Review

Relevance of Milk Protein for Muscle Hypertrophy, Fermented Milk Products for Gut Microbiome, and Milkfat for Cardiovascular Health: Facts and Controversies

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Relevance of Milk Protein for Muscle Hypertrophy, Fermented Milk Products for Gut Microbiome, and Milkfat for Cardiovascular Health: Facts and Controversies

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Abstract

Milk was designed by evolution to provide superior nutrition for support of growth and development of mammalian young**.** When humans domesticated dairy cattle about 10,500 years ago, they also adopted milk for adult consumption and learned to separate and utilize its constituent parts, milk proteins whey and casein for muscle growth, milk fermentation to make kefir, yogurt, and cheese, and milkfat to make butter. Research on how consumption of these different milk products affects human health has generated much factual data and some uncertainties and controversies about the extent it can be used to improve adult human body and overall metabolic health. This is important in the context of global burden of high cardiovascular morbidity and concerns about any impact of milkfat consumption on cardiovascular (CVD) and coronary heart diseases (CHD). The first theme of this review examines the involvement of milk proteins whey and casein on skeletal muscle hypertrophy (MHT). The major contribution of resistance training (RET) to MHT is contrasted to the lesser, but still important, contribution of protein supplementation (PS) and uncertainties about the efficacy of the milk proteins relative to plant proteins, along with dose, training status, and timing of PS relative to RET in producing MHT. The exceptionally rich concentration of essential and branched-chain amino acids makes whey protein and casein highly effective but not essential for MHT which can also be achieved with higher quality plant PS and is not critically dependent on either the timing of PS, the training status, or the age of users. The second theme examines the nature and importance of milk fermentation in production of full-fat and low-fat yogurt, kefir, and cheese in terms of bacteria involved, their metabolism in the gut, their beneficial influence on the gut microbiome (GM) and on overall as well as cardiovascular health. Lastly, milkfat as influence on cardiovascular health is discussed both from the perspective of its effects on blood lipids and cardiovascular physiology, but also as a component of the complex dairy matrices. As part of a rich nutrient matrix, milk products provide benefits to cardiovascular health because of their biologically active proteins and fatty acids which exert anti-inflammatory, anti-carcinogenic, antioxidative, and other beneficial actions, despite their high fat content and level of fat saturation. Fermentation usually lowers CVD and CHD risks of full-fat milk and its products, but health benefits often are greater when their fat content is reduced. Butter does not benefit from the biological activities of the milk proteins and is not fermented, so when consumed in large quantities, the balance of cardiovascular benefits shifts toward higher CVD and CHD risk. Three knowledge gaps need to be corrected for a better understanding of health benefits of consumption of milk products. Individual nutrient components in dairy food matrices need to be measured and recognized. Their identity needs to be linked to a better understanding of how they influence atherogenic lipoproteins and protein synthesis. And maximal consumption limits need to be defined for full-fat milk products to assure the benefits that their biologically active components offer, but also to reduce their detrimental effects on cardiovascular risk factors. Overall, as a food category, milk products justify acceptance as a healthy natural source of nutrition that was evolutionarily designed to support early growth and development of mammalian young but need to be prudently implemented for their lifelong consumption in adulthood.

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1. Introduction

Milk was evolutionarily designed to provide superior nutrition for support of growth and development of mammalian young**.** Abundant supply of milk from lactating mammals, including humans, during early development assures rapid growth of their young to optimal species-specific adult size. Insufficient supply of milk to mammalian young produces stunting and manifestations of metabolic abnormalities in adulthood [1] When humans changed from food gathering to food production about 10,500 years ago, they domesticated certain mammals such as the cow, sheep, goat, buffalo, yak, and camel [2] and adopted the milk from these animals for adult consumption They developed and learned to utilize its constituent parts, milk proteins whey and casein for muscle growth, milk fermentation to make kefir, yogurt, and cheese, and milkfat to make butter The consumption of milk and its products persists until this day. Given that the individual milk products have different nutrient compositions and can therefore affect human health differently, this review provides an overview of the facts, uncertainties, and controversies about their effects on different aspects of human health. The review outlines the relevance of cow's milk proteins to resistance-exercise-training (RET)-associated muscle hypertrophy (MHT) and increases in strength in section 3, relevance of milk products to gut microbiome (GM) in section 4, and relevance of milkfat to cardiovascular health (CVH) in section 5. This review integrates the information about these diverse milk food products that meet several different health needs.

2. Method

Considering the extensive scope of the review covering three different areas of interest and the diverse study methods and subject characteristics, selection of included data followed the accepted protocol of prioritizing randomized control studies (RCTs) with experimental manipulation of key variables and statistically justified meta-analyses and systematic reviews providing descriptive data but usually including large population cohorts followed for long periods of time. Also included were smaller studies with rigorous controls such as exercise trials engaging limbs unilaterally in short exercise bouts and using the inactive limb as sedentary control. Studies were largely limited to work published during the past two decades except for instances of historical significance. In section 3, the criteria for study inclusion were documentation of the duration, timing, and intensity of RET, the type and necessary concentration of protein supplementation (PS), and participant numbers that were appropriate to study design. In section 4, the health influence of fermented milk products was justified, in part, to their influence on gut microbiome. In section 5, the health effects of milkfat are presented both from the perspective of fat type and quantity in milk products as well as representing complex ingredient matrix in milk foods that contain it. Included also is the unresolved controversy over the relative suitability of nutritional advice and policy focusing on individual nutrient ingredients and properties such as saturation of milkfat as opposed to focusing on food matrices of milk products providing a spectrum of diverse health outcomes. PubMed Commons, Google Scholar, and bibliographies of representative recent meta-analyses and reviews were the source of data for this review.

3. Relevance of Milk Proteins for RET-Associated MHT and Increases in Strength

RET is the most potent nonpharmacological means of increasing skeletal muscle mass in adulthood by hypertrophy (MHT, [3]) after the capacity for prepubertal muscle cell proliferation has ended [4]. Mechanical loading of skeletal muscle is required to produce skeletal MHT which is manifested as an increase in axial cross-sectional area (CSA) of muscle and muscle fibers. To achieve

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that, RET requires consumption of protein for incorporation of amino acids into muscle protein. The first theme of this review examines the extent to which cow milk proteins contribute to RET-associated MHT in section 3. The features of cow's milk composition that support RET-associated MHT are outlined in section 3.1. Effective RET protocols for inducing MHT are outlined in section 3.2. Section 3.3 briefly outlines mechanotransduction hypotheses on how mechanical loading of muscles induces MHT. Milk proteins' effectiveness and specificity for RET-associated MHT is in section 3.4, and factors that modify RET-associated MHT and enhancement in strength are in section 3.5.

3.1. Milk Composition and Properties

Nutrient components of cow's milk have significant beneficial effects on human health [5]. Its nutrient composition is presented in Table 1.

Table 1. Nutrient components of bovine milk and of some of its fermented products per 100g of edible portion. Data adapted from USDA Food Data Central (https://fdc.nal.usda.gov/ (Accessed December 10, 2025).

Nutrient	Whole milk	Semi Skim milk	Skim milk	Whole natural yogurt	Whole Greek yogurt	Skimmed natural yogurt	Cheddar cheese	Swiss cheese	Ricotta cheese	Parmesan cheese
Energy (kcal)	63	48	38	60	95	50	409	393	158	420
Protein (g)	3.10	3.50	3.90	4	8.78	4.30	23.30	23.7	7.81	29.60
Fat (g)	3.80	1.60	0.20	2.60	4.39	0.32	34	31	11	28
SFA(g)	2.30	1.10	0.09	1.50	2.39	0.11	19.2	18.2	6.97	15.5
MUFA (g)	1.10	0.45	0.06	0.72	0.96	o.15	7.44	7.26	2.56	6.40
PUFA (g)	0.13	0.04	0.01	0.13	0.11	0	1.18	1.14	0.39	1.20
Cholesterol (mg)	14	6.30	2.60	10.20	17	1	100	93	48	87
Carbohydrate (g)	4.70	4.80	4.90	5.50	4.75	6.30	2.44	1.44	6.86	12.40
Water (g)	88.4	90.1	91	87.90	81.3 0	89.10	36.60	37.60	72.90	22.80
Calcium (mg)	124	125	121	142	111	140	707	890	224	884
Sodium (mg)	48	47	53	80	34	57	654	185	105	1750
Iodine (µg)	3.5 - 53									
Potassium (mg)	157	155	150	280	147	187	77	71	230	154
Phosphorus (mg)	92	91	97	170	126	109	458	574	162	634

Kcal= kilocalories, mg= milligram, µg= microgram, MUFA= monounsaturated fatty acids,, PUFA=polyunsaturated fatty acids, SFA= saturated fatty acids.

3.1.1. Milk Proteins

The bulk of bovine milk nitrogen is divided between casein (78%) and whey proteins (17%) [5]. Casein is formed as a curd either by milk acidification or by addition of the enzyme chymosin in the enzyme-rich rennet from the stomach lining of unweaned calves. Whey is a byproduct of milk processing that remains after casein coagulation during cheese production. Both casein and whey protein contain all nine essential amino acids (EAAs) (Table 2), and an abundance of branched-chain fatty acids (BCAAs leucine, isoleucine, and valine). Availability and proportions of nine EAAs is particularly important as they cannot be synthesized and need to be taken in the diet. Muscle and whole-body fractional protein synthetic rate are dependent on all nine amino acids being available and are limited by whichever EAA is in short supply. Whey protein has been favored for RET-associated MHT for both its amino acid composition that reflects that of the muscle [6–8] and for having, along with casein, the highest content of leucine, one of the EAAs and one of three BCAAs. Initially, leucine has received the most attention in MHT research because of its ability to stimulate the initial acute anabolic response in the muscle [9]. Whey protein and casein exceed by almost a factor of 2 the EAA criterion for muscle protein synthesis established by FAO/WHO [10]. Whey is particularly rich in BCAAs leucine and isoleucine, while casein has a higher proportion of EAAs histidine, methionine, and phenylalanine but a similar amount of the BCAA valine as whey. Concentrations of all EAAs in plasma except for lysine and threonine are below the FAO/WHO criterion and require EAA supplementation to stimulate muscle growth. Tryptophan concentrations

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were not reported for the muscle where this EAA produces a metabolite kenurenine for regulation of cellular energy and several other functions [11].

Table 2. Essential amino acids in plasma and muscle relative to WHO criteria, and in cow's milk and plant proteins.

EAA	Plasma (mg/dl)	Muscle (mg/dg W)	FAO/WHO′ (g/dg P)	Whey (g/dg P)	Soy (g/dg P)	Pea (g/dg P)′	Casein (g/dg P)	Milk SP (g/dg P
HIS	1.2	6.5	1.5	1.6	2.5	2.4	3.1	2.0
ISO	1.2	2.1	3.0	7.4	4.9	4.4	5.9	5.0
LEU	2.0	2.9	5.9	12.1	5.6	7.6	10.2	12.0
LYS	5.0	9.9	4.5	10.9	5.6	6.7	8.5	9.6
MET	0.6	1.4	1.6	2.5	1.4	0.9	2.9	2.1
PHE	0.9	0.6	2.5	3.8	5.5	5.7	5.5	3.8
THR	2.6	13.8	2.3	8.8	3.9	3.8	4.6	5.0
TRY	0.4	NA	0.6	1.7	1.3	0.9	1.4	2.1
VAL	2.9	4.2	3.9	6.9	5.1	4.9	7.6	5.1

Dg=100 g, dl=100 ml, EAA= essential amino acids, HIS= histidine, ISO=isoleucine, LEU= leucine, LYS= lysine, MET= methionine, NA= not available, P=protein, PHE= phenylalanine, SP= soluble protein, THR=threonine. TRY= tryptophan, VAL=valine, W= wet weight. Data from [5–8,10,12].

An important property of EAAs is that they are the primary regulators of the protein-mediated insulin response [13], the key hormone facilitating protein synthesis. Measured by stimulation of maximal plasma insulin concentration (Cmax), a 30-min intravenous infusion of leucine, lysine, phenylalanine, and arginine identified them as most effective EAA insulin secretagogues with their respective Cmax values of 193, 358, 172, and 567 pM. The insulinotropic properties of BCAA contribute to their stimulation of muscle protein synthesis (MPS).

The rate of protein digestion can influence the initial rate of MPS and is measured by the protein-digestibility amino-acid score (PDCAAS). Whey protein has fast digestibility as in its standard form it occurs within 2 to 2.5 hours, and its AA hydrolysate does so in 1 to 1.5 hours. Proteins that digest at medium speed of 3 to 3.5 hours include the egg, egg white, soy, and pea protein. Casein digestion is classified as slow as it requires greater than 4 hours. PDCAAS is ±100% for milk protein isolate, casein, egg white, whey preparations, and soy and pea protein isolates [14].

Milk also is a rich source of bioactive peptides (BPs) [5,15,16]. BPs are naturally present phosphorylated peptides derived from caseins, lysozyme, lactoferrin, immunoglobulins, or growth factors that are encrypted and inactivated in the primary structure of milk proteins. They are released after enzymatic hydrolysis or microbial fermentation of α, β, γ, and κ casein and whey proteins (β-lactoglobulin, α-lactalbumin, serum albumin, immunoglobulins, lactoferrin, and protein-peptone). Their active forms must resist digestion and be absorbed through the GI epithelium. BPs account for numerous health effects of milk due to their anti-thrombotic, antihypertensive, and anti-inflammatory as well as antioxidant, anti-microbial, or anti-obesogenic properties. Certain fragments of caseins and whey proteins called casoquinines and lactoquinines are found in milk and cheese and have antihypertensive actions of inhibiting the synthesis of angiotensin-converting enzyme (ACE). Milk-coagulating proteins, such as chymosin and κ–casein, related to thrombin and fibrinogen, affect blood coagulation and can suppress platelet aggregation ranging from anti-thrombotic, antihypertensive, and anti-inflammatory to antioxidant, anti-microbial, or anti-obesogenic actions.

