive tracts of ruminant animals, and is thus abundant in dairy products (Rainer and Heiss, 2004). CLA has been demonstrated in animal studies to reduce insulin resistance and increase fat oxidation (Nagao *et al.*, 2003). The effects of CLA in relation to improved body composition have been reported in animal studies, with a dose-dependent reduction of body fat reported in mice fed CLA independent of energy intake (DeLany *et al.*, 1999). Human studies have largely failed to replicate this finding, with Riserus *et al.* (2002) failing to find any significant reduction in body fat, saggital abdominal diameter, and body weight in a group of obese males who had consumed 3.4 g/day of purified CLA in comparison to placebo. Conversely, adverse health effects were observed in this treatment group, with an increase in insulin resistance and reduction in HDL cholesterol reported. This clearly suggests more needs to be known on the mechanisms of action of CLA. CLA is produced by the ruminant gut and has some biological role for cows. Understanding the details of this functionality and with a view to comparative physiology, may provide insights into the potential effects for humans.

The reductionist approach of isolating dairy product components including calcium, CLA, and trans-fatty acids in dietary interventions trials often yields inconclusive results. It is therefore highly plausible that dairy products exert maximum health benefits when consumed in their natural form. Because individuals do not generally consume these individual dairy components in isolation, examining the effects of these foods in their whole forms should be encouraged.

E. Saturated fats

Dairy products provide a source of dietary saturated fatty acids. Generally, saturated fatty acids have been reported in the literature to increase LDL-cholesterol (Katan *et al.*, 1994), a risk factor for CHD (Lamarche

et al., 1997). However, recent evidence suggests that perhaps the saturated fat within dairy products may not have the same health effects as other saturated fatty acids (Lock *et al.*, 2008).

Similarly, total LDL-cholesterol that was traditionally accepted as a risk factor for the development of CHD is now increasingly being categorized by size, with the smaller very low density lipoproteins demonstrating a greater risk to cardiovascular health than its larger more buoyant LDL-cholesterol counterpart (German *et al.*, 2009). Saturated fatty acids from dairy products have been demonstrated to have either a neutral effect on cholesterol, an increase in HDL-cholesterol, or a more favorable LDL profile overall. One cross-sectional study of 291 healthy adult males found an association between milk fatty acid intake and a reduction in small LDL-cholesterol particles reported by Sjogren *et al.* (2004).

It is important to bear in mind when discussing the effect of dairy fat in association to heart disease that dairy products contain many different saturated fatty acids that do not exert the same biological response in terms of, for example, cholesterol levels. The saturated fatty acids in milk fat include shorter and medium chain fatty acids (2:0–10:0), lauric acid (12:0), myristic acid (14:0), palmitic acid (16:0), and stearic acid (18:0). Other fatty acids in milk fat are oleic acid (18:1) and linoleic acid (18:2n-6) as indicated in Table 1.2.

The longer chained fatty acids, lauric, myristic, and palmitic acids are all cholesterol elevating fatty acids and it is possible that myrictic acid is the most cholesterol elevating fatty acid. Stearic acid is, however, different from the other longer chained fatty acids present in dairy fat since it may have neutral effects on cholesterol level (Katan *et al.*, 1994). The proportion of stearic acid in milk fat is about 11% (Lindmark-Månsson *et al.*, 2003).

In the Nurses' Health Study (Hu *et al.*, 1997), the dietary intake of short- and medium-chained saturated fatty acids (4:0–10:0) was not significantly associated with CHD (but other saturated fatty acids were). In an intervention study a higher intake of medium-chained triglycerides was found to significantly decrease total adipose tissue, subcutaneous adipose tissue, and upper-body adipose tissue stores in men compared to longer chained triglyceride consumption (St-Onge, 2005).

It seems that saturated fat from dairy products may not have the same atherogenic effect as saturated fatty acids consumed from other dietary sources. This may be due to the relatively high content of both stearic acid and saturated fatty acids with shorter chain length found within dairy fat. Parodi (2006) reports that fatty acids with shorter chain lengths and a lower carbon to oxygen ratio have a lower heat of combustion and thus contribute less energy than their longer chain counterparts. Similarly, fatty acids of carbon chain length shorter than 12 rapidly undergo

TABLE 1.2 Percentages of the different fatty acids in milk fat

Fatty acid	Trivial name	Percentage of total fatty acids	
4:0	Buturic acid	4.7	Total saturated fatty acids, >70%
6:0	Hexanoic acid	2.8	
8:0	Caprylic acid	1.5	
10:0	Capric acid	3.1	
12:0	Lauric acid	3.8	
14:0	Myristic acid	11.3	
15:0	Pentadecanoic acid	0.9	
16:0	Palmitic acid	30.3	
17:0	Heptadecanoic acid	0.4	
18:0	Stearic acid	11.5	
20:0	Arachidic acid	0.2	
18:1	Oleic acid	21.6	
18:2	Linoleic acid	1.5	

Adapted from Lindmark-Månsson *et al.* (2003).

