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Nutritional Significance of Milk Lipids

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17.1. Introduction

Fat is a concentrated source of energy that provides 30–40% of dietary calories in developed countries. Fat imparts palatability to food, serves as a vehicle for fat-soluble vitamins A, D, E and K and supplies essential fatty acids. The digestion products of fats, along with endogenously synthesized lipids, provide a diverse group of molecules that play a critical role in multiple metabolic processes.

Triacylglycerols are the major storage form of energy in animals. They also protect the body against both cold and heat loss and protect many organs from trauma due to external forces associated with day-to-day living. Lipids are vital components of cell membranes and take part in many inter- and intra-cellular signalling cascades. Lipids have multiple forms and functions, including vitamins, steroid hormones, and eicosanoids that are involved in many metabolic processes.

In the previous edition of this series, Gurr (1994) provided a chapter on the nutritional significance of lipids, which included aspects of their physiology and biochemistry. Since then, considerable advances have been made in lipid research, which Gurr *et al.* (2002) have covered recently in a very readable textbook. This chapter concentrates on the nutritional significance of milk lipids, a subject that has received scant attention in recent times. This omission is, no doubt, influenced by the negative nutritional image milk fat has acquired in recent decades due to its perceived association with coronary artery disease, and more recently to the belief that fat in general may be linked to cancer at some sites and to the current obesity epidemic.

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Advanced Dairy Chemistry, Volume 2: Lipids, 3rd edition.
Edited by P.F. Fox and P.L.H. McSweeney, Springer, New York, 2006.

17.2. Dietary Fat and Obesity

During the past two to three decades, the prevalence of obesity in most countries has increased dramatically. In affluent countries, it is estimated that 30% of adults are obese and another 35% are overweight. Apart from aesthetic concerns, excess adiposity can induce multiple metabolic abnormalities that contribute to a number of life-threatening diseases. Excess weight is responsible for 30–40% of heart disease, which no doubt occurs through linkage to other risk factors, such as hypertension, dyslipidemia, and type 2 diabetes with its associated insulin resistance and hyperinsulinemia. Other conditions associated with excess weight are cancer at several sites, including breast and colon, gall bladder disease, sleep apnea and an increased incidence of osteoarthritis (Willett, 2002).

Regional distribution of body fat can influence health risks. Subjects with upper-body obesity, where fat has accumulated in subcutaneous and visceral deposits are more prone to metabolic defects than those with peripheral obesity, where fat is stored in the buttock, thighs and lower abdomen (Lafontan and Berlan, 2003). Certain individuals have a genetic propensity to store excess energy as fat; others may harbour mutations in genes that regulate energy balance (Friedman, 2003). However, genetic changes are not responsible for the soaring incidence of obesity during the past 30 years. Rather, the incidence generally reflects an imbalance between energy intake and energy expenditure due to the combination of an abundant supply of cheap, palatable, energy-dense foods and an increasingly sedentary lifestyle.

The amount of fat in the diet has been blamed for the increase in obesity. There are two reasons for this. Firstly, the energy value of fat is 9.0 kcal/g compared to 4.0 kcal/g for protein and carbohydrate. Secondly, the prevalence of overweight individuals tends to be higher in affluent countries with a high fat intake than in underdeveloped countries with low fat intakes. However, these between-population (ecologic) comparisons are seriously confounded by differences in a number of lifestyle factors including types and availability of food and in physical activity. Moreover, the extensive increase in the incidence of obesity and overweight occurred during the period when there was a substantial decline in the percentage of energy derived from fat (Willett, 2002).

Within-population epidemiological studies can avoid confounding due to differences in lifestyle, ethnicity and food patterns found in ecological studies. Seidell (1998) and Willett (2002) have reviewed within-population studies on the relation between fat intake and obesity. Case-control studies have produced inconsistent results. This format can be prone to serious biases because obese subjects have been shown to underestimate their energy and fat intake by up to 50%. Non-obese subjects, on the other hand, tend to

have healthy lifestyles, aspects of which are difficult to control statistically. Prospective (cohort) studies should, but do not always, overcome these biases. Nevertheless, the outcomes of studies that investigated the association between percentage of energy from fat and weight gain were inconsistent. Thus, there is no conclusive evidence from epidemiological studies that intake of dietary fat promotes the development of obesity independently of total energy intake (Seidell, 1998; Willett, 2002).

17.2.1. Randomized Control Trials

The randomized clinical trial is the most reliable method for assessing the efficacy of diet or drug therapy. A number of systematic reviews have been conducted on randomized clinical trials that investigated low-fat diets versus other weight-reducing diets in overweight or obese individuals (Willett, 2002; Kris-Etherton *et al*., 2002; Pirozzo *et al*., 2003; Sanders, 2003).

Short-term dietary intervention studies on overweight and obese individuals show that diets with a lower percentage of calories from fat lead to modest weight losses (1–4 kg). However, in trials that lasted for one year or more, there were no significant differences in sustained weight loss between diets containing 18–40% of energy derived from fat and other weight-reducing diets. Furthermore, overall weight loss at the conclusion of these studies was of the order of only 2–4 kg. There is no evidence to suggest that low-fat diets are more beneficial than other diets of equal energy density for the prevention of weight regain.

A study using whole-body calorimetry showed that there was no significant difference in net fat accumulation in lean or obese women when fed controlled excess of dietary energy supplied by the monosaccharides glucose and fructose, the disaccharide sucrose, or fat (McDevitt *et al*., 2000).

17.2.2. Safety of Low-Fat, High-Carbohydrate Diets

Low-fat, high-carbohydrate diets have been shown to lower plasma low-density lipoprotein (LDL) cholesterol levels. At the same time, these diets decrease anti-atherogenic high-density lipoprotein (HDL)-cholesterol, and increase concentrations of plasma triglycerides, lipoprotein[a] (Lp[a]), and small dense LDL, plus increasing insulin resistance. Overall, these changes are likely to increase the risk of coronary heart disease (Willett, 2002; Kris-Etherton *et al*., 2002; Sanders, 2003).

17.2.3. Energy Value of Milk Fat

The energy value of a fatty acid is dependent on the ratio of its carbon atom content to its oxygen atom content. Stearic acid ($C_{18:0}$) has a high

carbon to oxygen ratio and the heat of combustion, used to calculate the energy value, is 9.48 kcal/g. On the other hand, butyric acid ($C_{4:0}$) has a low carbon to oxygen ratio and a heat of combustion of 5.92 kcal/g.

Furthermore, after digestion of milk fat, the fatty acids with chain lengths of less than 12 carbon atoms are absorbed from the intestine, pass to the portal circulation and are transferred to the liver, where they are rapidly metabolized by β-oxidation. In contrast, the longer-chain fatty acids are resynthesized into triacylglycerols in the enterocytes and are packed into chylomicrons that enter the lymph and are transported up the thoracic duct and emptied into the venous circulation. In the capillaries of extra-hepatic tissues, but mainly adipose tissue and muscle, lipoprotein lipase hydrolyses triacylglycerols of chylomicrons to fatty acids. Muscle cells consume these acids as fuel, whereas adipocytes incorporate them into triacylglycerols for energy storage. When triacylglycerols are required for energy their fatty acids are degraded by β-oxidation to acetyl-CoA and then to ATP. For saturated fatty acids, oxidation decreases with increasing carbon chain-length.

Up to one-third of the fatty acids in milk fat have a chain-length of 14 carbons or less. Because these acids are oxidized rapidly in the liver, have a lower energy value and are oxidized more readily than long-chain fatty acids, it follows that milk fat should contribute less to overweight than an equivalent amount of other dietary fats (Parodi, 2004). A study by Schneeman *et al.* (2003) showed that milk fat is a more potent stimulator of cholecystokinin than a blend of non-milk fat with a similar ratio of polyunsaturated to saturated fatty acids. Cholecystokinin is a "satiety" hormone released into the blood stream by the intestine during feeding and acts to suppress further eating.

17.3. Dietary Fat and Cancer

As recently as the early 1980s, public health organizations were recommending a reduction in fat intake for the prevention of cancer. This recommendation was based largely on evidence from early animal studies and from international comparison studies, which showed strong correlations between national *per capitut* fat consumption and the incidence of cancer (Willett, 2001a; Kushi and Giovannucci, 2002). However, other aspects of Western lifestyles, including positive energy balance and physical inactivity, seriously confound ecological studies; moreover, population correlations tell nothing about the diet of individuals who get cancer and those who do not.

On the other hand, within-population studies of a cross-sectional format have yielded conflicting results, but large prospective studies, which

provide the most rigorous evidence of association, have not supported an important role for total fat intake and cancer (Willett, 2001a; Kushi and Giovannucci, 2002). A brief outline of the role of dietary fat in the development of colon (colorectal), breast and prostate cancers—the major non-smoking related malignancies—follows. It should be appreciated that fat or a sub-type of fat is usually not consumed as a single unit, but rather as a component of a food item, that may contain both pro- and anti-cancer agents. For example, dairy products contain calcium, whey proteins as well as lipid components with anti-cancer potential (Parodi, 2001b).

17.3.1. Colon Cancer

International comparitive studies show a strong correlation between *per capitut* disappearance of fat and rates of colon cancer (Willett, 2001a; Kushi and Giovannucci, 2002). Early animal studies suggested that dietary fat plays an important role in the initiation and promotion of colon tumorigenesis. However, later evidence showed that total energy intake, rather than fat intake, was more likely to influence tumor development (Howe *et al*., 1997).

A number of case-control studies have shown an association between the risk of colon cancer and intake of fat. In many of these studies, a positive association between total energy intake and the risk of colon cancer was also noted, which is important because of the high correlation between fat intake and energy consumption. Other studies showed that physical inactivity or excess energy intake relative to requirements strongly increased the risk of this malignancy (Giovannucci and Goldin, 1997). Howe *et al*. (1997) combined the data from 13 case-control studies and found a significant association between total energy and the risk of colorectal cancer. However, neither total, saturated, mono- nor poly-unsaturated fat was associated with risk of colorectal cancer independent of total intake of energy.

Three of the four large prospective studies that adjusted for energy intake, the Netherlands Cohort Study (58,279 men, 62,573 women), the Iowa Women's Health Study (35,215 women), and the Health Professionals Follow-up Study (47,949 men), did not find an association between total fat intake or class of fat and the risk of colon cancer. The fourth study, the Nurses' Health Study (88,751 women) showed that animal fat, but not vegetable or fat from dairy products, was positively associated with the risk of colon cancer. Because intake of red meat has been associated with colon cancer in a number of studies, the increased risk may be linked to compounds like haem and heterocyclic amines formed during high-temperature cooking of meat rather than to fat (Giovannucci and Goldin, 1997; Willett, 2001a; Kushi and Giovannucci, 2002).

Overall, epidemiological evidence from within-population studies does not support an independent role for dietary fat or fat subclasses in the risk of colon cancer. However, the evidence does not preclude the possibility that certain fatty acids, such as ω-6s and ω-3s, may exert opposing influences.

17.3.2. Breast Cancer

There is an extensive literature on the association between fat intake and the risk of breast cancer. Many animal studies showed that diets high in fat are associated with an increased incidence of chemically induced tumor development. However, these associations may be due to the effect of total energy intake. The study of Ip *et al.* (1990), designed specifically to determine the effect of fat, independent of energy intake, showed that:

- Mammary tumorigenesis was very sensitive to the level of linoleic acid in the diet and increased proportionally in the range of 0.5–4.4% at which the effect leveled off
- There may be a small effect of fat distinct from the calorie effect
- Calorie restriction is more striking than a decrease in dietary fat in suppressing tumor development
- Reducing calorie intake can interrupt the promotion of mammary cancer by a high fat diet

High intake of total fat is correlated with an increased incidence of breast cancer in international comparative studies. In addition to the usual factors that confound associations between dietary fat and cancer in this type of study, countries with a high fat intake also have a lower age at menarche, later age at first birth, lower parity and higher post-menopausal body weight, which are risk factors for breast cancer (Willett, 2001b; Kushi and Giovannucci, 2002).

Evidence from case-control studies for an association between total fat intake and the risk of breast cancer is inconsistent (Willett, 2001b; Kushi and Giovannucci, 2002). On the other hand, prospective studies offer no support for an association between breast cancer risk and intake of total fat or specific types of fat. Hunter *et al.* (1996) conducted a collaborative-pooled analysis of original data from seven large prospective studies published up to 1995 that represented 4980 cases. The analysis found no evidence of an association between the intake of total, saturated, mono- or poly-unsaturated fat and the risk of breast cancer. There was no reduction in risk even among women whose energy intake from fat was less than 20% of total energy intake. What is more, for the small number of women reporting less than 15% energy from fat, the risk of breast cancer increased more than

twofold. A follow-up pooled analysis by Smith-Warner *et al.* (2001), which included 7,329 cases, supported the lack of association between total fat, fat class, animal or vegetable fat intake and the risk of breast cancer.