Milk proteins and peptides are also potent antioxidants that inhibit reactive oxygen species, act as pro-oxidant metal scavengers, and decrease hydroperoxide levels. Whey amino acids tyrosine, tryptophan, methionine, lysine, cysteine, and histidine, have antioxidant activity. The antimicrobial peptides present in milk act as powerful antioxidants and can inhibit a wide range of pathogenic bacteria, such as *Listeria, Salmonella, Escherichia, Staphylococcus, or Helicobacter,* filamentous fungi, and yeast. The antimicrobial potential of milk is linked to immunoglobulins, lysozyme, lactoferrin, and the lactoperoxidase system which, along with caseins, are the source of antibacterial peptides that also can stimulate the innate immune system to defend against the attack of pathogens.

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Some milk proteins have anti-cancer actions. The milk amino acids tyrosine, tryptophan, methionine, lysine, cysteine, and histidine derived from whey have antioxidant activity. Glutathione, β-lactoglobulin and α-lactalbumin decrease the toxic effects of cancer. Lactoferrin is effective in promoting apoptosis of intestinal cancer. Casein derived peptides in cheese enhance the human immune system by modulating immune cell responses and regulating inflammatory cytokine production. Cheeses displaying these properties are Parmigiano Reggiano, Chinese Rushan, Naizha, Goatskin Tulum, Edam, Gouda, Karish, and Ras.

3.1.2. Milk Fat

Cow's milkfat is present in the form of digestible micro fat globules containing over 400 different fatty acids, twelve of the more abundant of which are shown in Table 3 [5] Saturated fatty acids (SFAs) represent 60.5 (Table 1) to 64.3% (Table 3) of the total fatty acid content by weight of whole milk, with myristic, palmitic, and stearic fatty acids being most abundant.

Table 3. Select individual milk fatty acids as percent of total.

Fatty acid (number of carbons & saturation)	Percent of total milk fatty acids
Butyric 4:0	3.30
Capric 10:0	2.60
Caprylic 8:0	1.20
Caproic 6:0	1.90
Lauric 12:0	3.50
Linoleic 18:2	2.40
α-Linolenic 18:3	0.5
Myristic 14:0	11.50
Oleic 18:1	28.0
Palmitic 16:0	25.80
Palmitoleic 16:1	2.90
Stearic 18:0	11.0

FAs can be classified by their carbon-chain length into short-chain and long chain FAs. Short-chain FAs (SCFAs) have carbon chains between 2 and 6 carbons long. They include acetic acid (2:0), propionic (3:0), two FAs with 4:0 carbon chain (butyric, and isobutyric), as do two with 5:0 carbon chains (valeric and isovaleric) Caproic (hexanoic) FA has a 6:0 carbon chain. Long-chain FAs (LCFAs) have greater than 6 carbons in their molecule. Monounsaturated FAs (MUFAs) are approximately 27.3% of milkfat, with oleic acid being most abundant and palmitoleic present in lesser amount. Polyunsaturated FAs (PUFAs) constitute 3.97%, including the essential linoleic acid (LA) and α–linolenic acid (ALA), as well as EPA (eicosapentaenoic acid) and DHA (docosahexaenoic acid). Some short-chain FAs, such as butyric acid (C4:0), or odd-chain FAs, such as pentadecanoic (C15:0) plus heptadecanoic (C17:0), are present only in the fat of ruminants so, their presence in subcutaneous adipose tissue or in human serum constitutes a biomarker of bovine milkfat intake.

Conjugated linoleic acid (CLA, rumenic acid, the cis-9, trans-11 isomer of $C_{18}H_{32}O_2$) is present more abundantly in the milkfat of grass-fed ruminants rather than in ruminants fed a grain-based diet. It is converted first from the essential linoleic FA by biohydrogenation to vaccenic acid and next to CLA in the rumen. The concentration in cow's milk ranges from 2 to 37 mg/g of fat depending on diet. Health benefits are based on its anti-carcinogenic, anti-obesogenic, anti-diabetic, and anti-hypertensive actions. It exerts beneficial effects on obesity (especially if combined with exercise), inflammation, atherogenicity, immune-modulation, and anti-cancer and osteogenic properties, all mitigating the metabolic syndrome (MetSyn).

Trans FAs are present in milk in small amounts: trans MUFAs at 3.1%, trans PUFAs at 0.034%, and cis/trans and trans/cis PUFAs at 1.34%. They are derived from ruminant food sources, and they have different biological effects. Trans-vaccenic acid (TVA, trans-11-C18:1, or trans-C18:1 n-7) directly

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promotes effector CD8+ T-cell function and antitumor immunity in vivo. TVA inhibits cell proliferation and induces apoptosis in human nasopharyngeal carcinoma cells. Polar lipids (PLs) represent about 1% of milkfat (29.4–40 mg/100 g of raw cow milk) and include phosphatidylcholine, sphingomyelin, diacyl- and plasmalogen phosphatidylethanolamine, phosphatidylinositol, phosphatidyl serine, and glycosphingolipids. Despite their low concentration, PLs have a positive effect on neurological development, inflammation, cardiovascular disease, cholesterol absorption, and stress.

Unsaponifiable lipids (that are not FA esters) in the milk include sterols like cholesterol, β carotene, and fat-soluble vitamins A, D, E and K. Sterols make up about 0.2 to 0.55% of total cow milk lipids. Cholesterol represents about 95% of the milk sterols ranging from 271.4 mg/100 g of fat in conventional milk to 278.8 mg/100 g of fat in organic milk, while β-sitosterol, campesterol, and stigmasterol, are present in lower amounts.

3.1.3. Milk Carbohydrates

Lactose is the exclusive disaccharide of bovine milk composed of monosaccharides glucose and galactose and represents 4.7 % of its content [5]. Glucose, galactose, glycoproteins, and glycolipids form less than 100 mg of milk per liter. Cow's milk contains between 30 and 60 mature and largely indigestible milk oligosaccharides (MOs) at a concentration of 30 to 60 mg/L. When a portion of lactose that is undigested in small intestine arrives intact to the colon, lactic acid bacteria (LAB) internalize it through phosphotransferase systems to lactose-6-phosphate. This molecule is then transformed into glucose and galactose-6-phosphate via the action of phosphogalactosidase. Products of this process plus the MOs then stimulate the growth of *Bifidobacteria* and *Lactobacilli* in the intestine favoring the autochthonous intestinal microbiota. They inhibit colonization by enteropathogenic bacteria sensitive to antibacterial bacteriocins, lactic acid, and metabolites such as SCFAs including acetate, propionate, valerate, isovalerate, butyrate, and isobutyrate. In addition to their prebiotic effect, MOs are partially absorbed in the intestines and contribute to the development of molecular structures in the brain. They have anti-inflammatory effects, reduce the intestinal adhesion of pathogens, prevent respiration, and contribute to the development of the mucosa and immune system.

3.1.4. Milk Minerals and Vitamins

Milk and dairy products are generally excellent sources of minerals, especially calcium (Table 1), which can reach concentrations of 124 mg/100mL of milk. Adult women require 750 mg of calcium per day, so a 240-ml cup of milk would provide about 40% of the daily calcium requirement. However, phosphorus, zinc, sodium, potassium, iodine, selenium, and chromium also are relevant constituents of dairy, but milk is an iron-deficient food. Milk in industrialized countries provides on average 3.5 to 53 μg of iodine per 100 ml portion which amounts to 13 to 64% of the recommended daily iodine intake of 150 μg. [17]. Milk is an important iodine source in view of recommended dietary limit to 2.3 mg of iodized salt per day. Other dietary sources are eggs, fish, seaweed, and supplements. Source of iodine in the milk is grass or hay forage and sanitary udder teat dips in iodine-containing solutions before and after milking. Whole milk is considered a good source of fat-soluble vitamins A and D, with concentrations of 56 μg/100 g and 0.05 mg/100 g, respectively. Satisfaction of the vitamin requirements of a milk-consuming adult approaches 22 to 32% of the need for vitamin A, 34 to 100% of the need for vitamin D, 64 to 92% of the need for vitamin B2, 34 to 42% of the need for vitamin B5, and 84 to 112% of the need for vitamin B12.

3.2. RET Protocols for Inducing MHT and Increases in Strength

RET combined with dietary PS is commonly practiced by athletes and recreational exercisers with the goal of increasing skeletal muscle mass and strength. This strategy also is advocated for aging people to prevent or attenuate sarcopenia, muscle loss with age [18]. It should be noted that

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increases in muscle mass produced by RET are sustained only with continued muscle loading. Decreases in voluntary physical activity led to 3% to 8% loss of muscle mass per decade, accompanied by resting metabolic rate reduction and fat accumulation [19]. Similarly, absence of muscle loading during space missions in microgravity can reduce muscle mass in young astronauts by about 20% over 6 months [20].

To examine the effects of RET on skeletal MHT and increases in strength requires a brief explanation of the properties of muscle tissue involved. Muscle tissue is distinct in at least three ways. First, myocytes, the muscle cells, are usually several centimeters long and multinuclear as opposed to typical tissue cells measuring micrometers and containing a single nucleus. Second, myocytes are packaged in bundles which then form muscles, and all these elements appear striated. The striations reflect serial myocyte units the sarcomeres. Sarcomeres are delimited by z discs or z lines and contain two myofilaments, actin, the thin one, attached to either side of the z disc and extending halfway through the sarcomere, and myosin, a thick myofilament positioned in its middle. The third feature of myocytes is that they can exert force. They do that by virtue of cross-bridge binding sites on the two myofilaments which generate ratcheting movement by binding with the help of high-energy ATP molecules. ATP provides the energy for contraction, breaks down to ADP, adenosine diphosphate, while a phosphate bound to creatine, a product of protein metabolism, donates its phosphate to resynthesize ATP and perpetuate muscle contractions. Human skeletal muscles are differentiated in three different muscle fiber types: type I is slow and oxidative (SO), type IIa is fast, oxidative, and glycolytic (FOG), and IIx is fast and glycolytic (FG). SO fibers are red in color due to high density of capillaries, mitochondria, and the red blood pigment myoglobin. They generate ATP by aerobic respiration and oxidation of both lipids and carbohydrates, making them highly fatigue resistant and suitable for maintaining body posture and endurance exercise. FOG fibers are characterized by fast contraction speed, high glycogen content and intermediate fatigue resistance. They utilize both aerobic and anaerobic carbohydrate metabolism. FG IIx fibers are white with high glycogen content and low fatigue resistance. They produce the highest power but can sustain only short intense contractions. They also are most responsive to RET and engaged in generating MHT. However, FG IIx fiber type decreases the most with aging [21] making RET relevant as a defense against sarcopenia and loss of strength in the elderly. Most human skeletal muscles are a mixture of all three fiber types [22,23].

RET is usually carried out by shortening or concentric muscle contractions against external weight or resistance, but contractions can also be eccentric when a force applied to the muscle exceeds the momentary force produced by the muscle itself, resulting in the forced lengthening of the muscle-tendon system. RET contractions promote MHT by stimulating MPS and increases in strength, the ability to exert or resist a force. Physiological formula of strength is force = mass (m) x acceleration (a). Force is measured in Newtons (N), mass in kilograms, and acceleration in meters per second squared (m/s^2). Strength measurement unit is 1 repetition maximum (1 RM), the maximal load that can be resisted in one physical effort.

RET programs that have been developed for effective increases in MHT and strength provide protocols on the magnitude, frequency, and duration of muscle loading [24]. To optimize exercise-associated MHT, it is useful to understand how to apply and time muscle force. The relevant and most studied RET variables include the following. First is the load lifted per repetition which can be either high (H) or lower (L). Next, the number of sets specifies how many single (S) or multiple (M) movements of muscle groups should be engaged. Final element is set frequency, the number of resistance exercise sets or sessions completed in a day or per other unit of time.

RET protocols have been studied as multivariate RET prescriptions (RETx) to determine their effectiveness in increasing strength and MHT. Expressed as percent probability of obtaining improvement in strength, HM2 and HM3 protocols achieve, respectively, a success probability higher than 80% when M sets of heavy lifting (H) above 80% of 1 RM are applied 2 days, or greater than 3 days, per week, respectively. When applied one day/week, the probability of achieving increased strength is 60%. Only one H loading protocol, HM2, elicits MHT with 80% probability. In contrast to

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RETx H loading protocols designed to increase strength, L loading (less than 80% of 1 RM) can significantly increase MHT. With HM3 and HM1 protocols, MHT is achieved with 22 and 30% probability, respectively. Probability of MHT is 48% for LM1 and LM2 protocols, and 33% for LS2 performed twice a week. Since these RET protocols can be implemented to a variable extent, and may also involve different parts of the body, it is not possible to quantify the contribution of RET to MHT or strength increases in absolute terms.

3.3. Mechanotransduction Hypotheses for RET-Associated Increases in MHT and Strength

Mechanotransduction is the process through which RET produces MHT and increases in strength. It is evident that RET can increase skeletal muscle fiber size and strength, but the process and the specific signals guiding these changes are not fully understood. The product of RET-associated mechanotransduction is stimulation of MPS. At the molecular level, MPS involves activation of the mammalian target of rapamycin complex (mTORC), a kinase that acts as a central master regulator of protein synthesis, cell growth, and metabolism [3,25,26]. Besides sensing MPS-promoting stimuli, mTOR complex also controls lipid synthesis, autophagy, energy metabolism, cell survival, and cytoskeletal organization [27]. mTORC consists of mTORC1 which is sensitive to rapamycin inhibition and is a key player in stimulation of RNA translation, and of mTORC2 which is insensitive to rapamycin, controls cell migration and cytoskeleton rearrangement, and sustains stimulation of MPS and MH in later stages of exercise training. mTORC1 responds to upstream stimuli such as the mechanical aspect of muscle contraction, to nutrients such as the amino acid (AA) leucine but also to other AAs, to hormones such as insulin and growth factors such as IGF-1, and to cellular stress such as blood-flow restriction to the muscle which amplifies exercise-associated MPS [28]. MTORC 1 ultimately activates the ribosomal proteins eukaryotic translation initiation factor 4Ebinding protein 1 (4E-BP1) and p70 ribosomal S6 kinase 1 (S6K1) that are necessary for protein synthesis by engaging serine/threonine kinases to phosphorylate intermediate enzyme pathways activated by mechanical and nutritional stimuli [29,30]. Two other kinases, MEK (mitogen-activated protein kinase) and ERK (extracellular signal-regulated kinase) are activated by RET [3] and their actions may be necessary, along with mTORC, for maximal activation of the RET-associated MHT and increases in strength.