β-oxidation within the liver for energy utilization. Conversely, longer chain length fatty acids do not readily undergo this oxidation and are instead packaged into chylomicrons for distribution in the blood, potentially increasing the risk of CVD (Parodi, 2006).

It is also possible that the short chain fatty acids may affect the expression of the transcription factors NF-κB, which controls the expression of several genes involved in inflammatory and immunological reactions and cell proliferation. Butyric acid, for example, may inhibit the expression of NF-κB and this may be a beneficial influence on the relationship between dairy fat and CVDs (Reviewed in Bryngelsson, 2008).

F. Total dairy fats

It remains to be seen whether the observed advantageous health effects of milk or dairy consumption are affected by the level of fat within these food items. It had previously been hypothesized that saturated fat in milk would have unfavorable health effects, particularly in relation to cardiovascular health and obesity because of the observed links between dietary saturated fat and CVD. In the Hoorn study, however, low-fat dairy consumption was positively related, while high-fat dairy consumption

was inversely related to risk factors of the metabolic syndrome (Snijder *et al.*, 2007). Another study (Smedman *et al.*, 1999) of 62 elderly males also found an inverse relationship between milk fat intake and BMI, waist circumference, fasting plasma glucose, and LDL–HDL ratio. Similarly, the reported survival advantage of dairy intake in terms of metabolic outcomes was evident from meta-analyses of prospective cohorts that included time periods prior to the widespread replacement of whole milk varieties with reduced fat alternatives (Elwood *et al.*, 2008). Lamarche (2008) describes this as the counterintuitive nature of the beneficial cardiovascular effects of dairy consumption observed given the contribution of saturated fat to the diet that dairy products provide. This is particularly pertinent given that saturated fatty acids are known to increase LDL-cholesterol, a well-known risk factor for CHD (Lamarche, 2008). These paradoxical findings suggest the effect may lay in the food, not primarily the saturated fat component. It also demands research on the whole dietary (and lifestyle) contexts in which milk fat is consumed.

In the 1995 National Nutrition Survey (Australian Bureau of Statistics, 1995), Australian adults reportedly consumed approximately 17% of fat from dairy foods (inclusive of butter) and the corresponding figure has been reported to be 12.3% for total fat and 24.3% for saturated fat intake in the American diet (Huth *et al.*, 2006). This indicates that a large majority of dietary fat consumed in the diet is provided by food sources other than dairy foods. Dairy products are often perceived as weight inducing by individuals (Gulliver and Horwath, 2001) and the National Heart Foundation in Australia (National Heart Foundation (Australia), 2009) recommends low-fat dairy products in preference to whole varieties in individuals over the age of 2 years, as well as other governmental and scientific agencies in the world (National Cholesterol Education Program (NCEP) Expert Panel, 2002).

Finally, a novel aspect of dairy fat that has received attention in recent years is the milk fat globule membrane (MFGM). The triacylglycerols in milk are secreted in the alveolar lumina in the form of droplets, coated with a membrane, the MFGM. The MFGM contains a large array of bioactive components and it is possible that these can contribute greatly to the nutraceutical value of milk (Cavaletto *et al.*, 2008). New ''proteomics, functional genomics, and other ''omics'' techniques will advance this research area in the future (Cavaletto *et al.*, 2008; German, 2009; German *et al.*, 2002).

VII. EFFECTS OF INDIVIDUAL DAIRY FOODS

Dairy foods come in a range of forms with varying nutritional profiles and a history of various forms of processing and storage. It seems logical that this may influence effects on health. Despite all having several

homogenous qualities, including the provision of calcium, other minerals, vitamins, protein, and other bioactive components; items that are traditionally defined as ''dairy foods'' may vary considerably in terms of their effect on weight management and metabolic outcomes (Nestel, 2008).