The 14-year follow-up of the Nurses' Health Study, the largest of the prospective studies with 2,956 cases, also found no evidence of association between total or type of fat and breast cancer risk (Holmes *et al.*, 1999a). Further, no survival advantage was found for a low-fat diet or diets containing a particular fat type after diagnosis of breast cancer in this cohort of nurses (Holmes *et al.*, 1999b).

17.3.2.1. Specific Fatty Acids

Opposing effects of certain individual fatty acids could have influenced the lack of a relationship between dietary fat and fat type with the risk of breast cancer. Well-conducted animal studies suggest that linoleic acid promotes development of mammary tumors, whereas saturated, monounsaturated, and trans fatty acids have little or no effect. In many cases, ω-3 polyunsaturated fatty acids suppress tumor development. Conjugated linoleic acid (CLA) is the most potent anti-cancer fatty acid in that amounts of 1% or less of dietary fat can substantially inhibit the development of mammary tumors (Ip, 1997).

Nevertheless, at this time there is no persuasive evidence from epidemiological studies that any individual fatty acid is associated with the risk of breast cancer (Willett, 1997). A pooled analysis of nine prospective studies showed no association between the intake of various dairy products and the risk of breast cancer (Missmer *et al.*, 2002). However, in epidemiological studies there is often a high degree of correlation between individual fatty acids in the diet. This reduces the ability to detect an independent association between a single acid and cancer risk. Furthermore, dietary assessment during an epidemiological study may not reflect an individual's diet at the time of cancer initiation, which in the case of breast cancer may be in early life.

17.3.3. Prostate Cancer

Similar to cancer at other sites, there are strong correlations between *per capitut* fat intake and prostate cancer deaths in international comparative studies (Kolonel, 2001; Kushi and Giovannucci, 2002). Early case-control studies often showed positive associations between prostate cancer risk and total, animal, and saturated fat intake. However, in later studies in which energy intake was adjusted, only two of five found an increased risk for total fat intake, whereas two of eight found an increase in the risk of prostate cancer with animal fat intake (Kolonel, 2001).

There have been a number of prospective studies in which the relationship between fat intake and risk of prostate cancer was examined. Only four of these studies adjusted for energy intake. In these studies, there were no statistically significant associations between total fat, fat type or individual fatty acids and the risk of prostate cancer. An exception was a positive association with α-linolenic acid in the Health Professionals Follow-up Study (Kolonel, 2001).

Unlike colon and breast cancer, there is a lack of suitable animal models for mechanistic and dietary studies on prostate cancer. Studies that have investigated dietary fat intake and the role of individual fatty acids on prostate cancer risk have produced inconsistent results.

17.3.4. Comment

Evidence from well-conducted epidemiological studies does not support any meaningful associations between the intake of total fat, fat type or individual fatty acids and the risk of colon, breast or prostate cancer. Final proof of a null effect should come from randomized clinical trials. However, such trials seem unlikely because initiation of tumors may occur early in life, whereas the clinical symptoms arise late in life. The cost of appropriate trials would be prohibitive. Further advances, however, may come from improved dietary assessment and a better understanding of, and adjustment for, confounding factors in epidemiological studies.

17.4. Milk Fat and Coronary Heart Disease

By far the most telling negative nutritional aspect of milk fat is the belief that its content of saturated fatty acids and cholesterol elevate plasma cholesterol levels, which is a risk factor for coronary heart disease (CHD). During the early 1950s, it was found that the type of fat in the diet could influence plasma cholesterol level. Ahrens *et al.* (1957) showed that diets containing saturated fats, such as beef, lard and milk fat, produced higher plasma cholesterol levels than diets containing unsaturated fat like safflower and corn oil when they were fed under strict metabolic-ward conditions. Later, Connor (1961) reported that the level of cholesterol in the diet also influenced plasma cholesterol level.

Since then, there have been numerous studies that investigated the effect of different types and amounts of fat, individual fatty acids and other dietary components on plasma cholesterol level. It is now realized that all saturated fatty acids do not elevate plasma cholesterol levels to the same extent. The short-chain fatty acids, butyric ($C_{4:0}$), caproic ($C_{6:0}$), caprylic ($C_{8:0}$); the medium-chain capric ($C_{10:0}$); and stearic ($C_{18:0}$) acids,

like monounsaturated acids, have no discernible effect on plasma cholesterol level. On the other hand, the relative hypercholesterolemic effect of lauric ($C_{12:0}$), myristic ($C_{14:0}$) and palmitic ($C_{16:0}$) acids is controversial, but myristic acid may exert the greatest potency (Mensink *et al*., 2003).

17.4.1. Plasma Cholesterol and CHD

A number of studies, including several large prospective studies, such as the Framingham Study (Anderson *et al*., 1987), the Multiple Risk Intervention Trial (Stamler *et al*., 1986) and the Lipid Research Clinics Program (Pekkanen *et al*., 1990), as well as the Seven Countries Study (Verschuren *et al*., 1995) showed a positive correlation between levels of plasma cholesterol and mortality from CHD. However, epidemiological associations cannot prove causality and elevated cholesterol levels could be either a cause, a correlate or a consequence of CHD.

The early studies on CHD demonstrated that saturated fatty acids and cholesterol increase, whereas polyunsaturated fatty acids decrease plasma cholesterol levels, and that cholesterol levels are associated with CHD risk, led to the diet-heart or lipid hypothesis of CHD. Conversely, it followed that lowering the intake of saturated fatty acids and increasing polyunsaturated fatty acid intake will lower plasma cholesterol levels, which in turn will reduce the risk of CHD.

The studies that led to the lipid hypothesis measured plasma total cholesterol concentration. Cholesterol is insoluble in aqueous solution and needs to be combined with protein for transport in blood. These plasma lipoproteins are large heterogeneous aggregates that have different physical properties, such as density, chemical composition and metabolic function (Gurr *et al*., 2002).

Later epidemiological studies demonstrated that the low-density lipoprotein (LDL)-cholesterol, which is the predominant cholesterol carrier, like total cholesterol, was positively associated with the risk of CHD. On the other hand, HDL-cholesterol was negatively associated with the risk of CHD. Even between individuals having the same LDL-cholesterol level, those with a predominance of small, dense LDL particles have a much higher risk of CHD than individuals with a predominance of large, buoyant LDL particles (Gurr *et al*., 2002). It is notable that the $C_{12:0}$, $C_{14:0}$ and $C_{16:0}$ fatty acids that increase total and LDL-cholesterol the most, concomitantly increase the levels of anti-atherogenic HDL-cholesterol, such that there can be a beneficial decrease in the total:HDL ratio (Mensink *et al*., 2003).

Unfortunately, early studies that measured only levels of total cholesterol are still cited in reviews (e.g., Braunwald, 1997; Schaefer, 2002) to support the contention that restricting saturated fat and cholesterol intake

and increasing the intake of polyunsaturated fatty acids will reduce the risk of CHD. In addition, Ravnskov (1992) has pointed out that reviews supporting the lipid hypothesis written by distinguished scientific bodies often exhibited bias by ignoring non-supportive studies.

Since the time the lipid hypothesis was proposed, a number of other risk factors, that can be modified, for CHD have been reported. Of importance are hypertension, cigarette smoking, obesity and physical inactivity, diabetes mellitus, and elevated plasma levels of Lp(a) and homocysteine (Braunwald, 1997; Schaefer, 2002).

Data from the 25-year follow-up of the much-cited Seven Countries Study (Verschuren *et al*., 1995) showed that the level of plasma total cholesterol was linearly related to CHD mortality in all participating countries. However, the absolute levels of CHD mortality were strikingly different. At a serum cholesterol level of 5.2 mmol/L there was a fivefold greater mortality in Northern Europe than in Japan. These differences in mortality risk were established after adjusting for age, smoking and systolic blood pressure. This suggests that there are other powerful, as yet largely unknown, risk factors for CHD in industrialized communities. The WHO Multinational Monitoring of Trends and Determinants in Cardiovascular Disease (MONICA) Project measured CHD events and risk factors for 38 populations in 21 countries during a 10-year period (Kuulasmaa *et al*., 2000). The association between classic risk factors—namely, cigarette smoking, systolic blood pressure, BMI, and serum cholesterol—and trends in the incidence of coronary events was extremely weak or nonexistent.

Braunwald (1997) points out that fully half of all patients with CHD do not have any of the conventional risk factors (hypertension, hypercholesterolemia, cigarette smoking, diabetes mellitus, marked obesity and physical inactivity). Further, up to two-thirds of patients with CHD have what may be considered normal serum cholesterol levels (see references in Parodi, 2004). These facts suggest that the role of plasma cholesterol in CHD has been overemphasized and oversimplified.

17.4.2. Saturated Fatty Acids and CHD

Notwithstanding the fact that the consumption of certain saturated fatty acids can increase plasma cholesterol level, which is a risk factor for CHD, is there any direct evidence to indicate that saturated fatty acids are associated with the risk of CHD?

17.4.2.1. Epidemiology–International Comparative Studies

Most ecological studies showed a strong positive correlation between *per capitut* disappearance rates of dietary fat, dairy fat, saturated fat, or

cholesterol and mortality from CHD when simple regression analysis was employed (Ravnskov, 1998; McNamara, 2000; Schaefer, 2002). The inappropriateness of this type of study for attributing causality has been discussed in earlier sections.

17.4.2.2. Epidemiology–Case-Control Studies

Only a few case-control studies have investigated the association between CHD and the intake of saturated fat, polyunsaturated fat or total fat by CHD patients and by subjects free from CHD. Ravnskov (1998) lists six studies, none of which supported the hypothesis that saturated fat is associated with an increased risk, and polyunsaturated fat with a decreased risk of CHD.

17.4.2.3. Epidemiology–Prospective Studies

Ravnskov (1998) presented data for 28 cohorts from 21 prospective studies. In only three of these cohorts did the evidence show that saturated fat was associated with a statistically significant increased risk of CHD. CHD patients in three cohort studies had consumed significantly more polyunsaturated fat, and in only one cohort had CHD patients eaten less polyunsaturated fat than CHD-free participants. The cohorts included the Framingham Study and the large well-conducted Health Professionals Follow-up Study and the Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study. Since then, Hu *et al.* (1997) presented the 14-year follow-up data from the Nurses' Health Study. After adjustment for confounding variables in multivariate analyses, no statistically significant associations were found between intake of total fat, animal fat, or saturated fat and the risk of CHD.

Later, Hu *et al.* (1999) re-analyzed the fatty acid data from the 14-year follow-up results of the Nurses' Health Study. The saturated fatty acids were grouped as $C_{4:0} - C_{10:0}$, $C_{12:0} + C_{14:0}$, $C_{16:0}$, $C_{18:0}$, and $C_{12:0} - C_{18:0}$. After adjustments for confounding variables in multivariate analyses, none of the saturated fatty acid groupings was positively associated with the risk of CHD. The Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study also investigated the relationship between specific groups of saturated fatty acids and CHD risk (Pietinen *et al.*, 1997). After multivariate adjustment for energy and other confounding risk factors, the study found statistically significant inverse associations between the risk of CHD mortality and the intake of total saturated fatty acids and the so-called hypercholesterolemic fatty acids ($C_{12:0} - C_{16:0}$). What is more, this study showed positive associations between intake of polyunsaturated fatty acids and linoleic acid and the risk of death from CHD.

Epidemiological studies provide little, if any, evidence to support the hypothesis that saturated fatty acids, even those of chain length $C_{12:0} - C_{16:0}$ that can elevate serum cholesterol concentration, are associated with the risk of CHD. This may result from the increased plasma HDL-cholesterol concentration produced by saturated fatty acids largely compensating for the adverse effects of these fatty acids on LDL-cholesterol concentration (Hu and Willett, 2000). In addition, saturated fatty acids lower the level of plasma Lp[a], which is considered a significant risk factor for CHD (Mensink *et al*., 1992).

17.4.3. Dietary Cholesterol and CHD

17.4.3.1. Dietary Cholesterol and Serum Cholesterol

Clinical studies show that dietary cholesterol is a less potent regulator of plasma cholesterol than are saturated fatty acids. Results from meta-analyses predict that plasma cholesterol response to a 100 mg/day change in dietary cholesterol will be from 0.06 to 0.07 mmol/L. The data show that although dietary cholesterol elevates plasma total cholesterol and LDL-cholesterol level, it also increases the level of HDL-cholesterol such that there is little overall effect on the LDL:HDL ratio (McNamara, 2000).

17.4.3.2. Dietary Cholesterol and the Epidemiology of CHD

Numerous international comparative studies found significant positive correlations between *per capitut* consumption of cholesterol and CHD mortality using simple univariate regression analysis (McNamara, 2000).