There are at least four competing hypotheses regarding how mechanical muscle loading activates mTORC and MPS. One is that RET may activate mechanotransduction by producing muscle damage, cell swelling, and disruption of muscle-wide Z disc alignment. Cell swelling is proposed to produce metabolic stress and provide an anabolic stimulus for muscle growth particularly with the use of low mechanical loads [31] This hypothesis is countered by data indicating that this initial post-RET damage usually does not stimulate MHT until after tissue damage has healed [32].

The second hypothesis implicates muscle satellite cells (SCs) in RET-associated muscle damage and MHT [23,26]. A local pro-inflammatory response immediately follows myofilament damage after exercise where neutrophils and M1 macrophages mediate degeneration of damaged muscle fibers. During this stage of muscle repair, macrophages release pro-inflammatory cytokines like IL-6 and simultaneously stimulate SC activation and proliferation. SCs then either form small myocytes that integrate longitudinally into existing myocytes, or fuse into existing myocytes to contribute additional myocyte nuclei. Subsequently, macrophages change to an M2 phenotype and stimulate SC differentiation into functional muscle cell nuclei. Increased number of myocyte nuclei accelerates MPS. While at one time, the role of SCs in RET-induced MHT was considered essential, it is currently viewed as an innate mechanism responsible for the repair of muscle damage whatever its cause rather than a selective MHT stimulus specific to RET.

The third hypothesis implicates contractile myofilaments within the myocyte that are stretched during resistance exercise. The mechanosensitive myofilaments are in the costamere, a cluster of molecules physically associated with the Z disc which anchor myocytes to the extracellular matrix (ECM). The proteins involved are titin, vinculin, talin, integrin, filamins, desmin, and focal adhesion nonreceptor tyrosine kinase (FAK). Dystrophin and integrin transmembrane proteins penetrate

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sarcolemma to connect muscle cell interior with the cytoskeleton in the surrounding ECM. Dysfunction of dystrophin-glycoprotein complex is responsible for Duchenne muscular dystrophy. With the lengthening eccentric exercise, titin force increases and its molecule unfolds activating the FAK. Six weeks of multiple-set RET increase filamin C and desmin proteins in the costamere [22] but stretching of these myofilaments has not been implicated in RET-associated MHT.

The final mechanotransduction hypothesis implicates phosphatidic acid (PA) [33] and the integrin gene in hypertrophic signaling during eccentric exercise. Mechanical loads increase PA in the muscle which can activate the chief mTORC1 MPS pathway associated with RET, but the specific identity of initiating stimulus is not known. Thus, while muscle contractions can support MHT, none of the hypotheses on how they transduce the mechanical stimulus to MPS have been conclusively resolved.

3.4. Relative Effectiveness and Specificity of Cow's Milk Proteins for RET-Associated MHT and Strength Increases

Relative effectiveness of RET compared to cow's milk supplementation was presented in a meta-analysis and a meta-regression [34] that integrated the data from 1863 participants in 49 studies over a half a century since 1962. It included studies with average RET duration of 13 weeks, training at least twice a week, and on average 7 exercises per session in four sets. Protein supplementation (PS) was with whey in 23 studies, and a mixture of soy, pea, casein, and milk proteins was used in 6, 1, 3, and 10 studies, respectively. On average, 36 g of protein was supplemented daily, consumed either after RET in 40 studies, or both before and after, in 36. Supplemented group increased their daily protein intake by 23 g, while the control group did not, but daily energy intake between the two groups did not differ. Changes in strength and muscle mass in young individuals were reported both without, and with, PS. The meta-analysis showed that RET increased 1RM by 27 kg while PS contributed only an additional 2.49 kg. (9%). This indicates That RET by itself is a primary stimulus for increases in strength. Strength increases to PS during RET were more effective in trained individuals (4.3 kg) than in untrained subjects (1 kg). RET alone added 1.1 kg to fat-free mass (FFM) and increased fiber CSA by 808 μm^2 and mid-femur CSA by 52 mm^2. These increases were mostly in trained subjects (1.1 kg) while FFM declined by 0.02 kg in untrained ones. PS, on the other hand, augmented the RET-associated increase in FFM by 0.30 kg (27%), fiber CSA by 310 mm^2 (38%;), and mid-femur CSA by 7.2 mm^2 (14%). The comparison of the relative magnitude of PS to RET outcomes revealed that PS contributed to MHT increase about three times more (between 14 and 38%) than to increases in strength (9%). It thus appears that trained individuals have lower potential for MHT and may therefore be more responsive to PS. In addition, FFM increases to PS were diminished by increasing age by 0.01 kg, Finally, dose-dependent increase in muscle mass plateaued after PS of 1.6 g/kg/day and was greater in resistance trained (0.75 kg) than in untrained subjects. Current daily recommended dietary allowance for protein of 0.8 g/kg body weight was therefore found to be insufficient for those whose goal is to increase muscle mass and strength with RET. Instead, daily protein intake of between 20 and 40 g up to a maximum of 1.62 g/kg body weight, in separated doses over training day and combined with RET, was effective in achieving both goals.

The relative effectiveness of the two cow's milk proteins in stimulation of RET-associated MHT and increases in strength is examined in section 3.4.1, and the specificity of the effects between cow's milk proteins and select plant proteins, in sections 3.4.2 for milk and soy, and in section 3.4.3 for milk and pea proteins.

3.4.1. Relative Effectiveness of Cow's Milk Proteins Whey and Casein in Stimulation of RET-Associated Increases in MHT and Muscle Strength

Both whey and casein have similar EAA composition (Table 2) which is higher than EAA criterion concentration required for MPS or than their concentration in plasma but it approaches or exceeds EAA composition of the muscle (Table 2). Whey and casein differ mainly in their digestibility [14]. Whey is digested rapidly within 1.5 hours when hydrolyzed and in 2.5 hours as a protein. Casein

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digestion is slow and takes more than 4 hours. This digestibility difference between the two milk proteins affected their rate of incorporation into vastus lateralis thigh muscle immediately after a unilateral leg resistance exercise. Radioactive phenylalanine in the supplement served as a tracer. In the first study with 72-year -old men [35], PS was 20 grams of micellar casein or whey protein isolate. The casein supplement contained 8.2g EAAs, 4.0g BCAAs, and 1g leucine. Whey protein supplement contained 10.2g EAAs, 5.2g BCAAs and 2.8g leucine. MPS in the rested leg was higher after ingestion of whey (0.040 %/h) compared with ingestion of micellar casein (0.024 %/h). Similarly, MPS rate also was greater in the exercised leg after whey ingestion (0.059 %/h) compared to micellar casein (0.035 %/h). Similar results were obtained in the second study [36] where PS was carried out after unilateral leg resistance exercise by 23-year-old men. Here PS was a whey hydrolysate or micellar casein solution, each containing 10 g of EAAs. Mixed MPS in the nonexercised leg was approximately 93% greater after whey protein (0.091 %/h) than after casein (0.047 %/h). A similar result was observed after exercise MPS following whey consumption was approximately 122% greater in exercised leg after whey consumption than after casein.

The question of how prolonged RET affects the MPS comparison between whey, casein, and soy supplements was examined in two network meta-analyses (NMAs) which compared outcomes of both individual RCTs as well as meta-analyses of multiple RCT trials. NMA extends traditional pairwise comparisons used in standard meta-analyses to create rankings of multiple RCT treatments in a single analysis. It calculates a reference comparator (RC) EAA from multiple RCT studies to serve as the anchor point of the model [37].

The first NMA [38] used 116 RCTs with 4,711 participants and sought to identify effective types of PS that can improve muscle mass and strength after long-term RET with a rating of certainty of the evidence (RCE) for each outcome. Proteins that were compared were whey protein and casein in addition to six others. Mean differences (MD) were compared for several measures of body protein synthesis and increases in strength. MDs for casein were limited and showed inconsistency and variability for change in body-mass categories and strength-increase categories and in the RCEs. Whey had an MD of 0.42 kg for fat-free mass (FFM) with moderate RCE, MD of 2.88 kg for LBM with low RCE, MD of 0.11 kg for skeletal muscle mass (MM) with moderate RCE, and MD of 0.12 kg for appendicular MM with low RCE. A single casein MD available for comparison to whey protein, was 1.03 kg for LBM with moderate RCF offering a 2.8-fold difference in favor of whey relative to casein protein. Among effective PS for increases in strength with moderate or high RCEs were casein and whey protein for bench press with 6.5-fold greater MD for casein relative to whey protein (MDs 15.5 and 2.4 kg, respectively). On the other hand, whey protein MD for squats was 9.2 kg with moderate RCE compared to a negative MD of -13.9 kg NMA with moderate RCF for casein. Similarly, and unaccountably, NMA yielded negative MDs for leg extensions for whey protein (-4.66 kg) and casein (-0.76 kg) with moderate RCFs, in contrast to positive MDs for leg press changes after both milk proteins and the positive change for squat after whey protein only. No statistical difference was found for increases in muscle strength among different types of PS tested. Overall, whey PS in prolonged practice of RET appeared more effective than casein in this NMA analysis, but inconsistency in the MD and RCE changes for different measures of strength suggest caution in acceptance of these conclusions.

The second NMA [39] used 78 RCTs to compare the efficacy of: whey and casein, plus four other PSs in augmenting MHT and strength after long-term RET in middle-aged and older individuals. A standard mean difference (SMD) was a measure of treatment effects against the comparator. Whey PS most effectively augmented the efficacy of RET on muscle mass (SMD=1.29) and handgrip strength (SMD = 1.46) while the SMDs for casein was lower at 0.87. The SMD assessment of the relative efficacy of PS on hand-grip strength was similar for whey (1.46), milk (1.27) and casein (1.24). For leg strength, whey, milk, and meat had higher SMDs (1.41, 1.33, respectively) than casein (SMD=1.08). Therefore, this NMA suggests that whey protein and casein PS during prolonged RET has similar efficacy in stimulating MTH, but that whey protein is about 30% more efficient in stimulating increases in muscle strength.

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3.4.2. Relative Effectiveness of Milk and Soy Proteins in Stimulation of RET-Associated Increases in MHT and Muscle Strength

Soy protein has been sought as a plant-derived muscle-building supplement because of its generally good EAA and BCAA composition (Table 2). Its content of key AA leucine is 46% of the amount in whey protein and 55% of that in casein. Its content of the two additional BCAA isoleucine and valine is between 66 and 74 that of whey protein and between 83 and 67% of that of casein, respectively. Soy protein takes between 3 and 3.5 hours to digest, slower than required by whey but faster than casein, but its PODCAAS, amino-acid digestibility score is above 90% [14].

Because EAA leucine has the capacity to rapidly stimulate MPS [9], the concern about lower content of leucine in soy protein's capacity to increase MHT during RET has attracted attention. Two studies, one between whey and soy proteins, and the other one, between whey, casein and soy, compared their efficacy in stimulating RET-associated MPS rate. In the first whey-soy comparison [36], matching rates of protein digestibility between whey and soy explained the matching speeds of MPS after the two proteins were compared in this previously mentioned study. PS was a whey hydrolysate or soy protein isolate, each containing 10 g of EAAs and it was administered after unilateral leg resistance exercise by 23-year- old men. The rate of mixed MPS of soy supplement in the nonexercised leg was comparable to that of whey protein (0.08 versus 0.09 %/h, respectively). MPS after whey supplement was only 18% greater in exercised leg than after soy than whey despite soy having a moderately fast rate of digestion relatively to rapidly digested whey protein.

The second study was an RCT [40] which rated soy protein and whey PS as almost equaly efficient in increasing lean tissue mass and two measures of strength after a 6-week-long RET. Subjests were 23-year old men and women. Supplements were whey protein and soy protein, with maltrodextrin for placebo at 1.2 g/kg body mass. All three supplements were accompanied with sucros at 0.3 g/kg body mass and were presented in double-blind fashion. Increase in LBM was 1.5 times greater after whey protein (2.5 kg) than after soy protein (1.7 kg). and both were significantly higher than after the placebo (0.3 kg) while not being different between themselves. Squat 1-RM strength increase after whey PS (26.7 kg) did not differ from that after soy protein (23.7 kg), but both were significantly greater than after placebo (14.1 kg). Similarly, bench press 1-RM strength increase was only 11% higher after whey protein (8.2 kg) than after soy (7.6 kg), but significantly higher than after placebo intervention (4 kg).