Cheese, for example, has been demonstrated in clinical trials to have a smaller effect in raising LDL-cholesterol in comparison to butter and even milk (Tholstrup *et al.*, 2004). Cheese is unique from other dairy products due to its physical structure, whereby its fat content is encapsulated within a casein structure (Fellows, 2000).

Whether this structural anomaly may explain the largely null effect of cheese consumption on LDL-cholesterol remains a further area of research interest. Cheese also contains a large variety of bioactive peptides including angiotension-converting enzyme (ACE)—inhibitory effect, which may have hypertensive effects (Walther *et al.*, 2008). Despite this, analyses of cross-sectional data have suggested that cheese consumption may result in a greater risk of obesity and the metabolic syndrome (Nestel, 2008). Cheese intake was inversely related to a first myocardial infarction in a Norwegian study (Biong *et al.*, 2008), but in a Costa-Rican study, higher cheese consumption was associated with increased risk of myocardial infarction. In this same study, the consumption of low-fat milk produced no association (Kabagambe *et al.*, 2003). Thus, again, the observational research is inconclusive and the reasons may relate to the total diet and lifestyle context of cheese consumption.

On the other hand, fermented dairy products such as yoghurt have been hypothesized to reduce LDL-cholesterol due to their effects on encouraging a gut microbial environment to facilitate the production of short chain fatty acids and thus reduce the synthesis of cholesterol (Nestel, 2008). Fermented dairy has in fact been proposed as a nutraceutical with cholesterol-lowering potential (Chen *et al.*, 2008).

That not all dairy products have the same effect on disease risk has been shown in a study from Finland. In this study, reported cream intake was surprisingly inversely related to ischemic stroke, while intakes of other dairy products were not (Larsson *et al.*, 2009). Further analyses in the Rotterdam study revealed that milk and milk products (all kinds of milk, yogurt, coffee creamer, custard, curd, pudding, porridge, and cream) were inversely related to hypertension during follow-up, while high-fat dairy products (above 3.5% fat) were not (Engberink *et al.*, 2009).

When the relationship between dairy products and CHD has been studied in prospective cohort studies, the results have been mixed (Gibson *et al.*, 2009). These differences were to a large extent explained by different dairy products and possible differences in effects between the sexes. As pointed out by Tholstrup (2006), it is important to remember that different studies have used different methods to collect information on dietary intake. This makes it difficult to compare the results from one

study to another. It is always possible that the apparent beneficial associations between dairy products and CVD may be due to confounding by other healthy lifestyle choices among dairy eaters. Attempts are made to adjust for various confounding factors; however, there may still be other factors that have not been accounted for (Tholstrup, 2006).

VIII. BIOMARKERS OF MILK FAT INTAKE

To assess the impact of dairy food on health, measures to objectively assess intake are critical. As stated earlier in this chapter, one of the major limitations of nutrition research is the utilization of self-reported dietary intake data. An increase in awareness of healthy eating patterns as a result of advertising and nutrition education may exacerbate bias associated with self-reported dietary intake methodology (Johansson *et al.*, 1998). This is especially the case of at-risk groups. For example, obese people are more prone to underreporting dietary intakes (Livingstone and Black, 2003).

Fatty acid biomarkers reflecting milk intake help address issues associated with self-reported dietary intake data such as memory bias, over- and underreporting, and issues with methodological tools utilized to acquire dietary data (Trabulsi and Schoeller, 2001).

Data on the proportions of different fatty acids in plasma lipid esters (cholesteryl esters, phospholipids, free fatty acids, or triacylglycerol), erythrocyte membranes, or adipose tissue may provide a more objective and accurate path to evaluating dietary fatty acid composition (Arab, 2003; Baylin and Campos, 2006). The fatty acid composition in blood and body tissues reflects the fatty acid composition of the diet at different time points after ingestion. Short and medium-term changes in the composition of dietary fatty acid intake are reflected in plasma lipids and erythrocyte membranes, weeks and months after intake, respectively. The incorporation of fatty acids in adipose tissue reflects long-term changes in the diet (years) (Baylin and Campos, 2006; Katan *et al.*, 1997; Ma *et al.*, 1995; Zock *et al.*, 1997).