Ravnskov (1995) listed cholesterol intake for CHD patients and controls from 14 within-country longitudinal studies. In only two of these studies were CHD patients found to have a statistically significantly higher intake of cholesterol than control subjects.

Kritchevsky and Kritchevsky (2000) provided a summary of the evidence linking dietary cholesterol to the risk of CHD in 10 cohorts from eight large, well-conducted prospective studies that were reported since 1980, which included the Nurses' Health Study, the Health Professionals Follow-up Study and the Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study. In eight of the cohorts there was no statistical association between cholesterol intake and the risk of CHD. In one of the positive studies the association was established by simple univariate analysis and was not adjusted for other dietary variables. The other study adjusted only for fat intake. There is no compelling evidence from these epidemiological studies that dietary cholesterol is associated with the risk of CHD.

17.4.4. Intervention Studies for CHD Prevention

A consequence of the lipid hypothesis of CHD is that reduction of saturated fat and cholesterol and an increase of polyunsaturated fatty acids in the diet will lower plasma cholesterol level with a reduction in the risk of CHD. A causal relation between dietary fat and CHD can be established only by conducting randomized clinical trials.

17.4.4.1. Dietary Intervention Trials

There have been three primary and eight secondary prevention trials in which dietary change was the only variable. Dietary modification included reduction in total fat, substitution of saturated fat by polyunsaturated oils and reduction in cholesterol intake. These changes resulted in a reduction of saturated fat intake by 27–55% and reductions in plasma cholesterol of up to 18%. However, with the exception of one study, the Lyon Diet Heart Study (de Lorgeril *et al.*, 1994), neither total or CHD mortality was lowered significantly by the dietary interventions (Ravnskov, 1998; Parodi, 2004). In the successful Lyon Diet Heart Study, a Mediterranean-type diet was compared with the usual post-infarct prudent diet. Throughout this trial, plasma cholesterol levels were similar in both the treatment and control groups.

17.4.4.2. Multifactor Intervention Trials

Multiple studies have examined the effect of dietary modification plus various lifestyle changes or drug therapy on CHD outcome and total mortality. Muldoon *et al.* (1990), Ravnskov (1992), and Davey Smith *et al.* (1993) conducted meta-analyses of these trials. Overall, there was a small benefit for nonfatal CHD, which was more noticeable in high-risk patients, but not for CHD or total mortality.

17.4.4.3. Drug Intervention Trials

Bucher *et al.* (1999) conducted a systematic review of the benefit of different classes of cholesterol-lowering drugs for prevention of CHD and total mortality. Only the statin drugs showed a statistically significant reduction in mortality from CHD and from all causes.

Statin Drugs and CHD. Statin drugs are 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors that act by inhibiting HMG-CoA reductase, the rate-limiting step in cholesterol biosynthesis. The statins are now the most commonly prescribed drugs for the treatment of hypercholesterolemia. They can reduce plasma total cholesterol by 20–42%, LDL-cholesterol by 25–55% and triglycerides by 10–35%. In addition,

HDL-cholesterol levels are increased by 4–8% (Maron *et al*., 2000). These changes in plasma lipids are claimed to reduce the risk of major coronary events by around 30%. However, this value is misleading because it refers to the relative risk of CHD; the absolute risk reduction is only about 2%.

Parodi (2004) listed the six major statin intervention trials of five years duration or longer. The absolute risk reduction in CHD mortality ranged from 0.12 to 3.5% and in total mortality from −0.1 to 3.3% with weighted means of 1.3 and 1.7%, respectively. In the statin trials, the relative reduction in risk was independent of baseline levels of LDL-cholesterol (Sacks *et al*., 2000), whereas the absolute or percentage reduction in LDL-cholesterol had little relationship to coronary events (Sacks *et al.,* 1998). The risk of CHD events with statin therapy is considered less than with other drugs that produce comparable reductions in plasma cholesterol level. Moreover, angiography studies suggest that clinical improvement with statin therapy far exceeds changes in the size of atherosclerotic lesions (Takemoto and Liao, 2001).

It is now realized that statin drugs possess a number of important anti-atherogenic properties, independent of reduction in cholesterol level. Statin therapy can improve endothelial function, decrease oxidative stress and vascular inflammation and improve the stability of atherosclerotic plaques (Maron *et al*., 2000; Takemoto and Liao, 2001). Because of these pleiotropic effects, the contribution of reduction in plasma cholesterol level, if any, to reduction in the risk of CHD cannot be assessed and cannot be used to validate the lipid hypothesis. It is relevant that the hypolipidemic drug gemfibrozil and agents like calcium channel blockers, β-adrenergic blockers, angiotensin-converting enzyme inhibitors, and aspirin can reduce the risk of CHD events to about the same extent as statins without lowering plasma cholesterol levels (Parodi, 2004).

17.4.5. Comment

Evidence from well-conducted prospective epidemiological studies does not suggest that consumption of saturated fat and cholesterol is associated with an increased risk of CHD. Randomized clinical trials that reduced the intake of saturated fatty acids and cholesterol and increased the intake of polyunsaturated fatty acids to lower plasma cholesterol levels did not significantly improve CHD or total mortality. The minor improvement in CHD events for trials of the potent cholesterol-lowering statin drugs may result, to an unknown extent, from their pleiotropic effects and cannot be used to justify the lipid hypothesis.

Unfortunately, when saturated fatty acids are replaced by carbohydrate in the so-called low-fat, high-carbohydrate diets, a reduction in plasma

LDL-cholesterol levels is accompanied by an increase in small, dense LDL particles, triglycerides and insulin and a decrease in HDL-cholesterol level (Krauss, 2001), all of which are risk factors for CHD.

Individuals do not consume saturated fatty acids and cholesterol as dietary items, rather they are components of milk fat and other lipids and milk fat contains other components such as, sphingolipids and rumenic acid (RA; *cis*-9, *trans*-11-$C_{18:2}$), which may help prevent CHD. Similarly, milk fat is consumed as a component of dairy products and dairy product consumption has been associated with reduction in blood pressure, weight loss and a reduced incidence of the metabolic syndrome (a convergence of insulin resistance, glucose intolerance, hypertension, obesity and dyslipidemias), which are CHD risk factors (Parodi, 2004). Indeed, the Nurses' Health Study found no significant associations between intake of low-fat or high-fat dairy products and major CHD events (Hu *et al*., 1999), whereas a recent prospective case-control study found that the estimated intake of milk fat was negatively associated with the risk of CHD (Warensjo *et al.,* 2004). Elwood *et al*. (2004) identified ten prospective epidemiological studies that measured milk consumption and cardiovascular disease. A pooled estimate of risk found that drinking milk was associated with a small but worthwhile reduction in CHD and stroke risk.

17.5. *Trans* Fatty Acids and CHD

Early investigations into a link between dietary *trans* fatty acid (TFA) intake and plasma cholesterol level in clinical trials, and CHD in epidemiology studies provided conflicting results. This outcome resulted from small numbers of participants in the trials combined with poor experimental design.

17.5.1. Clinical Studies

In the early 1990s, a series of well-designed clinical studies convincingly demonstrated that TFAs increased plasma total and LDL-cholesterol to levels similar to those produced by saturated fatty acids. More than this, TFAs reduced plasma HDL-cholesterol level. The overall effect was that the ratio of LDL-cholesterol to HDL-cholesterol was approximately double that for an equivalent intake of saturated fatty acids (Ascherio *et al*., 1999). In addition, TFAs adversely affect other CHD risk factors. Plasma triglycerides and Lp[a] levels are increased (Ascherio *et al*., 1999) and it was shown recently that consumption of TFAs was associated with a deleterious increase in small, dense LDL particles (Mauger *et al*., 2003).

17.5.2. Epidemiological Studies

Early studies of the relation between intake of TFAs and the occurrence of CHD also produced conflicting results. However, the large, well-conducted prospective studies showed positive associations between TFA intake and risk of CHD. The Nurses' Health Study and the Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study produced statistically significant positive associations, whereas in the Health Professionals Follow-up Study the positive association did not attain statistical significance (Ascherio *et al.,* 1999). There was also a significant positive association between TFA intake and CHD risk in the smaller Zutphen Elderly Study (Oomen *et al*., 2001).

17.5.2.1. Differential Effect of Ruminant TFAs and TFAs from Hydrogenated Vegetable Oils

A case-control study (Ascherio *et al*., 1994), a cross-sectional study (Bolton-Smith *et al*., 1996) and three prospective studies; the Nurses' Health Study (Willett *et al*., 1993), the Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study (Pietinen *et al*., 1997), and the Zutphen Elderly Study (Oomen *et al*., 2001), separately assessed the effect of TFAs from hydrogenated vegetable oil and animal fat on the risk of CHD. With the exception of the small Zutpen Elderly Study (Oomen *et al*., 2001), the studies found that the positive association with the risk of CHD was explained entirely by the intake of TFAs from hydrogenated vegetable oil.

17.5.3. Biological Explanation for the Disparate Effects

17.5.3.1. Dietary TFAs

About one-fourth of the TFAs in ruminant fat is represented by RA, which is by far the predominant natural isomer of conjugated linoleic acid (CLA). The remaining TFAs are mainly *trans* monounsaturated acids of which vaccenic acid (VA; *trans*-11-$C_{18:1}$) is predominant. On the other hand, hydrogenated vegetable oils contain predominantly elaidic acid (*trans*-9-$C_{18:1}$) and have a more even distribution of the other *trans*-$C_{18:1}$ acids, but contain little or no RA (Parodi, 2004).

17.5.3.2. Bioconversion of VA to RA

Animal and human tissues contain the enzyme Δ^9-desaturase that can introduce a *cis*-double bond at carbon 9 in VA to produce RA. Santora *et al*. (2000) fed VA to mice and found that 12% of this VA or 50% of the VA stored in carcase adipose tissue was converted to RA. Humans fed increasing

amounts of VA exhibited linear increases of RA in their plasma. The mean conversion level was about 20% (Turpeinen *et al.*, 2002). Because the content of VA in ruminant fat can be several-fold higher than the content of RA, bioconversion of VA may effectively double the supply of RA to target tissues.

17.5.4. RA and Atherosclerosis

Several studies on animal models demonstrated that CLA could retard the development of atherosclerosis. The CLA used in these studies was a mixture of isomers in which RA and *trans*-10, *cis*-12-$C_{18:2}$ were predominant and present in near equal amounts. Because these two isomers can show identical biological effects in some tissues and dissimilar effects in other tissues, a benefit for RA in these studies cannot be assumed. Nevertheless, there is emerging evidence that RA has anti-atherogenic properties.

Kritchevsky (1999) reviewed studies conducted with colleagues, which demonstrated that dietary CLA could inhibit the development of cholesterol-induced atherosclerosis in hamsters and in rabbits. Reduction in the severity of pre-existing lesions was also noted. Recently, Kritchevsky (2003) fed rabbits a diet containing 1% of relatively pure RA. This diet significantly inhibited the formation of atherosclerotic lesions in the aortic arch and thoracic aorta, compared to a diet without RA.

The validity of extrapolating observations on animal models to human atherosclerosis is often questioned. However, during the last decade gene deletion technology has allowed the production of a variety of transgenic or knockout animal models that can closely resemble particular human lipoprotein disorders. One model that is now used extensively to study atherosclerosis is the apolipoprotein E-deficient mouse (apo $E^{-/-}$). In this model, mice develop severe hypercholesterolemia and atherosclerosis on a regular low-fat, low-cholesterol diet. The progression and histopathology of the lesions are similar to those that develop in humans. Recently, Toomey *et al.* (2003) reported that 1% RA fed to apo $E^{-/-}$ mice not only inhibited the development of atherosclerosis but also caused the regression of established lesions.

In most cases, the reduced incidence of atherosclerosis in animals fed CLA or RA was not accompanied by a decrease in plasma total or LDL-cholesterol level or an increase in HDL-cholesterol level. Thus, other mechanisms must be responsible for the prevention of atherosclerosis. Atherosclerosis is recognized nowadays as a chronic inflammatory disease, and RA has been shown to exhibit a number of important anti-inflammatory properties (Yu *et al.*, 2002):

- It reduces the expression of the inducible form of cyclooxygenase (COX)-2 and the end product prostaglandin E_2 (PGE_2). COX-2 is a rate-limiting enzyme in the conversion of arachidonic acid to series-2 prostacyclins, the vasoconstricting thromboxanes, and prostaglandins such as PGE_2.
- It decreases the production of inducible nitric oxide synthase, which is responsible for the release of pro-inflammatory nitric oxide.
- It reduces the production of the pro-inflammatory cytokines tumor necrosis factor α (TNF α), interleukin (IL)-1, and IL-6.

RA was also shown to inhibit the production of series-2 prostaglandins and thromboxanes in endothelial cells from human saphenous vein, which is the vein commonly used in coronary bypass surgery (Urquhart *et al.*, 2002).