A test of the soy, whey, and casein PS for increases in four different measures of LBMs and five different measures of increases in strength during prolonged RET was examined in the already mentioned NMA study [38]. None of the LBM changes produced positive MDs for all three proteins, and in three of those, soy MDs were negative for FFM, LBM, and appendicular MM (by -0.53, -2.0s, and -0.2 kg, respectively, all with moderate RCEs). In a single positive soy MD outcome, increase in skeletal MM to soy was about half as effective as that to whey (MD was 0.06 vs 0.11 kg, respectively, both with moderate RCF). In contrast to the absence of consistent body mass changes in soy MD measures, positive and parallel MD changes in soy, whey and casein proteins were present in two measures of strength increases, the bench-press strength (BPS) and the leg-press strengths (LPS). In the BPS, soy protein MD was 0.69 kg to whey protein MD of 2.39 kg with the same moderate RCF. Casein MD was 15.5 Kg with high RCF. In the LPS, soy protein MD was nearly identical to whey protein one (6.4 to 6.6 kg, respectively, with the same low RCF), and casein MD was 10.3 kg with moerate RCF. Two other strength comparisons had parallel positive MDs for soy and whey proteins. Increase in hand-grip strength (HGS) had MDs of 0.26 and 1.26 kg, for soy and whey, respectively (both with low RCFs), and increase in squat strength had MDs of 3.6 and 9.2 kg, respectively, both with moderate RCFs. Casein MDs in the two instances were 0 and -13.9 kg, resprctively, with moderate RCFs. This NMA suggests that soy supplementation during long-term RET is either not effective or is half as effective as whey sypplementation on measures of body mass changes. It also suggests that soy supplementation is equal to whey in one measure of strength increase (LPS) and between 21 to 39% as effective as whey in three other measures of strength increases (HGS, BPS, and squat).

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The near equality between soy and whey protein supplementation in the rate of MPS [36] was understandable due to the equality of their rates of digestion [14]. Similarly, a near equality between whey and soy stimulation of MPH and strength increases with a slightly greater whey protein to soy MPS efficacy was seen after an intermediate 6-week duration of RET [40]. However, a lower efficiency of soy protein relative to whey protein and casein in stimulating strength, and particularly MHT increases, was suggested after prolonged RET in the NMA [38]. This required testing the hypothesis that the inequality in the PS and RET outcomes reflected the relatively lower concentrations of leucine in soy protein relative to whey (Table 2), according to the "leucine trigger" property of this EAA or stimulation of MPS [9].

Two studies tested the relative leucine deficit hypothesis by comparing the relative effectiveness of soy and whey supplements after their leucine content was equalized. In the first study [41], 75 untrained men 21 years old were engaged in 12 weeks of RET with three PSs all of which contained 3 g of leucine. They were a whey protein concentrate (WC, 26.3 g/d), a whey protein hydrolysate (WH, 25.6 g/d), and soy protein concentrate (39.2 g/d). A equivalent amount of maltodextrin served as placebo. Percutaneous muscle biopsies from vastus lateralis sought histological changes in types I and II fiber CSAs. After engaging in similar volume and intensity of RET, increases in LBM determined by dual X-ray absorptiometry were similar in all leucine-containing groups at 2.2 kg. Changes in the increases in type I and II fiber CSAs also were similar after both PSs (+394 μm2 and +927 μm2). Strength increases in 3 RM also did not differ by group. They were 27 kg after leucine, 35 kg after WC, 37 kg after WH, 36 kg after soy concentrate, and 4.1 kg in the placebo group. The major unexpected effect of whey PS was a significant increase in the SC counts after WC (15 vs 8 per100 fibers) and WH (12 vs 12/100 fibers) while soy changes approached significance at 8 vs 14/100 fibers).

The second such study was an RCT [42], in which 61 untrained men and women were subjected to 12 weeks of RET three times a week. They were randomly assigned to PS of 1.3 g of protein per kg of body weight in the form of 19 grams of whey protein isolate or 26 grams of soy protein isolate, both containing 2 grams of leucine. Both groups showed similar increases in total body mass (0.68 kg) and in lean body mass (1.54 kg). Strength and peak torque were similar and increased in both groups. Thus, increases in LBM and strength (40.3 Nm in extensors and 20.4 Nm in flexors) in untrained subjects are comparable when soy or whey supplements are matched for leucine.

In conclusion, to produce equivalent increases in MHT and strength after prolonged exercise to match those produced by whey protein, soy protein supplement must either be large enough to provide 2 to 3 grams of leucine, or this amount of leucine needs to be added to the soy supplement, if its total amount is lower. A dose-response relationship between the relative total protein intake and the change in FFM related to prolonged RET was established as 1.62 g protein/kg /day [34]. This relationship was established by testing a diversity of proteins ranging from whey (n=23), casein (n=3), soy (n=6), pea (n=1), milk (n=10), whole food (n=7), and protein mixtures (n=13), and therefore justify adjustments to meet the 2 to 3 grams of leucine when choosing experimental PS.

3.4.3. Relative Effectiveness of Milk and Pea Proteins in Stimulation of RET-Associated Increases in MHT and Muscle Strength

Pea protein also has been used as an MHT supplement in RET interventions making comparisons of its EAA content and its relative digestibility to milk proteins relevant. Two of pea EAAs, histidine and phenylalanine, are 50% higher than in whey protein, but the other seven are represented at between 36 and 71% of the amount found in whey protein (Table 2). Of particular interest is that the leucine content of pea protein is 62 % of that found in whey protein. In contrast to rapidly digestible whey protein, pea protein has a moderate digestibility of 88.5% and requires between 3 and 3.5 hours to be absorbed [14].

Three RCTs compared the effectiveness of pea protein to whey protein in increasing MHT and muscle strength as supplements to RET. The first study [43] used a 10-day RET protocol in which 27 men and women performed unilateral knee extensions every other day. Muscle changes were studied with ultrasonography after deuterated water ingestion and by examination of myofibrillar MPS in

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muscle biopsies. RET significantly increased MPS in the trained leg with no significant difference between the whey (1.4%/day) and pea (1.5%/day) supplementation. Training and diet did not differentiate intracellular anabolic signaling, muscle architecture, strength, metabolic rate, renal function, or whole-body nitrogen balance between the two proteins.

In the second whey-pea protein comparison [44], 85 male and female sedentary subjects between 30 and 59 years old were assigned to 9 weeks of RET and supplemented with 20 g pea protein or whey isolate immediately after resistance exercise that involved both upper and lower body. There was no absolute change in muscle mass or differences in the change in whole-body muscle strength, which increased by 16% with pea supplements and by 11% with whey. Pea group improved their handgrip strength by 18.2 %, and whey group by 13%. Isometric leg strength increased by 4% in pea group, while whey increased it by 2.5%. Upper body strength increased 3.5% after pea supplementation, and 3 kg after whey.

A third whey-pea comparison [45] engaged 160 untrained males 18 to 35 years old in 12 weeks of RET and assigned them to three supplementation groups: 25 g pea isolate, 25 g whey concentrate, or maltodextrin twice/day. Vastus lateralis thickness (measured by ultrasonography) changed by 20.2% for pea, 15.6% for whey supplements, and 8.6% (24.9 mm to 27.3 mm) for carbohydrate placebo. Muscle circumference at rest increased from 32 to 32.4 cm in placebo, from 31.6 to 32.1 in whey, from 32.3 to 32,7 in pea. When contracted, it increased 32.7 to 33.7 cm in placebo, 32.4 to 33.4 after whey, and from 33.3 to 34.1 after pea supplementation. Maximal 1RM load in arm curl and muscle torque increased 46.1%. Torque increases were 8.8 Newton meters for placebo, 10.9 for whey, and 10.7 for pea supplements with no difference between supplement treatments.

Thus, overall, milk proteins did not appear to produce significantly higher MHT or increases in strength than soy and pea plant proteins when combined with RET. This was supported by a meta-analysis [46] which reviewed 9 studies involving 266 participants. RET was paired with soy supplementation in 5 studies and soy supplementation was compared to beef, milk, or dairy protein in 4 studies. There was an overall increase of 0.4 kg in LBM but no difference between the PS groups. There also were significant increases of 2 kg in 1 RM bench-press strength for all supplements and of 3.8 kg for squat, but the outcomes were not different between PS.

3.5. Factors That Modify RET-Associated Increases in MHT and Enhance Strength

Three modifying variables influencing RET-associated MHT and increases in strength have attracted substantial research interest. They are the influence of PS consumption timing relative to RET which is discussed in section 3.5.1, of the training status of study subjects examined in section 3.5.2, and the effect of subject age which was previously flagged for a significant decline in type IIx fast white muscle fibers responsible for strength and power [21], discussed in section 3.5.3.

3.5.1. Influence of PS Consumption Timing Relative to RET for MHT and Increases in Strength

A Window of anabolic opportunity (WAO) hypothesis largely influenced research regarding the possible role of PS timing relative to RET as a factor that may magnify the MHT and strength increases. While exercise-induced increases in markers of bone formation require a substantial meal by about an hour before exercise while the same meal following exercise by an hour does not stimulate anabolism [47], WAO hypothesis posited that post-exercise PS accelerated MPS due to increased RET-induced muscle sensitivity to provision of protein. The concept of WAO was supported by the reports [35,48] that post-exercise whey protein supplementation by itself triggers an increase in the plasma leucine concentration and the rate of MPS peak during the first post-exercise hour while gradually declining over the two post exercise days. RET-induced rates of myofibrillar MPS were greater than those observed after supplementation alone of either micellar casein or whey. However, whey protein ingestion stimulated MPS to a greater degree than micellar casein at rest in both supplemented and un-supplemented RET conditions.

It was promptly established that the MPS timing effect during the immediate post-exercise period depended on whether the supplementation was in the form of AAs or protein.

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Supplementation of EAAs (6 g with 35 g of glucose) immediately prior to an acute resistance exercise bout, elicited much higher rate of incorporation of radioactive phenylalanine into exercising vastus lateralis muscle of 6 volunteers than post-exercise administration [49]. Blood and the biopsied muscle phenylalanine concentrations increased by approximately 130% after amino acid supplement consumption in both pre-exercise and post-exercise trials. AA uptake by the muscle increased during exercise and remained elevated for 2 h after exercise in both trials. But phenylalanine uptake by the muscle was significantly greater in PRE (209 mg) than in POST trial (81 mg) indicating a greater MPS effectiveness of pre-exercise EAA supplementation. However, the timing effect was not present when the supplement was a protein [50]. In this study, 27-year-old volunteers consumed a whey protein solution either immediately before or after a bout of resistance exercise. The concentration of phenylalanine tracer in plasma increased by 50% in both trials. Muscle uptake of the AA tracer was not significantly different between PRE and POST when calculated from either the beginning of exercise or from the ingestion of each drink.

Difference in the design of two additional studies did not enhance clarity on the issue of supplement timing. In the first study [51], which tested the hypothesis that pre-exercise PS increased MHT, fractional MPS rate was measured during recovery 2 hours post exercise under two conditions. A bout of high-intensity leg exercise was performed by 22 young healthy subjects. Half of them were provided with an EAA plus carbohydrate solution one hour before exercise, and the other half exercised without this supplementation. Incorporation of tracer isotope in biopsied muscle was used to assess MHT, and immunoblotting measured signaling proteins involved in MHT. In the supplemented condition, fractional MPS rate increased immediately after consumption of PS but returned to basal values during exercise. In the non-supplemented condition, fractional MPS rate declined during exercise, but increased one-hour postexercise. Two hours post exercise, fractional MPS rate increased by 50% above basal in both conditions. Eukaryotic elongation factor 2 phosphorylation was reduced under both conditions. The study disproved the hypothesis that pre-exercise PS increases post exercise MPS and MHT.

The second study [52] tested the hypothesis of greater effectiveness of whey protein over casein in stimulation of MPS rate predicated on their difference in solubility and rate of incorporation into the muscle. To resolve this issue, eight healthy men received a whey PS either in the form of a 25 g bolus (PULSE) or in the form of ten 2.5 g aliquots every 20 minutes, immediately after a bout of high-resistance exercise. Single or delayed administration of supplement was intended to simulate fast (PULSE) versus slow (aliquots) delivery of nutrients. MPS and phosphorylation of signaling proteins were measured at rest and after RET. The outcome was a greater increase in blood EAAs (162% vs 53%), greater overall MPS (95% vs 42%), and greater changes in the phosphorylation of the Akt-mTOR pathway after bolus than after pulse PS administration. This demonstrated that in the short term, post-exercise timing of PS incorporation into the muscle may be influenced by the speed of PS digestibility but may not necessarily change the ultimate MHT.

Additional timing studies examined the possible influence of consuming PS before going to sleep after daytime RET or of circadian timing of RET and PS. The former study [53] reported that supplementation of 14 g of casein hydrolysate immediately before sleep during 12 weeks of RET increased strength and MHT in young adults compared to a noncaloric placebo. Finally, a meta-analysis [54] examined whether performance of RET at specific circadian times could enhance MHT and increase strength by virtue of a synchrony between exercise and endogenous metabolic rhythms. Eleven studies including 221 subjects were reviewed for comparison of the effects of morning vs evening RET. The main outcomes were (1) that at the baseline, significantly greater strength was observed in the evening hours; (2) RET in the morning hours may increase strength assessed in the morning to similar levels as strength assessed in the evening; (3) RET in the evening hours maintains higher strength in the evening hours; (4) time of day at which the training is performed affects increases in strength to a similar extent regardless of the time of day at which strength assessment is conducted. The conclusion was that increases in muscle size are similar irrespective of the time of day at which the training is performed.

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3.5.2. The Effect of Training Status on RET-Associated Increases in MHT and Strength

A systematic review [55] of 32 articles comparing the effectiveness of RET and PS in trained and untrained subjects concluded that for untrained subjects PS has little impact on lean mass and strength during the initial weeks of RET, but that increases take place after duration, frequency, and volume of interventions are consistently carried out over extended periods of time. This delay was attributed to MPS first being engaged in the repair of initial muscle damage caused by heavy resistance training. Only after the initial muscle damage has healed, can the new MPS can be registered [32].