Two saturated fatty acids, pentadecanoic acid (15:0) and heptadecanoic acid (17:0), in adipose tissue (Baylin *et al.*, 2002) and serum lipids (Smedman *et al.*, 1999; Sun *et al.*, 2007a; Wolk *et al.*, 1998) have been proposed and validated as biomarkers of dietary ruminant fat intake, that is, mainly from milk fat and to lesser extent from ruminant meat. The human body is unable to synthesize fatty acids with an uneven number of carbon atoms, whereas ruminal microbes of cows have this ability (Wu and Palmquist, 1991). To measure the content of 15:0 and/or 17:0 in plasma lipids or adipose tissue is consequently a way to estimate the milk fat intake. It is known that the proportion of 15:0 and 17:0

increase in plasma lipids as a response to a diet higher in dairy products (Warensjo *et al.*, 2008; Wennersberg *et al.*, 2009).

While the adipose tissue content of 15:0 has been reported as a highly valid marker of habitual dairy intake, reflecting long-term intake, in both Swedish men (r = 0.74) (Wolk *et al.*, 2001) and women (r = 0.60) (Wolk *et al.*, 1998), adipose tissue biopsies are costly and invasive. The validity of 15:0 in adipose tissue as a biomarker of dairy fat has also been reported by Baylin *et al.* (2002) (r = 0.31) and Brevik *et al.* (2005) (r = 0.28). In the study referred to above by Wolk *et al.* (2001), the sum of the biomarkers (15:0 + 17:0) measured in serum phospholipids correlated moderately with milk fat intake (r = 0.50). The relative content of 15:0 in serum cholesteryl esters in men was also moderately correlated (r = 0.46) to total dairy fat intake (Smedman *et al.*, 1999) and this was confirmed in a recent study from the United States (r = 0.36) (Sun *et al.*, 2007c). Plasma levels of 15:0 have been demonstrated to correlate significantly (r = 0.26) with dairy intake in a study of 200 Costa Rican men and women (Baylin *et al.*, 2005).

Consequently, a more objective way to measure the habitual intake of milk fat would be the fatty acid composition of adipose tissue. However, this is not routinely performed in larger cohort studies, due to cost and that the procedure is invasive and less tolerated by study participants. Analysis of plasma fatty acid composition is thus a more feasible option for examination to determine dairy intake in the study population. While some groups have separated plasma into its constituent phospholipids and cholesterol esters to analyze serum 15:0 and 17:0 as markers of dairy intake (Smedman *et al.*, 1999), Baylin *et al.* (2005) found that plasma that was not separated into its constituent cholesteryl ester, phospholipids, and triacylglycerols was still able to reflect habitual dairy intakes comparably to adipose tissue. Thus, whole plasma is an acceptable alternative to fractionated plasma in the absence of adipose tissue for analysis to reflect habitual dairy intakes and may be a cost effective option for consideration when conducting future intervention studies to assess the affect of dairy products on health outcomes.

Few studies have studied the association between the milk fat biomarkers and disease risk. The following section gives a comprehensive overview of studies conducted so far.

A. Coronary heart disease

The first study was published by Warensjö *et al.* (2004) and conducted in Sweden. In this prospective nested case–control study (78 cases and 156 controls), the cases and controls were matched for matched for age, sex, sampling time, and geographical region. The proportions of 15:0 and 17:0 and their sum (15:0 + 17:0) in serum phospholipids were associated with

a reduced risk to develop a myocardial infarction. Adjustment for metabolic risk factors removed this relationship and BMI, triglycerides, cholesterol, insulin, proinsulin, and leptin were negatively correlated to the estimated milk fat intake. The inverse correlations between risk factors and the milk fat biomarkers provided a potential causal link between estimated intake of milk fat and reduced heart disease risk. Further, many of these negative correlations remained after adjustment for BMI, which may indicate that these associations are related to central obesity than to overweight per se. This is an important observation in relation to the metabolic syndrome since central obesity is considered a main driver in the syndrome. Adjustment for lifestyle factors such as physical activity and dietary habits were not carried out in this study. It is thus possible that the observed association between milk fat and reduced risk of myocardial infarction might be related to a ''healthy food pattern,'' that is, high consumption of fruits, vegetables, and cereals with a potential health benefits.

The second case–control study from Norway (Biong *et al*., 2006) was published in 2006. In this study, 112 myocardial infarction cases and 107 controls were enrolled. Subcutaneous adipose tissue samples were drawn within 4 days of the myocardial infarction and the fatty acid composition of the adipose tissue was analyzed. The study reported that the proportion of 15:0 in adipose tissue was inversely related to the risk of a first myocardial infarction. Also, other fatty acids (14:0, 14:1, and 17:1) associated with the intake of milk fat were inversely related to a first myocardial infarction. Most of these relationships remained (all except between 14:0 and MI) after adjustment for age, sex, waist-to-hip ratio, smoking, and family history of CHD.