These anti-inflammatory properties, as well as certain other biological properties of RA, are similar to those seen with ligands for the peroxisome proliferator-activated receptors (PPARs), especially the PPARγ isoform. The PPARs are nuclear receptors primarily involved in the regulation of lipid and glucose homeostasis. Activation of PPARγ inhibits intracellular signalling cascades, such as for nuclear factor κB (NF-κB), a transcription factor that regulates cytokine production. A number of synthetic drugs used in the treatment of dyslipidemias, diabetes and obesity, that are risk factors for CHD, are PPAR agonists. At the molecular level PPAR agonists can regulate foam cell formation, plaque stability, and decrease the formation of pro-atherosclerotic proteins, such as fibrinogen and C-reactive protein (Desvergne and Wahli, 1999; Kersten *et al.*, 2000).

RA is a potent activator of PPARα and PPARγ (Belury *et al.*, 2002). Yu *et al.* (2002) found that inhibition of pro-inflammatory products by RA was associated with PPARγ activation. Likewise, Toomey *et al.* (2003) showed that the regression of preestablished atherosclerosis in apo $E^{-/-}$ mice fed RA is associated with an increased expression of PPARγ.

17.6. Anti-Cancer Agents in Milk Fat

The demonization of fat, especially milk fat, over the past several decades means that the diet has been deprived of several components with anti-cancer potential. Milk fat components, such as RA, sphingolipids, butyric acid, certain branched chain fatty acids, ether lipids, vitamins, and novel components introduced from feed, have been shown to prevent tumor development at a number of sites in animal models.

17.6.1. Rumenic Acid

17.6.1.1. Early Studies with Mixed Isomers of CLA

During the past 15 years, multiple studies have shown that CLA could inhibit the development of carcinogenesis in various experimental models. Physiological concentrations of CLA suppressed the proliferation in a wide range of human cancer cell lines that included breast, ovarian, prostate, colon, liver, mesothelioma, glioblastoma and leukemia. This growth suppression by CLA was in contrast to linoleic acid, which usually promoted growth. Mice fed CLA and inoculated with human breast or prostate cancer cells had reduced tumor growth and drastic reduction in metastases. CLA also inhibited chemically-induced skin, stomach and intestinal tumors (Parodi, 2004). However, the outstanding attribute of CLA is its ability to inhibit mammary tumor development (Ip *et al.*, 2003).

In the initial study by Ip *et al.* (1991), the authors fed rats a standard diet or that diet supplemented with 0.5, 1.0 or 1.5% CLA, 2 weeks prior to and following administration of the carcinogen 7, 12-dimethylbenz[a]anthracene (DMBA). At the end of the experiment, the total number of mammary adenocarcinomas in the groups fed 0.5, 1.0 and 1.5% CLA was reduced by 32, 56 and 60% compared to the control, respectively. Tumor incidence, tumor multiplicity (number of tumors per rat) and total tumor weight were reduced to a similar degree. This tumor inhibition by CLA is in contrast to linoleic acid, which promotes tumor development in this model.

This group subsequently conducted numerous studies to explore mechanisms contributing to the anti-cancer action of CLA (reviewed in Ip *et al.*, 2003). Notable findings were:

- At low carcinogen doses, there was effective inhibition of tumor development when the diet was supplemented with as little as 0.05% CLA.
- CLA was effective for both the direct-acting carcinogen methylnitrosourea (MNU) and the indirect-acting DMBA, suggesting the action of CLA was independent of carcinogen activation.
- CLA was equally effective in inhibiting mammary tumor development when it was part of a 5% low-fat diet or a 20% high-fat diet.
- Tumor inhibition was similar when CLA was part of 20% unsaturated-fat diet provided as corn oil or as a 20% saturated-fat diet provided by lard.
- Even though a diet containing 12% linoleic acid produced more mammary tumors than a diet containing 2% linoleic acid, CLA suppressed tumor development to the same degree with both diets.

- CLA was preferentially incorporated into the neutral lipids of mammary tissue. When CLA was removed from the diet, its disappearance from neutral lipids paralleled the rate of occurrence of new tumors.

The age of the rat when CLA supplementation is commenced can influence outcome. When rats were fed CLA from weaning at 21 days of age until day 51 only, then administered a carcinogen at day 57, they were protected from subsequent tumor development. However, when CLA entered the diet for the same period of time after carcinogen administration and when the animals were older, there was no protection against tumor development. A continuous intake of CLA was then necessary to obtain equivalent protection. The period from 21 to 51 days of age corresponds to development of the mammary gland to adult stage morphology.

Further studies showed that during the pubescent period, CLA reduced the development and branching of the expanding mammary ductal tree. There was also a reduction in the density and rate of proliferation of terminal end bud (TEB) cells. TEBs are the least differentiated and most actively growing glandular ductal structures, which are most abundant from weaning to puberty and are the site of chemically induced tumors (Ip *et al*., 2003).

17.6.1.2. Studies with Rumenic Acid

The early studies with CLA used a synthetic mixture of many isomers, but usually contained about 70–80% of near equal quantities of RA and *trans*-10, *cis*-12-$C_{18:2}$. Recently, a number of studies have demonstrated that both isomers can inhibit mammary tumor development (Ip *et al*., 2003).

In a novel experiment, Ip *et al*. (1999) demonstrated that rats fed a RA-enriched butter had reduced mammary epithelial mass, decreased size of the TEB population, suppressed proliferation of TEB cells, and inhibition of mammary tumor incidence and tumor number similar to animals fed an equivalent quantity of mixed CLA isomers. There was a consistently higher level of RA in the liver, mammary fat pad, peritoneal fat, and blood plasma of rats fed RA-enriched butter compared to CLA-fed rats. The elevated RA levels resulted, no doubt, from endogenous Δ^9-desaturation of VA, which was concurrently increased in the RA-enriched butter.

A follow-up study demonstrated that pure isomers of RA and *trans*-10, *cis*-12-$C_{18:2}$ were equivalent in preventing the development of both MNU-induced premalignant lesions, called intraductal proliferations (IDPs) and tumors in the mammary gland (Ip *et al*., 2003). Later, it was shown that feeding rats a diet containing 2% VA inhibited MNU-induced IDPs to the same degree as feeding a diet containing 1% RA (Banni *et al*., 2001).

17.6.1.3. Mechanisms for the Anti-Tumor Action of RA

Tumors grow when the rate of cell proliferation exceeds apoptosis. Apoptosis is the mechanism of programmed cell death by which the body eliminates unwanted or damaged cells. At the molecular level, a number of mechanisms by which RA exerts its antitumor effects have been demonstrated. RA, like mixed CLA isomers, inhibits the proliferation of malignant mammary tumor cells. In addition, RA inhibits proliferation in normal mammary epithelial cells. This reduction in proliferation was associated with decreased expression of the cell regulatory biomarkers, cyclin D1 and cyclin A. However, rapidly proliferating cells in young rats were more sensitive to RA treatment than more quiescent cells in older animals. When rats were fed RA, apoptosis was induced in MNU-induced IDPs, but not in normal TEBs or more differentiated alveoli structures. The induction of apoptosis in IDPs was associated with reduced expression of the anti-apoptotic regulatory protein bcl-2 (Ip *et al*., 2003).

Angiogenesis is the process of forming new blood vessels, which are necessary for the growth and spread of tumors. Masso-Welch *et al*. (2004) demonstrated that RA effectively inhibits angiogenesis in mice. This inhibition was related to reduced serum levels of vascular endothelial growth factor (VEGF). VEGF not only plays a central role in promoting angiogenesis, but can also stimulate the growth and invasiveness of breast cancer cells (Ip *et al*. 2003). Hubbard *et al*. (2003) reported that RA reduced pulmonary tumor metastasis in mice when mammary tumor cells were either transplanted into mammary fat pads or injected intravenously *via* the tail vein.

Two other, as yet largely unproven, mechanisms for the anti-tumorigenic action of RA are the reduction in COX-induced pro-tumorigenic eicosanoid species, and the activation of PPARs. RA may displace linoleic acid or its metabolite, arachidonic acid, from membrane phospholipids and from neutral lipids. This decreases available substrate for the tumor-promoting prostacyclins, thromboxanes and prostaglandins, generated by constitutive COX-1 and the inducible form COX-2. It is also possible that RA, or its metabolites, can directly inhibit COX activity (Belury, 2002; Parodi 2004). As discussed previously, RA can bind and activate PPARγ Moreover, RA can also increase the expression of PPARγ (Brown *et al*., 2003). Synthetic PPARγ agonists have been shown to protect against cancer at a number of sites including the breast (Desvergne and Wahli, 1999; Kersten *et al*., 2000).

17.6.1.4. Epidemiology

An initial case-control study on Finnish women found an inverse association between RA and breast cancer in post-menopausal women, but

not in pre-menopausal women (Aro *et al.*, 2000). After adjusting for known risk factors, post-menopausal women in the lowest quintile of dietary RA had a threefold greater risk of breast cancer compared to women in the highest quintile of intake. Similarly, women in the lowest quintiles of plasma RA and VA levels both had a fivefold greater risk of breast cancer.

Since then, three other epidemiological studies have failed to confirm a protective role for RA or VA in the development of breast cancer. A Dutch prospective study used dietary assessment, a French study compared RA levels in tissue adjacent to malignant tumors with levels adjacent to benign tumors, and the third study was a nested case-control study of Finnish women using fatty acid data from plasma collected prospectively. Recently, a fourth study showed that, although higher intakes of CLA were not related to overall breast cancer risk, risk of estrogen receptor negative breast cancer—the most aggressive form—in pre-menopausal women was reduced (Mc Cann *et al.*, 2004). Parodi (2004) has described methodological inadequacies in these studies.

17.6.2. Sphingolipids and Colon Cancer

Evidence suggests that dietary sphingolipids, acting through their hydrolysis products, ceramide and sphingosine, can inhibit the development of colon cancer (Duan, 1998; Vesper *et al.*, 1999). In milk fat, sphingomyelin is by far the predominant sphingolipid.

17.6.2.1. Mechanisms: the Sphingomyelin Signalling Pathway

Sphingomyelin is concentrated in the outer leaflet of cellular membranes and its function was thought to be solely associated with structural stability. However, an important sphingomyelin-signalling pathway has been established recently. Extracellular agonists such as TNF-α, interferon γ, IL–1, and 1,25-dihydroxyvitamin D_3 ($1,25(OH)_2D_3$) bind and activate their cellular receptors to stimulate the release of sphingomyelinase. The liberated sphingomyelinase cleaves sphingomyelin to produce ceramide. In turn, ceramide acts as a second messenger for the extracellular agonists, transmitting the signal through multiple downstream targets, such as protein phosphatases and protein kinases. These targets, in turn, regulate a number of transcription factors that control the expression of a range of genes responsible for inhibition of cell growth, cell cycle arrest, differentiation, and apoptosis.

Other agonists, like platelet-derived growth factor, can induce ceramidase, which hydrolyses ceramides to sphingosine. Sphingosine also acts as a second messenger in signalling cascades that regulate cell growth. Because of their biological activity in regulating cell growth, ceramide and

sphingosine are referred to as tumor suppressor lipids (Duan, 1998; Vesper *et al*., 1999).

17.6.2.2. Digestion and Tissue Enhancement

Unlike other dietary lipids, sphingolipids are poorly digested in the proximal small intestine, and digestion continues slowly in the distal small intestine and in the colon. The rate of sphingolipid digestion in the intestinal tract mirrors the distribution pattern for alkaline sphingomyelinase in the intestine. Studies in rats found that the digestion products of sphingolipids, mainly ceramides and sphingosine, are absorbed rapidly by intestinal cells where they can be degraded further or re-incorporated into sphingolipids that mostly remain associated with the intestine. Nevertheless, some labelled sphingoid base has been detected in lymph, plasma and the liver (Nyberg *et al*., 1997; Duan, 1998; Vesper *et al*., 1999). Nyberg *et al*. (1997) found that the ceramide content in the colonic mucosa increased with increasing sphingomyelin intake. Enhancement of colonic tissue with ceramide, as a result of dietary sphingolipid intake, could be important for the prevention of colon cancer.

17.6.2.3. Sphingomyelinase, Ceramide and Human Carcinomas

The importance of alkaline sphingomyelinase and ceramides in colon cancer is suggested by studies that compared their levels in tumors with levels in normal colonic tissue. Hertervig *et al*. (1997) found that the activity of alkaline sphingomyelinase was 75% less in tissue from human colon carcinomas than in normal colonic tissue. In familial adenomatous polyposis patients, there was a 90% decrease in alkaline sphingomyelinase activity in colonic adenomas and in surrounding mucosa compared to healthy controls (Hertervig *et al*., 1999). Human colon carcinomas had a 50% decrease in cellular ceramide content compared to normal colon mucosa (Selzner *et al*., 2001).