A different effect of the training status was reported in a previously discussed meta-analysis and a meta-regression that integrated the data from 1863 participants in 49 studies over a half a century since 1962 [34]. PS during RET was more effective in increasing 1RM in trained individuals (4.3 kg) than in untrained subjects (1 kg). RET increased 1RM by 27 kg, a 91% increase relative to only an additional 2.5 kg or 9% contribution by PS to strength enhancement. As for increases in fat-free mass (FFM), RET alone added 1.1 kg and increased fiber CSA by 808 μm2 and mid-femur CSA by 52 mm2. The increase in FFM was mostly in trained subjects (1.1 kg) while it declined by 0.02 kg in untrained. PS, On the other hand, PS augmented the RET-associated increase in FFM by 0.30 kg (27%), fiber CSA by 310 mm2 (38%;), and mid-femur CSA by 7.2 mm2 (14%).

3.5.3. The Effect of Subject Age on RET-Associated Increases in MHT and Strength

Regarding the effects of aging, a meta-analysis [34] documented that equivalent RET parameters and PS produced lower muscle mass and strength increases in older individuals compared to the young. In this study, in which RET alone added 1.1 kg of FFM, aging diminished it by 0.01 k. This phenomenon has been labeled age-related anabolic resistance [56] and attributed to age-associated blunting of MPS responses to a given exposure to RET or a dose of AAs and protein. Aging is associated with progressive decline in muscle mass and strength with 0.5% loss of skeletal muscle per year after the fourth decade of life escalating to about 1% to 2% annually after the age of 50. The rate of age-associated decline in muscle mass then increases exponentially to about 3% annually after the age of 60, at which age prevalence of sarcopenia (loss of muscle mass, strength, and performance) is about 10% in both men and women in the USA.

Age-associated muscle mass loss specifically impacts fast glycolytic white fibers IIx [21], the process that is infrequently studied and documented in RET-associated MHT studies. Aging reflects higher rate of muscle protein degradation compared to MPS. Studies examining the effect of aging on anabolic signaling report that when 70-year-old subjects are compared to 27-year-old ones, RET increases MPS signaling by phosphorylation of mTOR, ribosomal S6K1, eukaryotic initiation 4E-BP1, and extracellular signal-regulated kinase (ERK ½) only in young subjects [57]. One of the already reviewed studies [35] recruited 72-year-old men to RET with supplementation of 20 g of whey protein or casein. The study documented better muscle mass and strength increases after whey compared to casein. A systematic review [34] analyzed the extent to which experimental parameters, rather than absolute capacity of older individuals to respond to RET and PS, contributed to manifestations of age-related anabolic resistance. In 24 studies, of which 12 included some form of acute exercise stimulus, RET was utilized in 10 of the 12, and endurance exercise in two studies. Oral ingestion of amino acids/protein was reported in 15 of the 18 PS studies, while three studies administered amino acids through intravenous infusion. A total of six of the 24 studies combined exercise with oral or intravenous administration of amino acids/protein. While 18 study arms provided findings to support the presence of muscle anabolic resistance in older individuals, 30 study arms did not. Discrepancies could be explained by differences in exercise volume and intensity, the dose, source, and leucine content of amino acids vs protein provided, use of exercise or amino acid vs protein, administration vs feeding alone or in combination, and differences in experimental methodology and design [58]. When the volume of knee extension exercise at 40% of 1 RM in one study was doubled, age difference in MPS disappeared. In another study, the relative amount of protein required to maximally stimulate MPS was considerably greater in older adults (0.4 g/kg) than in the young (0.24

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g/kg), and no age-related deficit was found with supplementation of 30 g of protein. In yet another study, when 3 g of leucine, equivalent to that contained in 25 g of whey protein, were supplemented, again no age-difference in MPS was evident. MPS may be slower in old subjects as measuring it over the 6 hours post exercise period erased the age difference seen during the 3 hours post exercise. Thus, to prevent age-related anabolic resistance and stimulate age-independent MPS, PS needs to provide to elderly subjects rapidly digestible, 3-g leucine-rich proteins in doses of 0.4 g/kg body weight per meal, 1.6 g/kg protein/day in 20 to 25g supplements. Daily diet should also supply 8-9 g of EAAs plus a RET protocol of sufficient intensity and duration [59].

Several studies help summarize the overall influence of factors that modify RET-associated MHT and strength increases. In contrast to all the uncertainties and controversies about the effectiveness of specific proteins, timing of supplement intake, the training status of participants, and aging, that are related to RET and PS on increases in MHT and strength, three studies [58,60,61] concluded that these procedural details are inconsequential. In the first study [60], thirty untrained 66-year-old men engaged in 12 weeks of RET. The study examined whether 40 g of protein consumed post-exercise (PX) or shortly before sleep (PS) differentially enhances muscle thickness in conjunction with 12 weeks of RET. The muscle thickness of vastus lateralis, rectus femoris, and v. intermedius increased by 0.16, 0.13, and 0.18 cm, respectively, and their chest press strength by 10.9 kg, and leg press by 28.3 kg, with no timing-group difference. In the second study [61], 31 trained males engaged in 8 weeks of RET and were provided with 2 g/kg/day of whey protein concentrate (above the necessary upper threshold of 1.6 g/kg/day) at one of the 4 times, immediately before and immediately after exercise, or 3 hours before and after. All timing groups increased muscle mass by between 1.1 and 1.2 kg, and muscle strength, depending on the body part trained, between 25 and 44 kg (leg press) and 9.5 and 12.3 kg (chess press) with no significant timing difference. In the third study [58], 21 RET-trained men were provided with 25 g hydrolyzed whey protein and 1 g of carbohydrate immediately prior or post-exercise during a 10-week RET. Pre-exercise timing resulted in 3.7%, and postexercise one in 4.9%, increase in strength measure, and no significant increases in LBM or timing of supplementation. These findings provide evidence that the traditionally postulated “anabolic window” may not be as narrow as commonly proposed at least in trained participants. The total daily intake of any protein rich in EAAs and BCAAs, and appropriate intensity and volume of RET, appears to be the primary factor in facilitating MHT and strength increases induced by exercise irrespective of the timing of protein and exercise or specific type of PS.

4. Relevance of Fermented Milk Products for Gut Microbiome (GM): Facts and Controversies

Milk fermentation is one of the oldest methods used to extend the shelf-life of milk in the form of yogurt, traditional fermented milk, buttermilk, kefir, koumiss [62], and cheese [63]. Multiple fermented milk products were introduced and used by several ancient civilizations, mostly in the Middle East, Balkans, and India, and possibly at different times. It is widely believed that fermented milk products were discovered spontaneously, and that yogurt and cheese resulted from a fermentation process within the animal skin bags used for transportation of water and milk in regions with low humidity and high temperatures in Middle Asia and Middle East [64].These skin bags were usually made from the stomach of unweaned calves which contained a coagulation agent rennet, a cluster of enzymes featuring coagulator chymosin. Until the 1950s, yogurt was largely produced and consumed by ethnic groups in the Middle East, the Balkans, India, and Eastern Europe, but since then, fermented milk products have been distributed and marketed globally. In 2000, annual consumption of fermented milk products in kg per individual was about four times higher in Scandinavian countries than in USA, Canada, Britain and Australia [62].

The history of cheese-making [63,65] points to the early Neolithic period during the domestication of cows, sheep and goats. As was the case with fermented milk, cheese making also was probably discovered by transporting milk in skin bags made from stomachs of unweaned calves, a pattern that was maintained until the beginning of the 20th century in some Mediterranean

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countries. The exposure to rennet in the calf skins likely turned milk casein into a curd. Cheese-making entails preservation of casein, the solid milk component, in a less perishable and more transportable form by fermentation.

Cheese making involves an additional process of aging or ripening. Romans developed these techniques to extend different cheese flavors and characteristics, and they spread cheese consumption throughout the Roman Empire. During the Middle Ages, European monks further improved cheese ripening and aging techniques and developed several cheese varieties that still are marketed today. Cheese declined in popularity during the Renaissance but regained it in 19th century, when its production was industrialized. In 1860s, usage of milk and cheese became safer after Louis Pasteur discovered a way to destroy harmful bacteria by pasteurization. The World Wars and the Great Depression drove further innovation in cheese-making techniques to make this milk product an important dietary component worldwide. This review will list microorganisms facilitating milk fermentation and some methods of producing specific fermented milk products in section 4.1, gut microbiome (GM) composition and its effects on human health in section 4.2, the influence of probiotics, prebiotics, and symbiotics on GM in section 4.3. and the health benefits of fermented milk products in section 4.4.

4.1. Microorganisms Facilitating Milk Fermentation and Methods of Producing Some Specific Fermented Milk Products

Fermentation is an anaerobic metabolic process that converts sugars into energy, lactic acid, or alcohol and is mediated by enzymes from bacteria and yeast. Milk fermentation typically entails conversion of the milk sugar lactose to lactic acid by thermophilic lactic acid bacteria (LAB) like *Lactobacillus bulgaricus* and *Streptococcus thermophilus* and similar bacteria that belong to genera *Bifidobacterium* (7 species), *Enterococcus* (2 species), *Lactobacillus* (11 species), *Lactococcus lactis, Leucoinostoc, Pediococcus acidilactici*, and *Streptococcus* among others, and by the yeast *Saccharomyces boulardii* [62]. For production of yogurt and kefir, acidification by bacteria or by externally added acid triggers denaturation and clumping of total milk proteins. For production of cheese, the fermentation is primarily directed toward curdling the casein after decanting of the whey liquid. Milk fermentation improves milk product digestibility, increases its shelf life and bioavailability of certain nutrients, and produces probiotics, the bacteria that benefit GM, as well as general health.

Making yogurt or kefir [62,66] entails forming a milk base consisting of milk proteins and between 0.5 and 5 g/100 g of fat. The base is homogenized to reduce fat globules to less than 2 μm thus facilitating their incorporation into casein micelles to increase substrate viscosity. The milk base is then heated to between 85 and 95o C to destroy pathogens and denature ß-lactoglobulin, which forms a complex with k-casein. This is then cooled, and LAB organisms are introduced to create and acidify starter cultures and coagulate casein. At the pH of 4.6, the starter culture is cooled to 5o C to assure the appropriate cfu (colony forming unit) counts in manufacture, for instance, of probiotics.

Cheese fermentation [67] involves decanting whey liquid and then converting the milk casein to a semisolid curd with a coagulating agent rennet, acid, heat plus acid, or their combination. Microbial organisms in fermentation of cheese include LAB (usually added as starter culture), yeast, and some molds. The common LAB genera involved in the fermentation of cheese include *Lactococcus*, *Oenococcus*, *Lactobacillus*, *Leuconostoc*, *Pediococcus*, and some species of *Streptococcus* belonging to the phylum *Firmicutes* [68]. Bacterial communities differ among raw milk cheeses depending on the manufacturing process, but the bacteria in the cheese core are dominated by LAB genera *Lactococcus*, *Lactobacillus*, *Enterococcus*, *Streptococcus*, and *Leuconostoc.* An additional non-starter group of LAB (*lactobacilli, pediococci, enterococci,* and *Leuconostoc)* can also be present naturally in cheese. Artisanal cheeses manufactured from raw milk have complex microbiota that also include *Lactococcus spp., Leuconocstoc spp., Enterococcus spp., Streptocossus spp., Staphylococus spp., Micrococcus spp., Enterobacter spp., Citrobacter spp.*, and *Acinetobacter spp*.

Besides the fermentation, cheese manufacture involves cheese ripening. Cheese ripening is a complex, dynamic system which depends on diverse and complex proteolytic enzymes naturally

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present in milk as well as in LAB [67]. Starter cells may undergo lysis, non-starter LAB may grow, and a secondary microflora may develop. An example is growth of *Propionibacterium freudenrauchii* in Swiss cheeses, of molds in mold-ripened varieties, and of complex gram-positive bacterial flora in smear cheeses to contribute the texture and flavor of these varieties. LAB contain cell-envelope proteinases that contribute to the proteolysis of cheese proteins, breaking them down by the action of plasmin into oligopeptides. Oligopeptides can be subsequently taken up by cells via specific peptide transport systems or further degraded into shorter peptides and AAs through the collaborative action of various intracellular peptidase which may then accumulate during storage. These peptides, the rich complement of milk EAAs, and their derivatives, also contribute to the development of texture and flavor in a final cheese.

The process of ripening of the protein component of cheese [65,67] also elicits production of bioactive protein compounds (BPs) released mostly by LAB. BPs in cheese also include exopolysaccharides, organic acids, vitamins, including a neurotransmitter γ-amino-butyric acid (GABA). These compounds inhibit hypertension-causing angiotensin-converting enzyme (ACE) and exhibit antioxidant, anti-microbial, anti-inflammatory, immune-modulatory, analgesic/opioid activity, and anti-proliferative anti-carcinogenic activity. Some LAB also synthesize vitamins and antimicrobial peptides (bacteriocins). The above bioactivities lead to health-protective effects associated with a reduced incidence of CVD risk factors, such as obesity, dyslipidemia, and T2D as well as reduced incidence of MetSyn.

Ripe cheeses are a highly sought source of food globally prompting a brief survey of varieties and the final nutrient composition of cheeses [69,70]. It is estimated that there are more than 2000 varieties of cheese worldwide. They differ by source based on the species of lactating cattle such as bovine, caprine, ovine, and other dairy animal species. Other distinguishing factors are the cheese-making procedures used (milk pretreatments, the addition and the type of microbial starters, heating, acidification, renneting, curd cutting, cooking, pressing, salting), the form and size of the cheese, and ripening length and conditions. A list of some that are frequently consumed and used in meal preparation in the USA include, in alphabetical order: American, asiago, blue, brie, burrata. camembert, cheddar, Colby, cottage, cream, Edam, Emmental, feta, fontina, goat, gouda, gorgonzola, gruyere, halloumi, Havarti, Jarlsberg, limburger, mascarpone, Monterey jack, mozzarella, Muenster, Neufchatel, paneer, provolone, ricotta, Romano, string, Swiss, and queso fresco. Properties and nutrient composition are available [71] for a selection of out of 1050 different varieties of ripe cheeses. Manufactured by over 100 producers, they are grouped into 37 categories. Sixteen cheeses have protected designation of origin (PDO) which includes asiago and provolone. Four others fall into traditional cheese categories (TC), 3 in pasta filata cheese categories (PFC) that includes mozzarella, 5 into flavored cheese (FC) categories, 2 into goat milk categories, and 7 other categories ranging from very fresh (VF) to very hard (VH) cheeses. All are annually displayed on Caseo Veneti, a European cheese exhibition and competition. Some of the properties of several cheese varieties are shown in Table 4.