The third and the most recent study reported that a high estimated intake of dairy fat was associated with a greater risk of heart disease (Sun *et al*., 2007c). In this study, 166 cases and 327 controls were nested within the Nurses' Health Study Cohort. The cases were matched for age, smoking, fasting status, and date of blood drawing. Fatty acid composition was quantified in plasma and erythrocyte membranes and food and nutrient consumption was assessed by semiquantitative food frequency questionnaire. The positive associations between plasma 15:0 proportions and the risk of ischemic heart disease remained after adjustment for established dietary and lifestyle factors and other fatty acids in plasma. Women in the highest tertile of 15:0 in plasma had twice the risk of heart disease compared to those in the lowest tertile. The multivariate model in this study included BMI, postmenopausal status, postmenopausal hormone use, physical activity, alcohol intake, aspirin intake, and parental history of myocardial infarction before the age of 65, history of hypertension and diabetes, linoleic acid, and trans-fatty acids in plasma and erythrocytes.

Consistent with the former two studies, Sjogren *et al*. (2004) reported that 15:0 and 17:0 in plasma phospholipids to be associated with a less atherogenic LDL profile in a cross-sectional study from Sweden. Further, the proportion of 15:0 in plasma cholesteryl esters was negatively correlated with the serum cholesterol concentrations in both adolescent girls ($r = 0.32$) and boys ($r = 0.32$) (Samuelson *et al*., 2001).

Thus, the two case–controls studies from Scandinavian countries have reported beneficial associations between milk fat biomarkers and heart disease, while the study from the United States reported a positive association. The differences in results may well reflect the type of foods and the context in which the dairy food was consumed. In the United States, cheese and milk are commonly found in takeaway meals such as cheeseburgers and shakes, and often consumed with less healthy foods such as fries.

B. Stroke

The only study found here came from Scandinavia. The estimated intake of milk fat, mirrored as the proportions of 15:0, 17:0, and 15:0 + 17:0 in plasma phospholipids, was inversely related to a first stroke in a smaller case–control study. The odds ratio to have a first ever stroke was 0.41 95% CI [0.24–0.69] for each standard deviation increase of 17:0 in the plasma phospholipid fraction. These associations remained after adjustment for cardiovascular risk factors, other food groups, and physical activity. A similar, but nonsignificant, trend was seen in men (Warensjo *et al*., 2009). Again, this apparent protective effect may reflect the cultural diet and associated lifestyle.

C. Type 2 diabetes mellitus

Likewise in the single study involving type 2 diabetes, Krachler *et al*. (2008) measured 15:0 and 17:0 in erythrocyte membrane fatty acids and investigated their relation to the development of type 2 diabetes mellitus. The study included 159 individuals with type 2 diabetes mellitus and 291 sex- and age-matched controls. Higher proportions of 15:0 and 17:0 were associated with a lower risk of diabetes. After adjustment for BMI, HbA1c, alcohol intake, smoking, and physical activity, the association between the milk fat biomarkers and diabetes remained (Krachler *et al*., 2008).

Thus, studies involving milk fat biomarkers produced results that were similar to those using dietary intake measures. As the forms of error were different, the results provide some confidence in conclusions that can be drawn from the observed relationships.

IX. POSSIBLE MECHANISMS OF EFFECT

Given its nutrient rich nature, the compositional complexities of milk means the effects on health and disease are not likely to be due to a single mechanism (Elwood *et al.*, 2008), but several hypotheses have been put forward. Table 1.3 presents possible mechanisms that may be responsible for association between dairy food and weight management. These mechanisms are discussed in the following sections.

A. Calcium flux

One of the most frequently reported proposed mechanisms of action is hypothesized to be attributable to the relatively large proportion of the mineral calcium within dairy products. Zemel (2008) proposed an explanation for this phenomenon as being related to an overall decrease in lipogenesis and an increase of lipolysis. The bioactive form of vitamin D (1,25-dihydroxyvitamin D3) generally promotes the uptake of calcium into adipocytes, stimulating lipogenesis. However, the consumption of a large amount of calcium is said to depress the action of this bioactive vitamin D, inhibiting the influx of calcium and reducing the accumulation of lipid into fat cells.