17.6.2.4. Cell Culture Studies

Ceramides and sphingosine can inhibit cell growth, promote differentiation and induce apoptosis in a range of human cancer cell lines, including several types of colon cancer cells (Parodi, 2004). Selzner *et al*. (2001) established that ceramides induced cell death in cultured human SW403 colon cancer cells. Treatment of these cells with a ceramidase inhibitor, which prevented the catabolism of ceramides and thus increased its content in the cells, resulted in suppressed cell growth and induction of apoptosis. Selzner *et al*. (2001) then injected SW403 cancer cells and

human metastatic Lovo cancer cells into the portal vein of mice. Administration of a ceramidase inhibitor to these mice inhibited colon cancer metastases to the liver.

Bile acids that escape enterohepatic circulation and pass to the colon can be cytotoxic to colonocytes. Damaged cells undergo apoptosis and are shed into the lumen. To maintain cell homeostasis, new cells must be produced. This replacement can result in an increase in cell proliferation rate that can increase the risk of mutations in tumor-related genes and lead to carcinoma development. Moschetta *et al.* (2000) showed that sphingomyelin protected against bile acid-induced cytotoxicity in human CaCo-2 colon cancer cells, a common model for studying intestinal cell function.

17.6.2.5. Animal Tumor Studies

A series of studies show that sphingolipids derived from milk protect against development of colon tumors in mice. In the initial study (Dillehay *et al.*, 1994), mice fed diets supplemented with 0.025, 0.050 or 0.1% sphingomyelin had less than half the incidence of 1,2-dimethylhydrazine (DMH)-induced colon tumors than mice fed non-supplemented diets. In addition, a diet supplemented with 0.025% of the ganglioside GM_1 produced significantly fewer DMH-induced aberrant crypt foci (ACF) than control animals. ACF are microscopically determined pre-neoplastic lesions that can develop into colon tumors.

In a follow-up study (Schmelz *et al.*, 1996), mice were fed 0–0.1% sphingomyelin isolated from low-fat milk or buttermilk. Sphingomyelin from both sources reduced DMH-induced ACF by 70%. In a longer-term study, sphingomyelin had no effect on the incidence or multiplicity of chemically induced colon tumors. However, up to 31% of tumors in the mice fed sphingomyelin were adenomas, whereas all of the tumors in the non-supplemented mice were malignant adenocarcinomas. In order to eliminate the possibility that an unknown component isolated from milk along with sphingomyelin was the anti-tumor agent, Schmelz *et al.* (1997) compared the action of milk-derived sphingomyelin with a synthetically prepared product. Both sources of sphingomyelin reduced DMH-induced ACF formation to the same extent.

Schmelz *et al.* (2000) then isolated the complex sphingolipids, glucosylceramide, lactosylceramide and the ganglioside GD_3, from milk. These sphingolipids were added individually to the diet of mice at a level of 0.025 or 0.1%. All three sphingolipids reduced the number of DMH-induced ACF by about 40%, a reduction that was comparable to that obtained by sphingomyelin in earlier experiments.

The next study (Schmelz *et al*., 2001), used a different model, the $Apc^{Min/+}$ mouse. In this model the mice have a germline mutation in the adenomatous polyposis coli (*Apc*) gene and they spontaneously develop adenomas (polyps) throughout the intestinal tract, but preferentially in the small intestine. In humans, germline mutations in the *APC* gene (a tumor suppressor gene) are responsible for familial adenomatous polyposis, a syndrome in which patients develop multiple benign adenomas in the colon. If not resected, a proportion of these adenomas will progress to become malignant. In sporadic colon cancer, mutations in the *APC* gene initiate the development of most tumors.

$Apc^{Min/+}$ mice were fed a control diet or that diet supplemented with either 0.1% ceramide, a sphingolipid mixture of sphingomyelin, glucosylceramide, lactosylceramide and ganglioside GD_3 with a composition similar to that found in dairy products, or this mixture plus ceramide (60:40). All three diets significantly reduced the number of spontaneously developed tumors in all regions of the intestines (Schmelz *et al*., 2001).

In the preceding experiments, sphingolipid supplementation commenced after tumor initiation with a chemical carcinogen. Lemonnier *et al*. (2003) fed 0.05% sphingomyelin to a group of mice before tumor intiation and to another group after tumor initiation. Both dietary protocols drastically reduced tumor formation to the same extent, which suggests thst sphingolipids may have both chemopreventive and chemotherapeutic benefits. In this study, tumor inhibition was associated with normalization of cell proliferation and the rate of apoptosis.

This is an exciting area of research and further mechanistic studies leading to human intervention studies must follow.

17.6.3. Butyric Acid

Uniquely, milk fat of ruminants contains butyric acid, which is an important anti-cancer agent. Butyric acid is best known for its action in the colon where it is generated, along with other short-chain acids, by bacterial fermentation of dietary fiber and starch. Colonocytes utilize a portion of this butyric acid as a primary energy source, with the remainder delivered to the portal circulation and transported to the liver where it is metabolized rapidly.

A number of animal studies have shown that dietary fibers, which liberate a constant and elevated supply of butyrate to the colon, are the most effective for prevention of chemically induced colon tumors. Moreover, the level of butyric acid in the colonic lumen of patients with colorectal cancer and adenomas was found to be lower than that in healthy individuals (Parodi, 2004).

17.6.3.1. Mechanistic Studies

Low concentrations of butyric acid can inhibit growth in a wide range of human cancer cell lines, including prostate and several types of breast and colon cancer by a number of mechanisms (Williams *et al.*, 2003; Parodi, 2004). It is believed that central to the anti-cancer action of butyric acid is its ability to inhibit histone deacetylases, which results in histone hyperacetylation and destabilization of chromatin structure that facilitates transcription factor binding and activation of genes associated with cell growth. Histone hyperacetylation activates the *p21* gene, which inhibits the cell cycle regulators, cyclin D1 and cyclin B1. This results in the inhibition of cyclin-dependent kinase, which in turn prevents phosphorylation of retinoblastoma protein required for progress from the pre-synthetic G_1 phase of the cell cycle to the S phase. The arrested cells may then differentiate or undergo apoptosis (Davie, 2003). A summary of these related cell-growth inhibiting mechanisms induced by butyric acid (Williams *et al.*, 2003; Parodi, 2004) are outlined below:

- Inhibition of proliferation.
- Induction of terminal differentiation.
- Induction of apoptosis associated with increased caspase (cysteine protease) activity and decreased expression of the anti-apoptotic Bcl-2 protein along with increased expression of the pro-apoptotic Bak and Bax proteins
- Inhibition of angiogenesis associated with down-regulation of VEGF.
- Anti-inflammatory action. Chronic or recurrent inflammation probably has a role in many types of human cancer.
- Up-regulation of immunosurveillance.
- Increased expression of insulin-like growth factor binding protein-3 (IGFBP-3), the major plasma binding protein for insulin like growth factor-1 (IGF-1). Elevated levels of IGF-1 relative to levels of IGFBP-3 are implicated in the development of several human cancers, including colon, breast and prostate.
- Enhanced expression of glutathione *S*-transferase (GST). GSTs inactivate carcinogens by catalyzing their conjugation to glutathione, forming water-soluble metabolites that are easily excreted.
- Suppression of colonocyte NF-κB activation (Yin *et al.*, 2001). NF-κB is a transcription factor that regulates several signalling patterns involved in cell proliferation and apoptosis. In many tumors activation of NF-κB promotes proliferation and inhibits apoptosis. Activation of NF-κB is also associated with inflammatory response.

17.6.3.2. Butyric Acid as a Chemotherapeutic Agent

Early attempts to use butyrate in the treatment of human cancer—usually in patients refractory to other treatments—were unsuccessful (Pouillart, 1998). Failure was largely due to the very short half-life of butyrate in the circulation. The appeal of butyrate as a therapeutic agent lies in the absence of systemic toxicity, therefore a series of butyrate analogues—referred to as prodrugs—that increase the plasma half-life of butyrate were developed. One simple prodrug is the triacylglycerol, tributyrin. Orally administered tributyrin increased and extended plasma butyrate concentration in rats and mice (Egorin *et al*., 1999) and in humans (Conley *et al*., 1998). Thus, enhanced plasma butyrate levels may allow anti-cancer action at sites other than the colon. In milk fat, one triacylglycerol molecule in three contains butyrate (Parodi, 2004).

17.6.3.3. Mammary Tumor Prevention

Two studies have shown that dietary butyrate inhibits chemically-induced mammary tumor development in rats. First, Yanagi *et al*. (1993) showed that addition of 6% sodium butyrate to a basal diet containing 20% fat, supplied by a margarine made from safflower oil, significantly reduced the incidence of DMBA-induced mammary carcinomas and adenocarcinomas. In the second study, Belobrajdic and McIntosh (2000) fed rats a 20% fat diet consisting of either milk fat, sunflower seed oil (SSO), SSO + 1% tributyrin (butyric acid content equivalent to milk fat) or SSO + 3% tributyrin. At any period during the course of the experiment, there was a relative risk increase of 88% that rats consuming the SSO diet would develop MNU-induced mammary tumors compared to those in the milk fat group. The addition of 1% or 3% tributyrin to the SSO diets reduced tumor incidence by 20 and 52%, respectively, in comparison to SSO alone.

17.6.3.4. Synergy

There are a number of studies that show synergism between butyrate and other dietary components and common drugs in reducing cancer cell growth. This could result in lower plasma butyrate requirements for anti-cancer action (Parodi, 2004). In summary:

- Retinoic acid, at concentrations likely to be found in plasma, reduced by tenfold the level of butyric acid required to induce differentiation.
- Physiological concentrations of 1, $25(OH)_2D_3$ acted synergistically with butyrate to inhibit proliferation and induce differentiation.

- Resveratrol, a plant polyphenol found in red wine and grapes, enhanced the ability of butyrate to induce differentiation.
- A HMG CoA reductase inhibitor, used for treatment of hypercholesterolemia, enhanced butyrate-induced inhibition of proliferation.
- Aspirin, now commonly used for prevention of CHD and colon cancer, combined with butyrate exerted stronger anti-proliferate and pro-apoptotic effects than either alone.

17.6.4. 13-Methyltetradecanoic Acid

13-Methyltetradecanoic acid (13-MTDA) is a 15-carbon fatty acid with a terminal isopropyl group. It is synthesized by bacteria in the rumen, along with other branched-chain fatty acids. Yang *et al.* (2000) demonstrated that low concentrations of 13-MTDA could induce cell death in a range of human cancer cell lines. The cell lines tested were prostate, colon, lung (small cell), liver, and gastric carcinomas, mammary and pancreatic adenocarcinomas, and leukemia. 13-MTDA-initiated cell death resulted from rapid induction of apoptosis.

Human lung cancer cells and human prostate cancer cells were implanted into athymic nude mice, then harvested and implanted in the lung and prostate, respectively, of a MetaMouse orthotopic model. Feeding 13-MTDA for 40 days inhibited the growth of lung cancer implants by 65% and prostate cancer implants by 85% compared to control animals (Yang *et al.*, 2000).

Recently, Wongtangtintharn *et al.* (2004) showed that a series of *iso*- and *anteiso*-branched-chain fatty acids was cytotoxic to breast cancer cells. The highest activity was observed with *iso*-$C_{16:0}$ and the activity decreased as the chain-length increased or decreased from $C_{16:0}$. Cytotoxicity of branched-chain fatty acids was comparable to that of RA and *trans*-10, *cis*-12-$C_{18:2}$.

17.6.5. Ether Lipids

Milk fat contains small amounts of ether lipids. Synthetic ether lipids at low concentrations are potent anti-neoplastic agents. Cell culture studies showed that they inhibited cell growth, induced differentiation, promoted apoptosis and showed anti-metastatic activity. In human chemoprevention trials ether lipids have been administered parenterally. Although the ether bond is preserved when these lipids are delivered orally, no cancer chemoprevention studies have utilized dietary ether lipids (Parodi, 2004).

17.6.6. Cholesterol

Evidence of a role for dietary cholesterol in carcinogenesis, using animal models, is contradictory. This may result, in part, from contamination of

the test cholesterol with oxidation products that are carcinogenic and the use of supra-physiological doses. Nevertheless, dietary cholesterol was shown to inhibit chemically-induced colon tumors (El-Sohemy et al., 1996a) and mammary tumors (El-Sohemy et al., 1996b) in serum-cholesterol sensitive rats. The researchers consider that the accompanying elevated serum cholesterol levels triggered the inhibition of endogenous cholesterol synthesis by reducing the level of HMG-CoA reductase. HMG-CoA reductase is the rate-limiting enzyme in the cholesterol biosynthetic pathway that converts HMG-CoA to mevalonate, which is required for DNA synthesis during rapid cell proliferation associated with the early stage of tumorigenesis.