Table 4. Gross chemical composition of selected cheeses (from 71).

Cheese name (category)	Moisture %	Fat%	Protein %	WSN	Ash
Asiago-fresh (PDO)	41	29.9	23.5	5.3	1.9
Asiago-aged (PDO)	25.9	35.5	31.2	8.4	2.1
Provolone-bland (PDO)	40.8	28.6	25	6	2
Provolone-spicy (PDO)	37.2	30.9	25.3	6.8	2.1
Mozzarella (PF)	60.8	19.9	15.0	3	1.3
Goat	44.5 to 66.3	15.5 to 25.2	12.9 to 24.9		

WSN= water soluble nitrogen.

Milk and dairy fat are the most complex fats in the human diet as they consist of more than 400 distinct fatty acids (FAs). FA composition of cheese varies according to milk origin (e.g., dairy species

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and breed), rearing conditions (e.g., type of feed), and cheese-making technology (e.g., coagulation process, addition of salt, ripening period) Fatty acid composition of several categories of fresh or ripe cheese was reported recently [70]. The composition of FAs is reviewed [70,72] in eight categories of cheeses: curd cheese (CC, closest in FA composition to milk), processed cheese (PC, representing a blend of different cheeses), Dutch-type cheeses like edam and gouda (NED), Swiss type (SUI) like Emmental, lichen-blue cheese (LBC), Italian type cheeses like mozzarella, parmesan, ricotta. and Romano (ITA), hypertrophied blue cheese (HBC), and English type cheese (ENG) like cheddar, and cheshire.

Cheese also is an important source of a wide variety of biologically active substances among which specific FAs are of utmost importance such as conjugated fatty acids (CFA) are bioactive isomers present in the rumen of animal-derived milk and meat, which are widely studied because of numerous beneficial health properties [65–67,72]. Among them is the functional lipid conjugated linoleic acid (CLA) with anti-inflammatory and anti-carcinogenic biological activities. Other CFAs can be present in cheese fat because of the activity of ruminal microbiota as well as the activity of bacterial flora. Odd- and branched-chain fatty (OCFA and BCFA) are another important group of specific FAs. They are mainly saturated fatty acids with one or more methyl branches, largely derived from ruminal bacteria. OCFA and BCFA have anti-cancer activities. Due to ruminal bacterial activity, dairy fat is also the richest dietary source of natural trans FA isomers, mainly vaccenic acid (trans11C18:1) having beneficial properties, in contrast to artificial trans FA of partially hydrogenated oils.

SFAs constitute more than 60% of all cheese FAs [70] with slightly lower concentrations in curd and lichen-blue cheese (~63%) and highest in Italian-type cheeses (68%). MUFAs constitute about 30% of cheese fat with highest concentrations in curd and lichen-blue cheeses. PUFA concentration in cheese is about 20 to 30%. Palmitic acid (C16:0) predominates among saturated (SAT) FAs, and its highest amount was detected in ITA, in all types of ripening cheeses, and in CC and PC. Amount of myristic acid (C14:0) also was the highest in ITA. The highest content of C8:0 FA was in SUI but also predominated in LBC and ENG. Among ripening cheeses, the highest levels of C10:0 FAs were observed in ITA, SUI and HBC. Long chain SAT fatty acids were present only in trace amounts. Their content seemed to decrease with the increase in carbon chain length. C24:0 FA was present in higher amount only in ENG than in other examined types of cheese. Higher levels of C22:0 FAs were detected only in LBC and ENG. The highest amount of C20:0 FA was found in NED. ENG cheeses had the highest concentration of CLA at ~1,730 μg/g.

4.2. Gut Microbiome (GM) Composition and Its Effects on Human Health

GM term refers to bacteria that reside in the large intestine or colon. Human GM and cow's milk share many microorganisms. Milk consumption influences GM, and both have an impact on human health. Since the colon is the end point of a continuous gastrointestinal (GI) canal starting in the mouth, traversing stomach and small intestine, it is useful to understand to what extent the oral, esophageal, gastric, small intestinal, and colon microbiomes share bacteria species (Table 5) or exhibit distinct microorganism populations. All five regions of the oro-GI bacterial population have distinct bacterial populations [73].

Table 5. Bacterial profile of five oro-gastro-intestinal microbiomes (from 73).

Oral microbiome	Esophageal microbiome	Gastric microbiome	Small intestinal microbiome	Colonmicrobiome
Gemella		*Streptococcus*	*Lactobacillus*	*Lactobacillus*
Veillonella	*Streptococcus*	*Bacillus*	*Streptococcus*	*Streptococcus*
Neisseria	*Prevotella*	*Enterobacter*	*Prevotella*	*Prevotella*
Fusobacterium	*Veillonella*	*Leptotrichia*	*Veillonella*	*Lachnospiracea*
Streptococcus	*Haemophilus*	*Veillonella*	*Escherichia*	*Ruminococcus*
Prevotella	*Rothia*	*Pseudomonas*	*Enterococcus*	*Enterobacter*
Pseudomonas		*Helicobacter*	*Bacteroides*	*Bacteroides*

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Actinomyces	*Clostridium*	*Akkermansia*

Oral cavities have more than 1000 taxa of microorganisms [73]. Six phyla, *Firmicutes, Bacteroidetes, Proteobacteria, Actinobacteria, Spirochaetes,* and *Fusobacteria,* comprise 96 % of its bacterial taxa. In saliva, the predominant genera are *Gemella, Veillonella, Neisseria, Fusobacterium, Streptococcus, Prevotella, Pseudomonas,* and *Actinomyces*. The most abundant bacteria in the human esophagus belong to the phylum *Firmicutes* and the genus *Streptococcus*. Here, communities are dominated either by *Streptococcus* (*Streptococcus mitis/oralis/pneumoniae*), *Prevotella* (*Prevotella melaninogenica and P. pallens*), *Veillonella*, or *Haemophilus parainfluenzae* and *Rothia mucilaginosa*). In the stomach, growth of many bacteria is inhibited by the low-pH acid environment. The genera commonly found in the corpus and antrum include *Bacilliales incertae sedis*, *Streptococcaceae*, *Enterobacteriaceae*, *Leptotrichiaceae*, *Veillonellaceae*, and *Pseudomonadaceae.* In patients with abundant *Helicobacter pylori,* phylum *Proteobacteria (Succinivibrio, Cori-obacteriaceae, Enterococcaceae, and Rikenellaceae),* are prevalent, and overall alpha diversity of species and their distribution is reduced. In the small intestine, the rapidly dividing facultative anaerobes such as *Proteobacteria* and *Lactobacillales* prevail. *Streptococcus, Prevotella, Veillonella, Fusobacterium, Escherichia*, *Klebsiella,* and *Citrobacter* are abundant, whereas extreme anaerobes like *Alistipes, Ruminococcus,* and *Faecalibacterium* are not present. In jejunum and ileum, *streptococci, lactobacilli*, *Gammaproteobacteria,* the *Enterococcus* group, and the *Bacteroides* group are most common. In ileum, the distal small intestine, the bacteriome approaches that of the colon in terms of diversity and richness. The predominant colonic bacterial phyla are *Bacteroidetes*, *Firmicutes*, *Verrucomicrobia*, *Proteobacteria*, and *Actinobacteria.*

In the colon, the GI region implied by the generic term "gut microbiome" or GM, the mucus forms a stratified layer that is more defined than in the small intestine. Inner mucus layer physically excludes bacteria and contains immune effectors that target microbiota. The outer mucus layer is loose and serves as a colonization site for numerous microbes. Microbes that prioritize dietary starches and nutrients reside within the colonic lumen. Organisms that can utilize mucin such as *Akkermansia, Ruminococcus*, and some *Bacteroides* species reside within the outer intestinal mucus layer. The stable microbial core in the colon includes *Bacteroides*, *Eubacterium, Faecalibacterium, Alistipes, Ruminococcus, Clostridium, Roseburia*, and *Blautia,* while *Faecalibacterium prausnitzii, Oscillospira guillermondii, and Ruminococcus obeum* are the top three taxa shared by all adults.

GM is established by vaginal delivery and infant's consumption of breastmilk [1]. Breastfeeding exerts long-term effects on the microbiome and affects the immune system and GI tract through its enrichment throughout childhood and adolescence of *Bifidobacterium*, *Faecalibacterium,* and members of the *Lachnospiraceae* family. By contrast, the adult microbiome is more stable and is shaped more by environment (diet, antibiotics, smoking, exercise, geographic location) than by genetics.

With human aging, there are changes in the character of gut micro-biome [74].

Aging is associated with low-grade endotoxemia that contributes to chronic inflammation and is linked to metabolic diseases such as T2D and metabolic-dysfunction-associated steatotic disease (MASLD). Short-chain FAs (SCFAs) decline in elderly due to decline in specific SCFA-producing bacteria. With advancing age, microbial diversity declines and its structure shifts. Some centenarian studies show reduced *Bacteroides* and *Roseburia,* with enrichment of *Bifidobacterium* and *Akkermansia* linked to longevity. Others report increased abundance of taxa such as *Clostridium, Methanobrevibacter*, and *Synergistetes,* while *Eubacterium rectale* and *Faecalibacterium prausnitzii* appear relatively depleted. Some studies on healthy aging report a depletion of ubiquitous core taxa like *Bacteroides* while others show elevated presence of *Bacteroides*, *Alistipes,* and *Parabacteroides.*

4.3. The Influence of Probiotics, Prebiotics, and Symbiotics on Gut Microbiome

Probiotics represent beneficial LAB deliberately introduced into milk to elicit beneficial health outcomes [68,75]. The insight that introduction of beneficial bacteria in fermented milk products into human diet could improve human health was proposed hundred and fifteen years ago by Russian scientist Elie Metchnikoff who advocated consumption of lactic-acid producing bacteria. Several

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intestinal bacteria such as *Lactobacillus, Bifidobacterium*, *Enterococcus,* and some yeasts, help improve stability of gut microbiota. Identities of probiotic bacteria that survive stomach acidity, grow in the colon, and produce specific biological effects are reported in a systematic review [76] They include eight species of the genus *Lactobacillus (L.casei, L. rhamnosus, L. acidophilus, L. johnsoni, L. reuteri, L. paracasei, L. plantarum,* and *L. fermentum*) and several species of *Bifidobacterium.*

By contrast to probiotics, prebiotics are nondigestible food ingredients that benefit the host by providing some bacteria in the colon with the nutrients they can selectively utilize, thus stimulating their activity and growth [75]. Prebiotics are often short-chain carbohydrates which escape human digestion, including some undigested lactose, but are used as substrates for the growth of probiotics in the upper gastrointestinal tract and include inulin, and milk oligosaccharides (MOs) such as fructo-oligosaccharides, galacto-oligosaccharides, and oligofructose. They also are available in many fruits (apples, berries), legumes (peas, beans, and lentils), grains and cereals (oats, wheat bran, barley, and rye), and nuts and seeds (almonds, pistachios, and flaxseeds).

Symbiotics are mixtures of prebiotics and probiotics that improve human or animal health by allowing bacteria to use prebiotics for their growth. Their effectiveness is dependent on some species of bacteria and specific prebiotic substrates. An example of producing a symbiotic fermented product protecting the passage of a beneficial bacterium *Bifidobacterium animalis subsp. lactis in*to the gut utilized a process of encasing it in a microcapsule with a non-digestible milk MO [77]. In this procedure, bacteria were microencapsulated via internal ionic gelation utilizing sodium alginate as wall material, calcium carbonate as a cross-linking agent, and MOs as prebiotics. This produces tolerance to stomach gastric acid and positively modifies gut microbiota in that it reduces pathogenic gut bacteria species *Streptococcus anginosus* and *Eschericia coli*, and enriches the anti-inflammatory bacterium *Akkermansia muciniphila.*

4.4. Health Benefits of Prebiotics, Probiotics and of Fermented Milk Products

Many studies question whether milk fermentation conveys special benefits to human health. The evidence for the impact of probiotics and fermented milk products on GI health is examined in section 4.4.1, on inflammation, MetSyn, and T2D in section 4.4.2, on cancers in section 4.4.3, on bone health in section 4.4.4, and on mortality vs longevity in section 4.4.5.

4.4.1. The Impact of Probiotics and of Fermented Milk Products on GI Health

Probiotics in fermented milk products were reported to benefit several GI afflictions, among them lactose intolerance, (LI) which is the result of genetic deficiency of the intestinal digestive enzyme lactose. LI symptoms include abdominal bloating, diarrhea, flatulence, stomach cramps, and nausea. Beyond avoidance of consumption of unmodified milk, two systematic reviews reported moderation of LI symptoms with consumption of fermented milk products. In the first systematic review [78] intake of yogurt was associated with improved lactose digestion and lactose tolerance as LAB fermentation converts lactose to lactic acid. In the second systematic review of RCTs [79], nine studies reported that LI symptoms were alleviated by consumption of probiotics including *Lactobacillus acidophilus, L. reuteri, L. rhamnosus, L. bulgaricus, Steptococcus thermophilus, and Bifidobacterium longum*. *Bifidobacterium. animalis,* the most common bacterium in GM and the best studied probiotic bifidobacterium, had strong mucus adherence properties, inhibited pathogens, and improved intestinal barrier function. It also enhanced lactose digestion and increased transit time in patients with LI. Among the indigestible prebiotics, galacto-oligosaccharides were shown to increase the abundance of lactose-fermenting *Bifidobacterium, Faecalibacterium, Lactobacillus,* and *Roseburia* species in the gut to mitigate the symptoms of LI. A critical parameter when administering probiotics is the dose of administered microorganisms, with better results with doses as high as 108 cfus/day. Probiotic supplementation mitigated LI symptoms by decreasing the concentration of exhaled hydrogen and by reducing abdominal cramping, diarrhea, vomiting, bloating, and/or flatulence.