TABLE 1.3 Possible mechanisms responsible for association between dairy food and weight management

Mechanism	Action	Reference
Calcium flux	Decrease in lipogenesis and an increase of lipolysis	Zemel (2004)
Fecal fat excretion	Formation of insoluble calcium fatty acid soaps resulting in a greater overall fat excretion	Jacobsen *et al.* (2005)
Fat oxidation	Increased energy expenditure and a lowered respiratory quotient (higher oxidation of fat)	Cummings *et al.* (2006), Melanson *et al.* (2003)
Satiation	Increased release of cholecystokinin (CCK), a hormone related to satiation	Schneeman *et al.* (2003)
Nutrigenomic effects	Prevention of DNA damage	Fenech *et al.* (2005)
	Expression of genes	Boon *et al.* (2005)

More recently, Bortolotti *et al*. (2008) provided evidence to refute this calcium-mediated mechanism of weight loss, presenting results from a placebo-controlled crossover study of 10 obese adults with habitually low calcium intakes (<800 mg/day). Results indicated that dietary supplementation of 800 mg of calcium/day had no effect on circulating plasma free fatty acid concentrations or glycerol turnover. Theoretically, a calcium-mediated stimulation of lipolysis would have resulted in an increase in plasma free fatty acid concentrations and glycerol turnover, thus indicating a need for further research.

While the high proportion of the mineral calcium in dairy products has been hypothesized as the factor contributing to favorable metabolic outcomes (Zemel, 2001), several studies have identified more favorable health outcomes in intervention trials whereby calcium is administered in the form of dairy products in contrast to supplementation (Zemel, 2004, 2008). It may be that the calcium phosphate found in dairy products exerts a more significant weight loss effect as opposed to the calcium citrate or calcium carbonate utilized in supplements (Lorenzen *et al*., 2006).

Therefore, other bioactive components in dairy products may have an effect on the metabolic syndrome either independently or in addition to the calcium effect.

B. Fecal fat excretion

The role of calcium as a major component of dairy products in weight loss may be further explained by the positive correlation identified between dietary consumption of the mineral with fecal fat excretion (Jacobsen *et al*., 2005). This finding may be attributable to the formation of insoluble calcium fatty acid soaps upon consumption of calcium with a fat source, such as that found in dairy products resulting in a greater overall fat excretion in comparison to diets low in dairy calcium (Lorenzen *et al*., 2006). Dairy products have also been found to increase fecal energy, in a randomized crossover study of 10 healthy, moderately overweight adults, by Jacobsen *et al*. (2005), fecal fat excretion increased by 5.4 g from baseline following 1 week consumption of a diet providing an additional 1000 mg calcium from low-fat dairy products. Fecal fat and energy excretion was estimated at 350 kJ/day in this sample, which may have a significant effect on body mass in the long term.

C. Fat oxidation

Another possible mechanism to explain the potential effect of dairy products on weight loss derives from the observation that intakes of dietary calcium and dairy products have been associated with increases in energy expenditure and a lowered respiratory quotient. A lower respiratory

quotient indicates a higher oxidation of fat. Mechanisms to increase postprandial fat oxidation are advantageous for overweight individuals due to their reduced capacity to utilize fat as a fuel source (Blaak *et al*., 2006). Acute intakes of calcium both in supplemental form and from dairy products have been demonstrated to correlate positively with fat oxidation over a 24-h period ($r = 0.38$, $P = 0.03$) in 35 healthy weight adult subjects (Melanson *et al*., 2003). Similarly, Cummings *et al*. (2006) reported least suppression of postprandial fat oxidation following consumption of a high calcium intakes from a dairy meal in comparison with a high calcium intakes from a nondairy meal and a low calcium control meal in 10 overweight and obese adult subjects (BMI $= 32.5 \pm 0.97$ kg/m2).

While there is a lack of research regarding the effect of chronically high dairy product consumption on fat oxidation and energy expenditure, a small study by Gunther *et al*. (2005b) demonstrated that in healthy weight female volunteers consuming 1000–1400 mg of calcium from dairy products over a 1-year period, whole body fat oxidation had increased compared to controls with a habitually low dairy product intake (calcium $<$800 mg/day) regardless of the calcium content of the meal challenge.

D. Satiation

The potential for dairy products to be more satiating than their nondairy counterparts is another possible mechanism of action on weight loss. Evidence for the satiating effect of dairy products was provided by Schneeman *et al*. (2003) in a randomized crossover feeding study of 24 healthy adults, test meals containing two-thirds of energy from both full fat and low-fat dairy products increased concentrations of cholecystokinin (CCK) more significantly than nondairy test meals. Test meals providing full fat dairy products resulted in the greatest rise in CCK concentration in this group (Schneeman *et al*., 2003).