17.6.7. β-Carotene and Vitamin A

Milk fat supplies the diet with a substantial proportion of its daily β-carotene and vitamin A requirements. Dietary β-carotene is converted to retinal in the intestinal epithelium and in the liver by the enzyme β-carotene-15–15′-dioxygenase. The retinal formed is further metabolized to retinoic acid (vitamin A).

Epidemiological evidence suggests that diets rich in β-carotene—largely fruit and vegetables—and high plasma levels of β-carotene are inversely associated with the risk of cancer, especially esophagus, lung, stomach, colorectal, breast and cervical cancers (Cooper, 2004).

Both β-carotene and vitamin A can inhibit growth in a large range of human cancer cell lines (Krinsky, 1993; Niles, 2000). Several animal studies have shown that vitamin A deficiency promotes the development of spontaneous and chemically-induced tumors, whereas dietary supplementation with vitamin A can prevent chemically-induced tumor development (Niles, 2000). Likewise, β-carotene protects against tumor development in animal models (Krinsky, 1993; Cooper, 2004; Russell, 2004). Nevertheless, β-carotene is preferred for human studies because blood and tissue levels increase in proportion to dietary intake, whereas vitamin A level does not increase in a linear manner because of homeostatic regulation; high levels of vitamin A are toxic (Cooper, 2004).

There has been some success with vitamin A and its derivatives for treatment of certain types of cancer (Niles, 2000). However, the use of β-carotene as a therapeutic agent suffered a setback when the results from two of three large human intervention studies indicated that high doses of β-carotene caused an increased risk of lung cancer in smokers and subjects exposed to asbestos. This increased risk is thought to be due to metabolites associated with high doses of β-carotene in the presence of smoke (Russell, 2004).

17.6.8. Vitamin D and its Metabolites

Milk fat is not a rich source of vitamin D, but in some countries dairy products are fortified with this vitamin. Vitamin D, through its metabolites, is an anti-cancer agent of increasing importance. Vitamin D results from ultraviolet light-catalyzed conversion of 7-dehydrocholesterol in the skin. The pre-vitamin D_3 thus formed is transported to the liver, where it is converted to 25-hydroxyvitamin D_3 [$25(OH)D_3$], which is the main circulating form of the vitamin. The circulating $25(OH)D_3$ is subsequently converted to 1, $25(OH)_2D_3$ in the kidneys by the enzyme 25-hydroxyvitamin D-1α-hydroxylase. This conversion is regulated by physiological requirements (Hansen *et al*., 2001; Zitterman, 2003; Holick, 2004). It is now realized that a number of tissues, including colon, breast and lung contain 1α-hydroxylase and can produce 1, $25(OH)_2D_3$, which acts in an autocrine manner to regulate cell growth (Holick, 2004).

1, $25(OH)_2D_3$ inhibited proliferation and induced differentiation and apoptosis in human colon, breast, prostate and gynecological cancers as well as several forms of hematopoietic cancer (Studzinski and Moore, 1995; van Leeuwen and Pols, 1997). Experimental animal studies show that 1, $25(OH)_2D_3$ inhibited chemically-induced breast, colon, and skin tumors. The growth of colon, breast and prostate cancer cells, as well as melanoma and retinoblastoma cells implanted into rodents was retarded by treatment with 1, $25(OH)_2D_3$ (Studzinski and Moore, 1995; van den Bemd *et al*., 2000).

Epidemiologic evidence suggests that low exposure to sunlight, low dietary intake of vitamin D and low plasma levels of $25(OH)D_3$ and 1, $25(OH)_2D_3$ increase the risk of developing colon, breast and prostate cancer (Studzinski and Moore, 1995; van den Bemd *et al*., 2000; Zittermann, 2003). There is evidence that vitamin D deficiency can attenuate the beneficial effect of dietary calcium for the prevention of colonic adenoma and carcinoma (Parodi, 2001a).

17.6.9. Anti-Cancer Agents from Feed

The cow has a remarkable capability to extract biological components from its feed and transfer them to its milk. The best-known example is β-carotene from pasture, a portion of which is converted to vitamin A *in vivo*, so that milk fat contains both these anti-cancer compounds. Vitamin E can also be obtained from the cow's feed. Cows fed cottonseed meal transfer the polyphenol, gossypol, to milk, and alfalfa or lucerne provides β-ionone. Both gossypol and β-ionone, an HMG-CoA reductase inhibitor, are demonstrated anti-cancer agents (Parodi, 2004).

Milk fat contains small quantities of phytanic and pristanic acid. Phytanic acid is produced by bacterial cleavage of the phytol side chain of plant chlorophyll in the rumen. Some phytanic acid is converted to pristanic acid by α-oxidation in the liver. Both of these branched-chain acids are agonists for PPARα at physiological concentrations (Parodi, 2004). Milk fat from cows fed cannery fruit and vegetable waste no doubt contains other interesting phytochemicals with anti-cancer potential.

17.6.10. Milk Fat and Cancer

Milk fat contains a number of components with anti-cancer potential, but many of these components are present at levels lower than those shown to produce a benefit in *in vitro* and *in vivo* experimental models. Nevertheless, as outlined in Section 17.6.3, synergy between anti-cancer components in milk fat and with other components from dietary items can lower several fold the concentration required to produce a physiological effect.

A role for milk fat in cancer risk has not been examined adequately in epidemiological studies, because milk fat is not consumed as a single dietary item, but as a component of dairy products and dairy products also contain non-lipid components with anti-cancer potential (Parodi, 2001a, b). On the other hand, seven studies were found in the literature, where milk fat or butter diets were compared with diets containing equal amounts of polyunsaturated vegetable oils or margarine in animal models of colon, breast, and skin cancer. All seven studies showed that there was less tumor development with milk fat-based diets (Parodi, 2004).

17.7. Other Nutritional Benefits

When milk fat is included in the diet it can confer a number of additional health related benefits. These aspects of milk fat nutrition have recently been reviewed in some detail (Parodi, 2004) and are summarized briefly below:

- Children fed low-fat milk had up to a fivefold greater incidence of acute gastrointestinal illness than children who were fed whole milk.
- Studies *in vitro* and in rats showed that short and medium chain fatty acids and monoacylglycerols hydrolyzed from milk triacylglycerols and digestion products of sphingolipids possess strong anti-bacterial and anti-viral properties.
- Milk fat is not a rich source of linoleic (ω-6) and linolenic (ω-3) acids; however, the ratio of ω-6: ω-3 is close to unity, which is considered to be ideal for good health. Rats fed diets high in milk fat had a beneficial long-chain polyunsaturated fatty acid profile in plasma

and aortic phospholipids, where arachidonic acid levels were reduced and the levels of eicosapentaenoic and docosahexaenoic acids enhanced. An equivalent benefit was not observed when rats were fed the same level of vegetable oils or lard.
- Milk phospholipids were shown to protect against stress, bacterial, and chemical-induced gastric mucosal damage.
- Milk fat may promote bone formation.
- There was less plaque formation on human tooth surfaces in subjects who drank whole milk than in subjects who drank skim milk or water.
- There is accumulating evidence that consumption of whole milk compared to skim milk and the consumption of butter compared to margarine is associated with a lower incidence of asthma and other allergic disorders.

17.8. Conclusions

The demonization of fat during the past two decades is unwarranted. There is no compelling evidence that fat is responsible for the current obesity epidemic or is implicated in weight gain independent of energy density. Evidence from well-conducted animal and epidemiological studies does not support a role for fat in the etiology of breast, prostate and colon cancer, the major non-smoking-related cancers.

Even though milk fat contains some fatty acids that may elevate plasma total and LDL-cholesterol levels, which are risk factors for CHD, this effect is balanced by concurrent increases in levels of anti-atherogenic HDL-cholesterol. In addition, saturated fatty acids reduce plasma levels of atherogenic Lp[a] and produce a less atherogenic LDL particle size. Dietary intervention studies, where there was a substantial reduction in saturated fat intake and plasma cholesterol levels, did not produce an improvement in CHD or total mortality. Prospective epidemiological studies provide no evidence that saturated fatty acids are a risk factor for CHD. Indeed, in two large studies, saturated fatty acids were inversely associated with risk.

Milk fat contains several compounds that have demonstrated anticancer activity in animal models. The more important ones are rumenic acid, a potent inhibitor of mammary tumorigenesis, sphingomyelin and other sphingolipids that prevent the development of intestinal tumors and butyric acid, which prevents colon and mammary tumor development. Emerging evidence suggests that milk fat can prevent intestinal infections, particularly in children, prevent allergic disorders, such as asthma and improve the level of long-chain ω-3 polyunsaturated fatty acids in blood.

Fat is an essential component of the diet, and inclusion of milk fat as part of a balanced diet should be advantageous rather than detrimental.

Bibliography

Ahrens, E.H., Hirsch, J., Insull, W., Tsaltas, T.T., Blomstrand, R., Peterson, M.L. 1957. The influence of dietary fats on serum-lipid levels in man. *Lancet*. **1**, 943–953.

Anderson, K.M., Castelli, W.P., Levy, D. 1987. Cholesterol and mortality. 30 Years of follow-up from the Framingham Study. *J. Am. Med. Assoc*. **257**, 2176–2180.

Aro, A., Mannisto, S., Salminen, I., Ovaskainen, M-l., Kataja, V., Uusitupa, M. 2000. Inverse association between dietary and serum conjugated linoleic acid and risk of breast cancer in postmenopausal women. *Nutr. Cancer*. **38**, 151–157.

Ascherio, A., Hennekens, C.H., Buring, J.E., Master, C., Stampfer, M.J., Willet, W.C. 1994. *Trans*-fatty acids intake and risk of myocardial infarction. *Circulation*. **89**, 94–101.

Ascherio, A., Katan, M.B., Zock, P.L., Stampfer, M.J., Willett, W.C. 1999. Trans fatty acids and coronary heart disease. *N. Engl. J. Med*. **340**, 1994–1998.

Banni, S., Angioni, E., Murru, E., Carta, G., Melis, M.P., Bauman, D., Dong, Y., Ip, C. 2001. Vaccenic acid feeding increases tissue levels of conjugated linoleic acid and suppresses development of premalignant lesions in rat mammary gland. *Nutr. Cancer*. **41**, 91–97.

Belobrajdic, D.P., McIntosh, G.H. 2000. Dietary butyrate inhibits NMU-induced mammary cancer in rats. *Nutr. Cancer*. **36**, 217–223.

Belury, M. 2002. Inhibition of carcinogenesis by conjugated linoleic acid: potential mechanisms of action. *J. Nutr*. **132**, 2995–2998.

Bolton-Smith, C., Woodward, M., Fenton, S., Brown, C.A. 1996. Does dietary trans fatty acid intake relate to the prevalence of coronary heart disease in Scotland? *Eur. Heart J*. **17**, 837–845.

Braunwald, E. 1997. Cardiovascular medicine at the turn of the millennium: triumphs, concerns, and opportunities. *N. Engl. J. Med*. **337**, 1360–1369.

Brown, J.M., Boysen, M.S., Jensen, S.S., Morrison, R.F., Storkson, J., Lea-Currie, R., Pariza, M., Mandrup, S., McIntosh, M.K. 2003. Isomer-specific regulation of metabolism and PPARγ signalling by CLA in human preadipocytes. *J. Lipid Res*. **44**, 1287–1300.

Bucher, H.C., Griffith, L.E., Guyatt, G.H. 1999. Systematic review of the risk and benefit of different cholesterol-lowering interventions. *Arterioscler. Thromb. Vasc. Biol*. **19**, 187–195.

Conley, B.A., Erorin, M.J., Tait, N., Rosen, D.M., Sausville, E.A., Dover, G., Fram, R.J., Van Echo, D.A. 1998. Phase 1 study of the orally administered butyrate prodrug, tributyrin, in patients with solid tumors. *Clin. Cancer Res*. **4**, 629–634.

Connor, W.E. 1961. Dietary cholesterol and the pathogenesis of atherosclerosis. *Geriatrics*. 407–415.

Cooper, D.A. 2004. Carotenoids in health and disease; recent scientific evaluations, research recommendations and the consumer. *J. Nutr*. **134**, 221S–224S.

Davey Smith, G., Song, F., Sheldon, T.A. 1993. Cholesterol lowering and mortality: the importance of considering initial level of risk. *Br. Med. J*. **306**, 1367–1373.

Davie, J.R. 2003. Inhibition of histone deacetylase activity by butyrate. *J. Nutr*. **133**, 2485S–2493S.

de Lorgeril, M., Renaud, S., Mamelle, N., Salen, P., Martin, J-L., Monjaud, I., Guidollet, J., Touboul, P., Delaye, J. 1994. Mediterranean alpha-linoleic acid-rich diet in secondary prevention of coronary heart disease. *Lancet*. **343**, 1454–1459.

Desvergne, B., Wahli, W. 1999. Peroxisome proliferator-activated receptors: nuclear control of metabolism. *Endocr. Rev*. **20**, 649–688.