A review of RCTs [80] reported that kefir consumption reduces some pathological bacteria associated with *Streptococcus mutans* and dental caries risk and can eradicate the pathological

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bacterium *Helicobacter pylori*. Another RCT showed that the patients with Crohn's disease, an inflammatory bowel disease (IBD), but not the patients with ulcerative colitis, had significantly higher *Lactobacillus* levels after consuming 400 ml of kefir twice a day than patients in the control group. The conclusion was that kefir has a unique beneficial microbiological profile. Another RCT [81] evaluated the effect of 4-month daily consumption of 400 m of kefir per day on the fecal microbiota and symptoms of Crohn's disease. Stool *Lactobacillus kefiri* after kefir consumption contained 5 x107 cfu/ml in subjects with ulcerative colitis (UC) and Crohn's disease, while the bacterial load of feces in control subjects was between 104 and 106 cfu/g. Erythrocyte sedimentation rate and high-sensitivity C-reactive protein (hs-CRP), an inflammation marker,-decreased in patients with Chron's disease. Conclusion was that kefir consumption may modulate *Lactobacillus kefiri* in the gut and reduce IBD symptoms.

Finally, in a double-blind RCT [82], consumption of a symbiotic yogurt containing both a probiotic *Lactobacillus acidophilus, Lactobacillus bulgaricus*, and *Streptococcus thermophiles* with 7 mg/kg of *L. plantarum* ST- III along with a prebiotic inulin at two doses (1% or 1.5%), alleviated both functional constipation (FC) and functional diarrhea (FD). The numbers of *Bifidobacteri*a and *Lactobacillus* in feces of the participants significantly increased after 14-day consumption of probiotic at either concentration of the inulin prebiotic in a dose-dependent manner. At the same time, the numbers of the pathogens *Clostridium perfringens* and *Escherichia coli* were significantly reduced. This suggested that consumption of symbiotic yogurt can improve problems in the digestive system.

4.4.2. Impact of Probiotics and of Fermented Milk Products on Inflammation, Oxidative Stress, Metabolic Syndrome, and Type 2 Diabetes

Systemic inflammation appears to be involved in metabolic disorders such as obesity, insulin resistance, hepatic steatosis, MetSyn and T2D. A systematic review [83] examined the effects of probiotics and prebiotics on obesity, T2D, and cancers. The review summarized health-promoting benefits of fermented milk beverages as being mediated by gut LAB and probiotic bacteria in yogurts and kefirs. Both low-fat and high-fat milk and fermented milk products displayed anti-inflammatory activity. The conclusion was that these microorganisms regulate the immune system, maintain the integrity of the intestinal epithelium, protect the host body from pathogens and protect human health by preventing civilization diseases.

Two studies indicated that fermented milk products can mitigate systemic inflammation. The influence of fermented milk products on inflammation was reported in a cross-sectional analysis [84]. It measured the associations between the serum hs-CRP and consumption of fermented and non-fermented dairy products and butter in a population with high dairy intake. Intakes of fermented and non-fermented dairy products and butter in 1338 generally healthy Finnish men aged 42-60 years were 189, 522, and 33 g/d, respectively. Higher intake of total dairy, total non-fermented dairy, total milk and butter were associated with higher concentration of the inflammation marker serum hs-CRP, but fermented dairy was not. Only higher butter intake remained significantly associated with increased serum hs-CRP after additional analysis. The odds ratio for elevated hs-CRP (>3 mg/L) in the highest vs. the lowest quartile of total dairy, total non-fermented dairy, total milk and butter was 2.50. Therefore, high intake of butter, but not of other dairy products or of fermented dairy, may be associated with increased low-grade inflammation.

In a review [85] that examined 52 clinical trials since 1990, inflammatory markers were compared to the consumption of dairy products using an inflammatory score (IS). Thirty-two studies with high-fat dairy products, and 20 studies with low-fat products, were examined. The fat content of milk products did not affect the inflammatory status, and consumption of dairy products did not cause adverse effects on biomarkers of inflammation in this and another study. Anti-inflammatory activity was higher in obese subjects with MetSyn indicating a strong benefit of milk consumption for a condition that is linked to low-grade systemic chronic inflammatory state and production of inflammatory cytokines. By product category stratification, both low-fat and high-fat products, as well as fermented products, displayed anti-inflammatory activity. This indicates that dairy products,

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in particular fermented products, have anti-inflammatory properties in humans, especially in those with metabolic disorders.

Systemic inflammation is frequently associated with oxidative damage. The hypothesis that rich bioactivities of Mexican rangelands for the goat forage relative to indoor dry diets may influence the antioxidant properties of goat milk was tested in a study [86] in which antioxidant activity in the two diets and the goat milk was assessed by the DPPH+ radical (synthetic free-radical reference tool) and by total blood concentrations of flavonoids and polyphenol. Milk concentration of PUFA, MUFA, and n-3 fatty acids from grazing goats (respectively 4.7%, 25.2%, and 0.94% of fatty acid methyl esters) was higher in milk and cheese from grazing goats than from goats fed indoor diets. Total polyphenol content in cheese from grazing goats (300 mg/kg of gallic acid equivalent (GAE) was 5 times higher than in cheese from goats fed indoor diet (60 mg of GAE/kg). *Acacia* pods are a semiarid rangeland feed resource that inhibits in vitro formation of TBARS lipid peroxidation and diminishes the damage induced by reactive oxygen species. It increases free radical scavenging as per DPPH, oxygen radical absorbance capacity, and anti-inflammatory activity. The results highlight superiority of grazing/browsing practices over indoor feeding to promote the transfer of bioactive compounds from vegetation to animal tissue and subsequently to humans.

A systematic review [83] examined the effects of probiotics and prebiotics on obesity, T2D, and cancers. It listed evidence that bacteria in fermented milk products sustain the integrity of the intestinal epithelium, protect the host body from pathogens, prevent development of civilization diseases, and produce metabolic benefits for human health. The review listed studies suggesting that consumption of yogurt, probiotics, low-fat milk and other low-fat dairy products lower risk of MetSyn [87] and T2D risk factors [88,89]. Finally, fermented food products were shown as able to prevent or mitigate T2D in additional studies. A review of observational studies [90] summarized the relationship between consumption of fermented milk and several aspects of cardiometabolic health. It provided evidence for beneficial effects of consumption of at least 200 grams of yogurt per day. There was a generally inverse association between consumption of fermented milk, yogurt, and cheese with prevention of T2D, cardiometabolic syndrome including hyperglycemia, hypertension and hyperlipidemia, and possibly also overweight. The possible effect of fermented milk on mitigation of T2D, was also re-ported in a meta-analysis [91] that examined 7 cohort studies. In two studies, there was a 14% lower risk of T2D associated with 200 g of yogurt intake per day. In 9 prospective cohorts, consumption of 244 g of yogurt per day reduced recent risk of T2D by 18%. Another study found a nonlinear association between consumption of 80 g/d of yogurt and a 14% reduced risk of T2D. Additionally, moderate yogurt consumption (1 to <3 servings per week) was associated with lower risk of developing prediabetes during a 12-year follow-up study in the Framingham Heart Study Offspring Cohort. Higher consumption of cheese and whole-meal bread was associated with a lower risk of diabetic retinopathy (DQ) in Australian subjects with T2D [92]. The measurement was with a diagnostic retinal photocoagulation test (RPC). DQ was analyzed over a period of 8.6 years in 8,122 57-year-old subjects with T2D of whom 314 received the test. DQ was inversely associated with cheese and whole-meal bread consumption with a HR=0.64 demonstrating a significant lowering of the risk of DQ by consumption of cheese and whole-meal bread.

4.4.3. Impact of Probiotics and of Fermented Milk Products on Cancer

Several studies have examined the relationship between fermented milk product consumption and cancers of the bladder, prostate, colon, rectum, and breast. A systematic review of 108 selected studies on the effects of yogurt on human health published between 1979 and 2017 [78], reported reduced risk of breast and colorectal cancer (CRC) with fermented milk consumption in one study. In addition, an association was reported between reduced incidence of prostate cancer with dairy product consumption in general, with no difference between fermented and unfermented products. A large longitudinal trial and a meta-analysis [93] from the Nurses' Health Study on 83,054 46-year-old women and on 43,269 52-year-old men in the Health Professionals Follow-Up Study who were followed up for 32 and 26 years, respectively, examined the role of yogurt consumption on CRC

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incidence and mortality. Baseline yogurt consumption was associated with a reduced risk of colon cancer after adjusting for calcium and fiber intake, and the results were restricted to the cancer in proximal colon. Hazard ratio for consumption of 1+ servings per week of yogurt was 0.84. The most important window of opportunity for regular yogurt consumption to prevent CRC was the prior 16- to 20- year period. No trend was observed between yogurt consumption and the CRC mortality. Another meta-analysis [94] examined the association between milk and dairy products on one hand and bladder cancer, on the other, in 26 cohort and case-control studies. Medium, compared with low, consumption was associated with lower risk of bladder cancer. For total dairy products RR, was 0.90, for fermented dairy products RR was 0.87, and the effect was stronger in Asians (RR = 0.79). Lower cancer risk was associated with high consumption of milk (RR = 0.89) and with high consumption of fermented dairy products RR was 0.78 compared with low consumption. Thus, decreased risk of bladder cancer was associated with moderate consumption of total dairy products and with medium to high consumption of milk and fermented dairy products.

An observational study [95] compared the association between dietary consumption of milk and dairy products including high-fat cheese with incidence of CRC in 308 Spanish subjects to 308 age- and sex-matched controls. There was a direct association between milk and dairy products consumption, in particular high-fat cheeses, with the increase in the CRC with the odds ratio (OR=1.87). On the other hand, consumption of fiber-containing foods, especially whole grains and fatty fish, reduced CRC risk to RR=0.62 and 0.53, respectively. The conclusion was that high-fat cheese was associated with increased risk and fiber-containing foods and fatty fish, with decreased risk of CRC. A systematic review [72] reanalyzed meta-analysis data on cheese consumption to arrive at RRs for estrogen-receptor breast cancer mortality of 0.89. The overall conclusion from the above studies as that cheese consumption exerted neutral to moderately positive influence on incidence of cancer.

4.5. Impact of Probiotics and of Milk Products on Bone Health

Bone mass, dimensions, and mineral density (BMD) decrease after human midlife [96] and this often leads to osteopenia, osteoporosis, and bone fractures. It is therefore not surprising that programmed age-associated changes in bone mass and structure influence efforts to find nutritional ways to reduce the rate of BMD loss, ensure its maintenance and prevent bone fractures. Sixty percent of bone mineral is hydroxyapatite, a crystalized form of calcium phosphate ($Ca_{10}(PO_4)_6(OH)_2$). This led to development of daily nutritional requirements [97] to include 1,000 to 1,200 mg of calcium, 600 to 1,000 IU of vitamin D (some of it supplemented in cow's milk), and phosphorus, magnesium, Vitamin K2, and protein. With its abundance of calcium and phosphorus (Table 1) and EAAs (Table 2), milk and its fermented products, with the help of LAB as a source of probiotics, provide these essential nutrients for the maintenance of BMD especially in circumstances of postmenopausal (PM) osteopenia or osteoporosis that follow the decline in estradiol.

Nine RCTs provide facts about the effects of milk products on the rate of BMD loss, changes in BMD, and markers of bone turnover. Three cohort studies recorded in a meta-analysis document the associations between probiotics and fermented milk products on bone fractures. Two reviews discuss suggested mechanisms of nutrient interactions with bone mass and structure. The first three RCTs document the effects of milk products on the rate of BMD losses. The first RCT [98] tested the effectiveness of 12 months of consumption of 1010 cfus of *Lactobacillus reuteri* 6475 on the total tibia volumetric BMD (vBMD) in 70 postmenopausal (PM) Japanese women. Probiotic consumption reduced loss of total vBMD by 0.83% compared to placebo group loss of -1.86%. The second study was a multicenter RCT [99] that compared BMD losses in PM women receiving the probiotic *Lacticaseibacillus paracasei* LPC100 and *Lactiplantibacillus plantarum* LP140. Smaller T-score declines (0.19) were observed after the probiotic compared to the -0.08 T-score in the placebo group, and there was no significant decline in blood concentrations of vitamin D. In the third RCT [100], an isoflavone-rich red-clover extract combined with a heterogeneous culture of probiotic LAB (RCE) was consumed daily for 12 months by one half of 78 PM women with the other half receiving a placebo. All also received 1,200 mg calcium, 550 mg of magnesium, and 25 μg of vitamin D. RCE use significantly

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attenuated loss of BMD at the L2-L4 lumbar spine vertebrae (-0.99%), femoral neck (-1.4%) and trochanter (-0.67%) compared with the controls who lost -2.2%, -3.05%, and -2.79%, respectively.

Actual changes in BMD as a function of milk product consumption were documented in two RCTs. In the first of these studies [101], daily consumption of spore-containing tablets of *Bacillus subtilis* C-3102 for 24 weeks, significantly increased total hip BMD (2.53%) vs placebo (0.83 %) in 76 PM women. The second 6-month-long RCT [102] measured the effect of bone turnover markers and BMD in 27 58-year-old PM women receiving the probiotic *Lactobacillus fermentum* SRK414 and in 26 placebo controls. BMD was maintained and femoral neck increased in subjects using the probiotic, but not in the controls.