Despite the fact that each of these proposed mechanisms may provide an insight into the action of dairy products on health, it is likely a complex interplay between all of these identified factors exists. Furthermore, as nutrition science continues to evolve consideration of the effect of dairy products on genome health may further explain proposed mechanism of action for favorable health outcomes reported.

E. Nutrigenomic effects

Dairy foods present a unique opportunity from a nutrigenomics perspective, given the nature of mammalian milk as a complete source of nutrition which has been adapted over time to meet the needs of particular animals (German, 2009). The potential for dairy products to prevent DNA damage was reported by Fenech *et al*. (2005), who identified a reduced

rate of genome damage in lymphocytes in individuals with higher consumptions of micronutrients predominantly found in dairy products. Boon *et al*. (2005) reported a significant reduction in the expression of fatty acid synthase mRNA in the adipose tissue of healthy weight subjects consuming 2500 mg of calcium from dairy products per day.

While such work is still in its infancy, exposing the position of dairy products on genome health may complete the ''mechanistic puzzle'' regarding dairy products and health.

X. CONCLUSION

Dairy foods may well play a very significant role in the prevention and amelioration of obesity-related chronic diseases. As these conditions are affected by the whole diet, however, the effects of dairy foods will always be relative. The challenge for future research is to work with the substantial body of knowledge that has emerged in recent years to better expose how these effects might be navigated.

Obesity-related chronic diseases represent a gradual decline of the metabolic system, with body fat overload likely to be the key feature (Lebovitz, 2006). Evidence for these effects can be shown through intermediary biomarkers such as circulating lipid levels and hormone levels addressing glucose homeostasis, inflammation, and appetite control. End points can be seen as changes in body weight, development of disease (such as hypertension, type 2 diabetes, and CVD), and incident mortality. In the case of dairy foods, evidence for the relationship between their consumption and these variables tends to suggest a protective and supportive role for dairy foods, but the landscape is problematic. The mechanistic effects of the component parts of dairy foods suggest that the positive effects are plausible, yet a distinction clearly lies between the effects of single nutrients, whole foods, and whole diets.

If body fat is indeed the offending component of the disease process, it is well to acknowledge that it goes on slowly and then responsive mechanisms come into play. Food may not just supply calories that lead to body fat (or its reduction), but it may also affect mediators for these mechanisms. There is some evidence, for example, that dairy foods may help to improve metabolic variables reflected in measurements of hypertension, body composition/fat mass, insulin resistance, and energy expenditure (German *et al*., 2009). The effects of dairy foods on weight loss are inconclusive, but weight loss is a total diet effect. The main effect of dairy foods may be to provide metabolic advantage under adverse conditions of energy overload and/or dietary restraint. This might prove beneficial in preventing weight regain may also explain the apparent role in protection against type 2 diabetes.

The situation with CVD is more problematic and this is where closer attention to the type of dairy foods may be required.

Given research to suggest that small incremental weight gains affect a large proportion of the population and are a significant contributor to the obesity epidemic, perhaps more emphasis should be placed on the dietary factors which may ameliorate such a phenomenon. Perhaps, future intervention studies examining the effect of dairy on body mass should be designed to detect this potential effect.

It is clear that evidence to support role of dairy on weight management is a key research area, and while calls have been made for further substantiation to determine whether a true food effect is at play (Lamarche, 2008), it might also be important to discern how a ''food effect'' occurs. This is not likely to be the same as a pharmaceutical effect. A review by Parikh and Yanovski (2003) identifies the need for large, population based, clinical trials assessing the effect of dairy products on body weight, but there needs to be some development of the theoretical framework in which this might be assessed.

Paradoxically, evidence seems to suggest a consistent association with dairy product intake and improved metabolic profiles. Given the lack of consensus regarding the role of dairy products as a facilitator for weight loss and the difficulty in controlling all other potential confounding factors to explain this effect perhaps this is the area that demands the greatest research interest.

While weight loss is important in terms of improving health and ameliorating metabolic risk factors, the emerging evidence to indicate a positive effect of dairy in terms of improving weight maintenance following weight loss, prevention of weight gain, and preservation of fat free mass should not be underestimated (Major *et al.*, 2008). In contrast to more novel foods which have similarly demonstrated health effects, dairy products are consumed habitually several times a day by a large proportion of the population, thus having a unique opportunity to positively influence the health of many. Perhaps, more effort should be made to consolidate the position of dairy products as an integral component of a healthy diet in this regard. When trying to identify favorable health outcomes associated with dairy intake, it is important to note that it is widely hypothesized that much of the observed metabolic benefits of dairy may be attributable to a healthier lifestyle overall. Thus, while it may not be possible to separate out a ''dairy effect,'' it may be possible to consider the ''dairy contribution.''