Dillehay, D.L., Webb, S.K., Schmelz, E-M., Merrill, A.H. 1994. Dietary sphingomyelin inhibits 1,2-dimethylhydrazine-induced colon cancer in CF1 mice. *J. Nutr*. **124**, 615–620.

Duan, R-D. 1998. Sphingomyelin hydrolysis in the gut and clinical implications in colorectal tumorigenesis and other gastrointestinal diseases. *Scand. J. Gastroenterol*. **33**, 673–678.

Egorin, M.J., Yuan, Z-M., Sentz, D.L., Plaisance, K., Eiseman, J.L. 1999. Plasma pharmacokinetics of butyrate after intravenous administration of sodium butyrate or oral administration of tributyrin or sodium butyrate to mice and rats. *Cancer Chemother. Pharmacol.* **43**, 445–453.

Elwood, P.C., Pickering, J.E., Hughes, J., Fehily, A.M., Ness, A.R. 2004. Milk drinking, ischaemic heart disease and ischaemic stroke. 2. Evidence from cohort studies. *Eur. J. Clin. Nutr.* **58**, 718–724.

El-Sohemy, A., Kendall, C.W.C., Rao, A.V., Archer, M.C., Bruce, W.R. 1996a. Dietary cholesterol inhibits the development of aberrant crypt foci in the colon. *Nutr. Cancer*. **25**, 111–117.

El-Sohemy, A., Bruce, W.R., Archer, M.C. 1996b. Inhibition of rat mammary tumorigenesis by dietary cholesterol. *Carcinogenesis*. **17**, 159–162.

Friedman, J.M. 2003. A war on obesity, not the obese. *Science*. **299**, 856–858.

Gillman, M.W., Cupples, L.A., Gagnon, D., Millen, B.E., Ellison, R.C., Castelli, W.P. 1997. Margarine intake and subsequent coronary heart disease in men. *Epidemiology*. **8**, 144–149.

Giovannucci, E., Goldin, B. 1997. The role of fat, fatty acids, and total energy intake in the etiology of human colon cancer. *Am. J. Clin. Nutr*. **66**, 1564S–1571S.

Gurr, M.I. 1994. Nutritional significance of lipids. In: *Advanced Dairy Chemistry: Lipids*, Vol. 2, 2nd edn (P.F. Fox, ed.), pp. 349–402, Chapman and Hall, London.

Gurr, M.I., Harwood, L.L., Frayn, K.N. 2002. *Lipid Biochemistry*. 5th edn, Blackwell Science, Oxford.

Hansen, C.M., Binderup, L., Hamberg, K.J., Carlberg, C. 2001. Vitamin D and cancer: effects of 1, 25$(OH)_2D_3$ and its analogs on growth control and tumorigenesis. *Front. Biosci*. **6**, 820–848.

Hertervig, E., Nilsson, A., Bjork, J., Hultkrantz, R., Duan, R-D. 1999. Familial adenomatous polyposis is associated with a marked decrease in alkaline sphingomyelinase activity: a key factor to the unrestrained cell proliferation? *Br. J. Cancer*. **81**, 232–236.

Hertervig, E., Nilsson, A., Nyberg, L., Duan, R-D. 1997. Alkaline sphingomyelinase activity is decreased in human colorectal carcinoma. *Cancer*. **79**, 448–453.

Holick, M.F. 2004. Vitamin D: importance in the prevention of cancers, type 1 diabetes, heart disease, and osteoporosis. *Am. J. Clin. Nutr*. **79**, 362–371.

Holmes, M.D., Hunter, D.J., Colditz, G A., Stampfer, M.J., Hankinson, S.E., Speizer, F.E., Rosner, B., Willett, W.E. 1999a. Association of dietary intake of fat and fatty acids with risk of breast cancer. *J. Am. Med. Assoc*. **281**, 914–920.

Holmes, M.D., Stampfer, M.J., Colditz, G.A., Rosner, B., Hunter, D.J., Willett, W.C. 1999b. Dietary factors and the survival of women with breast carcinoma. *Cancer*. **86**, 826–835.

Howe, G.R., Aronson, K.J., Benito, E., Castelleto, R., Cornee, J., Duffy, S., Gallagher, R.P., Iscovich, J.M., Deng-ao, J., Kaaks, R., Kune, G.A., Lee, H.P., Lee, M., Miller, A.B., Peters, R.K., Potter, J.D., Riboli, E., Slattery, M.L., Trichopoulos, D., Tuyns, A., Tzonou, A., Watson, L.F., Whittemore, A.S., Wu-Williams, A.H., Shu, Z. 1997. The relationship between dietary fat intake and risk of colorectal cancer: evidence from the combined analysis of 13 case-control studies. *Cancer Causes Control*. **8**, 215–228.

Hu, F.B., Stampfer, M.J., Manson, J.E., Ascherio, A., Colditz, G.A., Speizer F.E., Hennekens, C.H., Willett, W.C. 1999. Dietary saturated fats and their food sources in relation to the risk of coronary heart disease in women. *Am. J. Clin. Nutr*. **70**, 1001–1008.

Hu, F.B., Stampfer, M.J., Manson, J.E., Rimm, E., Colditz, G.A., Rosner, B.A., Hennekens, C.H., Willett, W.C. 1997. Dietary fat intake and the risk of coronary heart disease in women. *N. Engl. J. Med*. **337**, 1491–1499.

Hu, F.B., Willett, W.E. 2000. Protein, fat, and ischemic heart disease. *Am. J. Clin. Nutr*. **71**, 848–849.

Hubbard, N.E., Lim, D., Erickson, K.L. 2003. Effect of separate conjugated linoleic acid isomers on murine mammary tumorigenesis. *Cancer Lett*. **190**, 13–19.

Hunter, D.J., Spiegelman, D., Adami, H-O., Beeson, L., van den Brandt, P.A., Folson, A.R., Fraser, G.E., Goldbohm, R.A., Graham, S., Howe, G.R., Kushi, L.H., Marshall, J.R., McDermott, A., Miller, A.B., Speizer, F.E., Wolk, A., Yaun, S-S., Willett, W.C. 1996. Cohort studies of fat intake and the risk of breast cancer–a pooled analysis. *N. Engl. J. Med*. **334**, 356–361.

Ip, C. 1990. Quantitative assessment of fat and calories as risk factors in mammary carcinogenesis in an experimental model. In: *Recent Progress in Research on Nutrition and Cancer* (C.J. Mettlan, T. Aoki, eds.), pp. 107–117, Wiley–Liss, New York.

Ip, C. 1997. Review of the effects of *trans* fatty acids, oleic acid, n-3 polyunsaturated fatty acids, and conjugated linoleic acid on mammary carcinogenesis in animals. *Am. J. Clin. Nutr*. **66**, 1523S–1529S.

Ip, C., Chin, S.F., Scimeca, J.A., Pariza, M.W. 1991. Mammary cancer prevention by conjugated dienoic derivative of linoleic acid. *Cancer Res*. **51**, 6118–6124.

Ip, C., Banni, S., Angioni, E., Carta, G., McGinley, J., Thompson, H.J., Barbano, D., Bauman, D.E. 1999. Conjugated linoleic acid-enriched butter fat alters mammary gland morphogenesis and reduces cancer risk in rats. *J. Nutr*. **129**, 2135–2142.

Ip, M.M., Masso-Welch, P.A., Ip, C. 2003. Prevention of mammary cancer with conjugated linoleic acid: role of stroma and the epithelium. *J. Mammary Gland Biol. Neoplasia*. **8**, 103–118.

Kersten, S., Desvergne, B., Wahli, W. 2000. Roles of PPARs in health and disease. *Nature*. **405**, 421–424.

Kolonel, L.N. 2001. Fat, meat, and prostate cancer. *Epidemiol. Rev*. **23**, 72–81.

Krauss, R.M. 2001. Dietary and genetic effects on low-density lipoprotein heterogeneity. *Ann. Rev. Nutr*. **21**, 283–295.

Krinsky, N.I. 1993. Actions of carotenoids in biological systems, *Ann. Rev. Nutr*. **13**, 561–587.

Kris-Etherton, P.M., Binkoski, A.E., Zhao, G., Coval, S.M., Clemmer, K.F., Hecker, K.D., Jacques, H., Etherton, T.D. 2002. Dietary fat: assessing the evidence in support of a moderate-fat diet; the benchmark based on lipoprotein metabolism. *Proc. Nutr. Soc*. **61**, 287–298.

Kritchevsky, D. 1999. Conjugated linoleic acid and experimental atherosclerosis in rabbits. In: *Advances in Conjugated Linoleic Acid Research*, Vol. 1 (M.P. Yurawecz, M.M. Mossoba, J.K.G. Kramer, M.W. Pariza, G.J. Nelson, eds.), pp. 397–403, American Oil Chemists' Society Press, Champaign, IL.

Kritchevsky, D. 2003. Conjugated linoleic acid in experimental atherosclerosis. In: *Advances in Conjugated Linoleic Acid Research*, Vol. 2 (J-L. Sebedio, W.W. Christie, R.O. Adlof, eds.), pp. 292–301, American Oil Chemists' Society Press, Champaign, IL.

Kritchevsky, S.B., Kritchevsky, D. 2000. Egg consumption and coronary heart disease: an epidemiologic overview. *J. Am. Coll. Nutr*. **19**, 549S–555S.

Kushi, L., Giovannucci, E. 2002. Dietary fat and cancer. *Am. J. Med*. **113**, 63S–70S.

Kuulasmaa, K., Tynstall-Pedoe, H., Dobson, A., Fortmann, S., Sans, S., Tolonen, H., Evans, A., Ferrario, M., Tuomilehto, J. 2000. Estimation of contribution of changes in classic risk factors to trends in coronary-event rates across the WHO MONICA project populations. *Lancet*. **355**, 675–687.

Lafontan, M., Berlan, M. 2003. Do regional differences in adipocyte biology provide new pathophysiological insights? *Trends Pharmacol. Sci*. **24**, 276–283.

Lemonnier, L.A., Dillehay, D.L., Vespremi, M.J., Abrams, J., Brody, E., Schmelz, E.M. 2003. Sphingomyelin in the suppression of colon tumors: prevention versus intervention. *Arch. Biochem. Biophys*. **419**, 129–138.

Maron, D.J., Fazio, S., Linton, M.F. 2000. Current perspectives on statins. *Circulation*. **101**, 207–213.

Masso-Welch, P.A., Zangani, D., Ip, C., Vaughan, M.M., Shoemaker, S.F., McGee, S.O., Ip, M.M. 2004. Isomers of conjugated linoleic acid differ in their effects on angiogenesis and survival of mouse mammary adipose vasculature. *J. Nutr*. **134**, 299–307.

Mauger, J-F., Lichtenstein, A.H., Ausman, L.M., Jalbert, S.M., Jauhiainen, M., Ehnholm, C., Lamarche, B. 2003. Effect of different forms of dietary hydrogenated fats on LDL particle size. *Am. J. Clin. Nutr*. **78**, 370–375.

McCann, S.E., Ip, C., Ip, M.M., McGuire, M.K., Muti, P., Edge, S.B., Trevisan, M., Freudenheim, J.L. 2004. Dietary intake of conjugated linoleic acid and risk of premenopausal and postmenopausal breast cancer, Western New York Exposures and Breast Cancer Study (WEB Study). *Cancer Epidemiol. Biomark. Prev*. **13**, 1480–1484.

McDevitt, R.M., Poppitt, S.D., Murgatroyd, P.R., Prentice, A.M. 2000. Macronutrient disposal during controlled overfeeding with glucose, fructose, sucrose, or fat in lean and obese women. *Am. J. Clin. Nutr*. **72**, 369–377.

McNamara, D.J. 2000. Dietary cholesterol and atherosclerosis. *Biochim. Biophys. Acta*. **1529**, 310–320.

Mensink, R.P., Zock, P.L., Katan, M.P., Hornstra, G. 1992. Effect of dietary *cis* and *trans* fatty acids on serum lipoprotein[a] levels in humans. *J. Lipid Res*. **33**, 1493–1501.

Mensink, R.P., Zock, P.L., Kester, A.D.M., Katan, M.B. 2003. Effects of dietary fatty acids and carbohydrates on the ratio of serum total to HDL cholesterol and on serum lipids and apolipoproteins: a meta-analysis of 60 controlled trials. *Am. J. Clin. Nutr*. **77**, 1146–1155.

Missmer, S.A., Smith-Warner, S.A., Spiegelman, D., Yuan, S-S., Adami, H-O., Beeson, W.L., van den Brandt, P.A., Fraser, G.E., Freudenheim, J.L., Goldbohm, R.A., Graham, S., Kushi, L.H., Miller, A.B., Potter, J.D., Rohan, T.E., Speizer, F.E., Toniolo, P., Willett, W.C., Wolk, A., Zeleniuch-Jacquotte, A., Hunter, D.J. 2002. Meat and dairy food consumption and breast cancer: a pooled analysis of cohort studies. *Int. J. Epidemiol*. **31**, 78–85.

Moschetta, A., van Berge-Henegouwen, G.P., Portincasa, P., Palasciano, G., Groen, A.K., van Erpecum, K.J. 2000. Sphingomyelin exhibits greatly enhanced protection compared with egg yolk phosphatidylcholine against detergent bile salts. *J. Lipid Res*. **41**, 916–924.

Muldoon, M.F., Manuck, S.B., Matthews, K.A. 1990. Lowering cholesterol concentrations and mortality: a quantitative review of primary prevention trials. *Brit. Med. J*. **301**, 309–314.

Niles, R.M. 2000. Vitamin A and cancer. *Nutrition*. **16**, 573–576.

Nyberg, L., Nilsson, A., Lundgren, P., Duan, R-D. 1997. Localization and capacity of sphingomyelin digestion in the rat intestinal tract. *J. Nutr. Biochem*. **8**, 112–118.

Oomen, C.M., Ocke, M.C., Feskens, E.J.M., van Erp-Baart, M-A.J., Kok, F.J., Kromhout, D. 2001. Association between *trans* fatty acid intake and 10-year risk of coronary heart disease in the Zutphen Elderly Study: a prospective population-based study. *Lancet*. **357**, 746–751.

Parodi, P.W. 2001a. An assessment of the evidence linking calcium and vitamin D to colon cancer prevention. *Aust. J. Dairy Technol*. **56**, 38–58.

Parodi, P.W. 2001b. Cow's milk components with anti-cancer potential. *Aust. J. Dairy Technol*. **56**, 65–73.

Parodi, P.W. 2004. Milk fat in human nutrition. *Aust. J. Dairy Technol*. **59**, 3–58.

Pekkanen, J., Linn, S., Heiss, G., Suchindran, C.M., Leon, A., Rifkind B.M., Tyroler, H.A. 1990. Ten-year mortality from cardiovascular disease in relation to cholesterol level among men with and without preexisting cardiovascular disease. *N. Engl. J. Med*. **322**, 1700–1707.

Pietinen, P., Ascherio, A., Korhonen, P., Hartman A.M., Willett, W.C., Albanes, D., Virtamo, J. 1997. Intake of fatty acids and risk of coronary heart disease in a cohort of Finnish men. The Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study. *Am. J. Epidemiol*. **145**, 876–887.

Pirozzo, S., Summerbell, C., Cameron, C., Glasziou, P. 2003. Should we recommend low-fat diets for obesity? *Obesity Rev*. **4**, 83–90.

Pouillart, P.R. 1998. Role of butyric acid and its derivatives in the treatment of colorectal cancer and hemoglobinopathies. *Life Sci*. **63**, 1739–1760.

Ravnskov, U. 1992. Cholesterol lowering trials in coronary heart disease: frequency of citation and outcome. *Br. Med. J*. **305**, 15–19.

Ravnskov, U. 1995. Quotation bias in reviews of the diet-heart idea. *J. Clin. Epidemiol*. **48**, 713–719.

Ravnskov, U. 1998. The questionable role of saturated and polyunsaturated fatty acids in cardiovascular disease. *J. Clin. Epidemiol*. **51**, 443–460.

Russell, R.M. 2004. The enigma of β-carotene in carcinogenesis: what can be learned from animal studies. *J. Nutr*. **134**, 262S–268S.

Sacks, F.M., Moye, L.A., Davis, B.R., Cole, T.G., Rouleau, J.L., Nash, D.T., Pfeffer, M.A., Braunwald, E. 1998. Relationship between plasma LDL concentrations during treatment with pravastatin and recurrent coronary events in the cholesterol and recurrent events trial. *Circulation*. **97**, 1446–1452.

Sacks, F.M., Tonkin, A.M., Shepherd, J., Braunwald, E., Cobbe, S., Hawkins, C.M., Keech, A., Packard, C., Sims, J., Byington, R., Furberg, C.D. 2000. Effect of pravastatin on coronary disease events in subgroups defined by coronary risk factors. *Circulation*. **102**, 1893–1900.

Sanders, T.A.B. 2003. High-versus low-fat diets in human diseases. *Curr. Opin. Clin. Nutr. Metab. Care*. **6**, 151–155.

Santora, J.E., Palmquist, D.L., Roehrig, K.L. 2000. *Trans*-vaccenic acid is desaturated to conjugated linoleic acid in mice. *J. Nutr*. **130**, 208–215.

Schaefer, E.J. 2002. Lipoproteins, nutrition, and heart disease. *Am. J. Clin. Nutr*. **75**, 191–212.

Schmelz, E.M., Dillehay, D.L., Webb, S.K., Reiter, A., Adams, J., Merrill, A.H. 1996. Sphingomyelin consumption suppresses aberrant colonic crypt foci and increases the proportion of adenomas *versus* adenocarcinomas in CF1 mice treated with 1,2-dimethylhydrazine: implications for dietary sphingolipids and colon carcinogenesis. *Cancer Res*. **56**, 4936–4941.

Schmelz, E.M., Bushnev, A.S., Dillehay, D.L., Liotta, D.C., Merrill, A.H. 1997. Suppression of aberrant colonic crypt foci by synthetic sphingomyelins with saturated or unsaturated sphingoid base backbones. *Nutr. Cancer*. **28**, 81–85.

Schmelz, E.M., Sullards, M.C., Dillehay, D.L., Merrill, A.H. 2000. Colonic cell proliferation and aberrant crypt foci formation are inhibited by dairy glycosphingolipids in 1, 2-dimethylhydrazine-treated CF1 mice. *J. Nutr*. **130**, 522–527.

Schmelz, E.M., Roberts, P.C., Kustin, E.M., Lemonnier, L.A., Sullards, M.C., Dillehay, D.L., Merrill, A.H. 2001. Modulation of intracellular β-catenin localization and intestinal tumorigenesis *in vivo* and *in vitro* by sphingolipids. *Cancer Res*. **61**, 6723–6729.

Schneeman, B.O., Burton-Freeman, B., Davis, P. 2003. Incorporating dairy foods into low and high fat diets increases the postprandial cholecystokinin response in men and women, *J. Nutr*. **133**, 4124–4128.

Seidell, J.C. 1998. Dietary fat and obesity: an epidemiologic perspective. *Am. J. Clin. Nutr*. **67**, 546S–550S.

Selzner, M., Bielawska, A., Morse, M.A., Rudiger, H.A., Sindram, D., Hannun, Y.A., Clavien, P-A. 2001. Induction of apoptotic cell death and prevention of tumor growth by ceramide analogues in metastatic human colon cancer. *Cancer Res*. **61**, 1233–1240.

Smith-Warner, S.A., Spiegelman, D., Adami, H-O., Beeson, W.L., van den Brandt, P.A., Folsom, A.R., Fraser, G.E., Freudenheim, J.L., Goldbohm, R.A., Graham, S., Kushi, L.H., Miller, A.B., Rohan, T.E., Speizer, F.E., Toniolo, P., Willett, W.C., Wolk, A., Zeleniuch-Jacquotte, A., Hunter, D.J. 2001. Types of dietary fat and breast cancer: a pooled analysis of cohort studies. *Int. J. Cancer*. **92**, 767–774.

Stamler, J., Wentworth, D., Neaton, J.D. 1986. Is relationship between serum cholesterol and risk of premature death from coronary heart disease continuous and graded? *J. Am. Med. Assoc*. **256**, 2823–2828.

Studzinski, G.P., Moore, D.C. 1995. Sunlight—can it prevent as well as cause cancer? *Cancer Res*. **55**, 4014–4022.

Takemoto, M., Liao, J.K. 2001. Pleiotropic effects of 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors. *Arterioscler. Thromb. Vasc. Biol*. **21**, 1721–1719.

Toomey, S., Roche, H., Fitzgerald, D., Belton, O. 2003. Regression of pre-established atherosclerosis in the apoE$^{-/-}$ mouse by conjugated linoleic acid. *Biochem. Soc. Trans*. **31**, 1075–1079.

Turpeinen, A.M., Mutanen, M., Aro, A., Salminen, I., Basu, S., Palmquist, D.L., Grinnari, J.M. 2002. Bioconversion of vaccenic acid to conjugated linoleic acid in humans. *Am. J. Clin. Nutr*. **76**, 504–510.

Urquhart, P., Parkin, S.M., Rogers. J.S., Bosley, J.A., Nicolaou, A. 2002. The effect of conjugated linoleic acid on arachidonic acid metabolism and eicosanoids production in human saphenous vein endothelial cells. *Biochim. Biophys. Acta*. **1580**, 150–160.

van den Bemd, G-J.C.M., Pols, H.A.P. and van Leeuwen, P.T.M. 2000. Anti-tumor effects of 1, 25-dihydroxyvitamin D_3 and vitamin D analogs. *Curr. Pharm. Des*. **6**, 717–732.

van Leeuwen, J.P.T.M., Pols, H.A.P. 1997. Vitamin D: anticancer and differentiation. In: *Vitamin D* (D. Feldman, F.H. Glorieux, J.W. Pike, eds.), pp. 1089–1105, Academic Press, San Diego.

Verschuren, W.M.M., Jacobs, D.R., Bloemberg, B.P.M., Kromhout, D., Menotti, A., Aravanis, C., Blackburn, H., Buzina, R., Dontas, A.S., Fidanza, F., Karvonen, M.J., Nedeljkovic, S., Nissinen, A., Toshima, H. 1995. Serum total cholesterol and long-term coronary heart disease mortality in different cultures. Twenty-five-year follow-up of the Seven Countries Study. *J. Am. Med. Assoc*. **274**, 131–136.

Vesper, H., Schmelz, E-M., Nikolova-Karakashian, M.N., Dillehay, D L., Lynch, D.V., Merrill, A.H. 1999. Sphingolipids in food and the emerging importance of sphingolipids to nutrition. *J. Nutr*. **129**, 1239–1250.

Warensjo, E., Jansson, J-H., Berglund, L., Boman, K., Ahren, B., Weinehall, L., Lindahl, B., Hallmans, G., Vessby, B. 2004. Estimated intake of milk fat is negatively associated with cardiovascular risk factors and does not increase the risk of a first acute myocardial infarction. A prospective case-control study. *Br. J. Nutr*. **91**, 635–642.

Williams, E.A., Coxhead, J.M., Mathers, J.C. 2003. Anti-cancer effects of butyrate: use of micro-array technology to investigate mechanisms. *Proc. Nutr. Soc*. **62**, 107–115.

Willett, W.C. 1997. Specific fatty acids and risks of breast and prostate cancer: dietary intake. *Am. J. Clin. Nutr*. **66**, 1557S–1563S.

Willett, W.C. 2001a. Diet and cancer: one view at the start of the millennium. *Cancer Epidemiol. Biomark. Prev*. **10**, 3–8.

Willett, W.C. 2001b. Diet and breast cancer. *J. Intern. Med*. **249**, 395–411.

Willett, W.C. 2002. Dietary fat plays a major role in obesity: no. *Obesity Rev*. **3**, 59–68.

Willett, W.C., Stampfer, M.J., Manson, J.E., Colditz, G.A., Speizer, F.E., Rosner, B.A., Sampson, L.A., Hennekens, C.H. 1993. Intake of *trans* fatty acids and risk of coronary heart disease among women. *Lancet*. **341**, 581–585.

Wongtangtintharn, S., Oku, H., Iwasaki, H., Toda, T. 2004. Effect of branched-chain fatty acids on fatty acid biosynthesis of human breast cancer cells. *J. Nutr. Sci. Vitaminol*. **50**, 137–143.

Yanagi, S., Yamashita, M., Imai, S. 1993. Sodium butyrate inhibits the enhancing effect of high fat diet on mammary tumorigenesis. *Oncology*. **50**, 201–204.

Yang, Z., Liu, S., Chen, X., Chen, H., Huang, M., Zheng, J. 2000. Induction of apoptotic cell death and *in vivo* growth inhibition of human cancer cells by a saturated branched-chain fatty, 13-methyltetradecanoic acid. *Cancer Res*. **60**, 505–509.

Yin, L., Laevsky, G., Giardina, C. 2001. Butyrate suppression of colonocyte NF-κB activation and cellular proteasome activity. *J. Biol. Chem.* **276**, 44641–44646.

Yu, Y., Correll, P.H., Vanden Heuvel, J.P. 2002. Conjugated linoleic acid decreases the production of pro-inflammatory products in macrophages: evidence for a PPARγ-dependent mechanism. *Biochim. Biophys. Acta*. **1581**, 89–99.

Zittermann, A. 2003. Vitamin D in preventive medicine: are we ignoring the evidence? *Br. J. Nutr*. **89**, 552–572.