Individuals with higher intakes of dairy have been hypothesized as more likely to be better educated and participate in physical activity more frequently, although few studies have tested this theory. Because overall health is largely influenced by level of education, socioeconomic status (Adler and Newman, 2002), and level of physical activity (Warburton

et al., 2006), it is difficult to make meaningful assumptions about the role of dairy in weight management and favorable metabolic profiles. However, in studies where the relationship between dairy intake and disease risk have been investigated and an attempt to adjust for potential healthy lifestyle characteristics have been carried out, the results remain in some instances. This rule consequently out the possibility that healthy lifestyle factors have mediated the effects. Even though adjustment for lifestyle factors relies on self-reported data which is prone to reporting bias, it does suggest that dairy does contribute in a significant way.

From the total diet perspective, Azadbakht *et al.* (2005) reported that individuals in the highest quartile of dairy intake also consumed a higher proportion of nutritionally advantageous foods such as whole grains, fiber, fruit, and vegetables. Thus, while it is difficult to isolate dairy products as being the sole cause of the improved health profile, it certainly appears part of the team. Similarly, Kant *et al.* (2000) identified a lower risk of mortality in women who consumed a diet similar to dietary guideline recommendations encouraging intake of fruits, vegetables, whole grains, and low-fat dairy products. This ''healthy person'' theory is often presented when mechanistic studies with dairy components as the nutrient of interest fail to show a clinically significant improvement in metabolic risk factors. For example, in a further cross-sectional study of 827 healthy subjects, Azadbakht *et al.* (2005) reported an inverse association between dairy consumption and the risk of acquiring the metabolic syndrome, as measured by waist to hip circumference. This association was declared to be markedly less significant in those consuming a high-calcium diet from nondairy sources, an outcome consistent with the findings of Zemel (2004).

The metabolic effects of dairy food consumption have been further supported in a recent study by Wennersberg *et al.* (2009). Here, a possible threshold effect of the impact of dairy products on markers of metabolic risk was identified in an intervention study of 121 overweight subjects. Following 6 months of consumption of 3–5 serves of dairy products per day, no significant improvement in blood pressure, adiponectin, body fat, body composition, and several other metabolic markers was evident between the intervention and a control group. However, post hoc analyses indicated that in individuals with habitually low baseline calcium intakes ($<$700 mg/day), significant reductions in waist circumference ($P = 0.003$) and saggital abdominal diameter ($P = 0.0034$) were observed in the intervention group. The compliance with the milk intervention in this study was confirmed with an increased proportion of the milk fat biomarker 15:0 measured in serum cholesteryl esters. The authors concluded that the study did not support the evidence that dairy products or calcium in dairy have a favorable effect on body weight as suggested by Zemel (2008), but it did support the findings of a 1-year trial by Gunther

et al. (2005a) that reported no effect on body fat mass or weight in women receiving either an isocaloric control diet, or medium, or high content dairy diet.

Thus, it appears there are no real ''weight loss'' foods, which should be obvious given that foods deliver calories and nutrients. Dairy foods, however, supply significant nutrients that are highly relevant to metabolic processes that may assist with energy balance and the prevention of insulin resistance and risk factors for CVD (German *et al*., 2009). Not all dairy foods appear to be the same, and their effects may be different for different stages of metabolic dysfunction. There may be extra advantages in fermented products such as yoghurt, and the effects of cheese need to be studied in more supportive dietary contexts. Low-fat milk might be more beneficial in some contexts than others. Mechanisms of effects for further research include calcium flux, fecal fat excretion, and effects on satiation and gene–nutrient interactions. Component parts of dairy foods, such as peptides and the types of fats delivered, offer both promise for the future and conundrums for elemental versus whole food research. This research will benefit greatly from studies of dairy intake biomarkers. This research has provided a novel and informative contribution to the body of knowledge on the effects of dairy food consumption on obesity-related chronic disease. From a clinical and public health perspective, however, the most important point is that these effects are contextual. Dairy foods need to be consumed in the company of other foods that also deliver supportive components, and that together provide just enough calories as the human body requires.

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