Four RCTs documented changes in markers of bone turnover in response to probiotics or fermented milk products. In the first of these [101], 38 PM women received daily spore-containing tablets of *Bacillus subtilis* C-3102 for 24 weeks. The probiotic significantly reduced the bone resorption marker urinary type I collagen cross-linked N-telopeptide (uNTX) but not in 38 control subjects. The second RCT was 6 months long [102] and measured bone turnover markers in 27 58-year-old PM women receiving daily the probiotic *Lactobacillus fermentum* SRK414, and in 26 controls who did not receive the probiotic. Osteocalcin levels increased in parallel to *L fermentum* concentration in GM in the treatment group while they decreased in the control group. In the third RCT [103], 20 subjects received daily *Bifidobacterium lactis* Probio-M8, and 20 received placebo. Probiotic improved bone metabolism, reflected by an increased vitamin D3 level and procalcitonin levels in serum. It also significantly increased GM microbial genes encoding some carbohydrate metabolism pathways (including ABC transporters, the phosphotransferase system, and fructose and mannose metabolism) and a choline-phosphate cytidyltransferase. A 6-months-long RCT [104] measured the effects of daily consumption of 16 g of kefir and 15 g of calcium bicarbonate (CaCO3) on bone metabolism in 40 subjects with osteoporosis. Placebo group consumed only calcium bicarbonate. Bone turnover markers and BMD at the spine, total hip, and hip femoral neck were assessed at 1, 3, and 6 months. The marker of bone resorption β carboxyterminal telopeptide of type 1 collagen (β-CTX) significantly decreased after three-month treatment in osteopenic subjects with T-scores of > -1. Six months of kefir consumption increased the bone formation markers osteocalcin and serum PTH, but both remained low or decreased in the control group.

Bone fractures, particularly of the femoral neck of the hip bone, can lead to life-altering disabilities or death and have been studied in the context of consumption of milk products or pre- and probiotics. A systematic review and meta-analysis of RCTs [105] examined 518 articles about the relation between fermented milk consumption and bone fracture incidence, BMD, BMD T-scores, and percentage change in bone turnover markers in PM women. Three prospective cohorts, and 3 case-control studies involving 102,819 subjects suggest that higher yogurt consumption was associated with reduced relative risk of hip fracture (RR=0.76). A minor inverse relationship in hip fracture risk was found after higher compared to lower cheese consumption (RR=0.89). Case-control studies revealed that cheese intake had either a null or a protective effect against osteoporosis (BMD T-score ≤-2.5). Daily yogurt or cheese consumption (<2 months) decreased bone resorption marker CTX but had no effect on bone formation markers. In PM women, only greater yogurt consumption was associated with a reduced risk of hip fracture compared with low or no intake. The conclusion of these studies is that daily fermented milk product consumption may be associated with increased bone calcium absorption, BMD, and overall bone health.

Data from the original Framingham cohort prospective trial [106] examined the association between consumption of milk, yogurt, cream, and milk and yogurt on one hand, and hip fractures on the other, in 85- men and women over a mean follow up time of 11.6 years. The mean servings at baseline were for milk=6, yogurt=0.4, cheese=2.6, and cream=3.4. Participants with medium (>1 and <7) servings per week, or higher (>7 servings per week) of milk and yogurt tended to have lower fracture risk than those with low, <1 serving per week. The high vs low consumption HR was 0.58, and for medium vs low intake HR was 0.61. There appeared to be a threshold for milk with 40% lower

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risk for hip fracture for those with medium relative to low intake. No associations were found for other milk products.

A cohort study with Swedish women produced a unexpected negative outcome regarding the association of milk consumption and hip fractures [107]. In a population-based Swedish Mammography Cohort study, 61,240 women periodically answered food questionnaires (FQs) and were followed over 22 years. Compared with a low intake of low-fat milk (<1 glass/day) and high intake of fruits and vegetables (>5 servings/day), a high intake of milk (>3 glasses/day) combined with concomitant low intake of fruits and vegetables (<2 servings/day) resulted in a high risk of hip fractures (HR=2.49.) Pairing fruits and vegetables with fermented milk (yogurt and soured milk) yielded lowest hip fracture rates from highest consumption compared with lower consumption of both (HR=0.81). The extraordinary results of increased hip fracture with high milk intake may be attributable to low vitamin D content of low-fat milk in Sweden during data collection years between 1987 and 1990 coupled with low exposure to sunshine at that geographic latitude [108]. The conclusion is that fermented yogurt and soured milk overcame whatever caused the unfermented milk matrix food consumption to increase the risk of hip fracture.

Another cohort study [109] compared two large US cohorts, the Nurses' Health Study (NHS) of women and the Health Professionals Follow-up Study (HPFS) of men to the results from Swedish women's cohort study. In these two cohorts, 80,699 PM women and 43,306 men were followed for up to 32 years, Participants reported their frequency of consumption over the previous year for specified serving sizes of more than 130 food items by selecting from among nine categories: never or < 1/month, 1-3/month, 1/week, 2-4/week, 5-6/week, 1/day. 2-3/day, 4-5/day, and - 6/day. Skim, low-fat, and whole-fat varieties of milk were assessed separately and reported per 1 cup (240 ml) serving. Each serving of milk was associated with a significant 8% lower risk of hip fracture in men and women combined (RR=0.92). A suggestive inverse association was found for cheese for women only (RR=0.91). Total dairy food intake of which milk contributed about half, was associated with a significant 6% lower risk of fracture per daily serving in men and women (RR=0.94). Calcium, vitamin D, and protein from non-dairy sources did not modify the association between milk and hip fractures, nor was it explained by contribution of these nutrients from milk alone. This study confirms that the outcome of the Swedish study [107] was most likely confounded by insufficient availability of vitamin D in the low-fat milk food matrix they were exposed to.

Two reviews interpret the reasons for the beneficial effects of milk and its fermented products on bone health. The first one [110] suggests that prebiotics including undigested lactose and indigestible milk MOs like galactooligosaccharide increase probiotic bifidobacteria in the gut as well as improve mineral balance and bone properties. Milk sugar lactose promotes intestinal calcium absorption in mammals. Hydrolyzed lactose forms simple sugars with organic acids which reduce the intestinal pH thereby enhancing the absorption and transport of calcium ions as calcium gluconate. Prebiotics as well as probiotic bacteria *Lactobacillus, Bifidobacterium* as well as LAB and non-LAB bacteria improve mineral balance and bone properties by lowering intestinal pH and converting lactose to lactic acid. Milk prebiotics, probiotics, and symbiotics, can improve bone biomarkers and bone mineral density through increased intestinal absorption of minerals, especially calcium, phosphorus and magnesium. Casein phospho-peptides prepared from β-casein enhance the absorption of calcium by increasing the soluble calcium concentration in the small intestinal lumen. Fat globules of milk are a source of vitamin K2 which activates osteocalcin to bind calcium to bone matrix. Lactoferrin is an iron-binding milk glycoprotein capable of stimulating bone-forming osteoblasts while inhibiting osteoclasts.

The second review [111] compares the association of probiotics, usually *Lactobacillus, Bifidobacterium, Escherichia, Enterococcus*, and *Bacillus subtilis*, as well as the yeast *Saccharomyces* with fermented milk products, as beneficial to bone health. It explains that fermented milk products represent a food matrix that supplies not only the cluster of probiotic bacteria but also essential bone minerals, nutrients, and vitamins, some of which have important benefits. In reviewing the effects of probiotics on bone, it points out that probiotics confer health benefit on the host when administered

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in adequate amounts. The effective probiotic bacteria concentration is approximately 10e7 to 10e8 for every gram of a serving size of 100 to 200 mg, but a dose-response between probiotics and bone outcomes has not been systematically studied. As to the mechanisms by which probiotics and fermented milk products achieve beneficial bone effects, six hypotheses are proposed: reduced gut permeability, interaction with dietary prebiotics, effects on immune system, and interactions with estrogen, vitamin D receptor, and calcium. Administration of probiotics prevents increases in gut permeability and lowers production of osteoclastogenic cytokines. Dietary prebiotics can stimulate growth and/or metabolism of bacteria in GM and increased calcium absorption. Prebiotics stimulate GM bacterial fermentation and production of SCFAs. SCFAs, in turn, inhibit osteoclast number, differentiation, and activity, and thereby bone resorption. This reduces biochemical markers of bone resorption and prevents menopause-induced, as well as inflammation-dependent, bone loss and bone resorption. SCFA's propionate or butyrate promote an increase in trabecular bone volume by activating Wnt10b signaling in bone marrow stromal cells, leading to their proliferation and differentiation into osteoblasts. Butyrate is also associated with higher bone sialoprotein and osteoprotegerin production. A beneficial influence of probiotics on BMD involves the GM contribution to the immune system. The regulation and production of T-lymphocytes is mediated, at least partially, by factors secreted by the probiotic strain of *Lactobacillus reuteri* in the colon. Probiotic strains such as *Lactobacillus rhamnosus* and *Lactobacillus plantarum* increase vitamin D receptor expression in human intestinal epithelial cells and are protective against *Salmonella*-induced colitis. All of this compelling evidence supports mediation by probiotics and fermented milk products in improving bone health.

4.6. Impact of Fermented Milk Products on All-Cause Mortality and Longevity

In view of the anti-inflammatory, antioxidative, antihyperglycemic, anti-thrombotic, anti-carcinogenic, and bone supportive actions of fermented milk products, an interest in the possible influence of their consumption on all-cause mortality is not surprising. Four studies have produced evidence of this influence, and a fifth study proposes a hypothetical mechanism for its existence. A meta-analysis of prospective longitudinal studies [112] investigated the association of total dairy, total fermented dairy, and different dairy subtypes (total/high-fat/low-fat milk, yogurt, cheese, butter, and cream) with the risk of CVD, CHD, bone fracture and all-cause mortality among 1746 Danish healthy men and women 30 to 60 years old. During a mean follow-up of 30 years, CVD was reported in 904 cases, CHD in 332, bone fracture in 447, and all-cause mortality in 680. Highest tertile intake of fermented milk was associated with reduced bone fracture risk (HRs =0.67), CVD (HT=0.84). CHD (HR=0.82) and risk of all-cause mortality (HR=0.77).

A systematic review [72] reanalyzed data from previous meta-analyses on cheese consumption to arrive at relative risks for all-cause mortality of RR=0.95, cardiovascular mortality of RR=0.93, incident cardiovascular disease and CHD RR= 0.92, stroke of RR=0.93, estrogen-receptor negative breast cancer of RR=0.89, T2D of RR=0.93, total bone fracture of RR=0.90, and of dementia RR=0.81. Based on differences in the quality of evidence, the conclusion was that cheese consumption has neutral to moderate benefits for reducing several types of human mortality.

A previously cited trial and a meta-analysis [93] presented evidence for beneficial effects of fermented milk on cancer in the Nurses' Health Study and the Health Professionals Follow-Up Study on 83,054 46-year-old women who were followed for 32 years and on 43,269 52-year-old men who were followed for 26 years. The studies examined the role of yogurt consumption on colorectal cancer incidence and mortality. Baseline yogurt consumption was associated with a reduced risk of colon cancer after adjusting for calcium and fiber intake and were restricted to cancer in proximal colon. The incidence hazard ratio (HR) for consumption of 1+ servings per week of yogurt was 0.84 but no trend was observed between yogurt consumption and the colorectal cancer mortality.

A meta-analysis [113] examined the association between dairy product consumption and all-cause mortality risk in eight meta-analyses each of which included from 6 to 26 cohort studies, with data from 6 to 28 populations. The sample sizes varied across studies from 24,466 participants

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reporting 5092 mortality cases to 938,817 participants reporting 126,759 mortality cases. They found no association between dairy product consumption (including total, high-fat, low-fat, and fermented dairy products) and all-cause mortality for intakes between 200 and 240 g/d of milk, 10 to 50 g/d of cheese, or from 50 g/d of yogurt.

A longitudinal study [114] with 79,618 Swedish men and women 61 years old who consumed substantial amounts of whole milk or its fermented form and who were followed for up to 18 years and monitored for the cerebrovascular events. There were 9735 cases of total stroke, of which 7573 were cerebral infarctions, 1470 hemorrhagic strokes, and 692 unspecified strokes. Higher long-term milk consumption was directly associated with hemorrhagic stroke with HR=0.98 for 0 g/day, 1.02 for 200 g/day, 1.07 for 400 g/day, 1.13 for 600 g/day, and 1.19 for 800 g/day. The above study is a contrast to data showing generally beneficial health effects of yogurt in a majority of epidemiological and clinical studies.

A speculative hypothesis proposed a potential molecular aging mechanisms affecting human mortality [115]. The hypothesis links potential atherogenicity of non-fermented milk consumption to mTORC1 signaling. Nonfermented pasteurized whole milk has high bioavailability of insulinotropic BCAAs, abundance of lactose, and bioactive exosomal microRNAs (miRs). All of these enhance mTORC1 signaling, which is proposed to shorten human lifespan and increases all-cause mortality. The increase in human mortality is therefore possibly attributable to consumption of persistently large quantities of nonfermented pasteurized cow's milk. Additional detrimental changes are postulated to include introduction of heat processing in general, of pasteurization and refrigeration of milk, all initiated after the industrial revolution, which restrict the action of beneficial milk-fermenting bacteria in their degradation of milk's BCAAs, galactose and bioactive miRs. Consumption of fermented milk products allows fermentation-associated LAB to metabolize BCAAs and degrade galactose (which induces oxidative stress and activates mTORC2) and milk exosomes including their mTORC1-activating microRNAs. Consumption of fermented milk products thus appears to mitigate mTORC1-driven diseases of civilization, reduced lifespan and increased mortality.

5. Relevance of Milkfat to Cardiovascular Health: Facts and Controversies

Of all the topics on milk products examined in this review, the issue of the effect of milkfat on cardiovascular health (CVH) is beset with most controversies. Part of the problem is that measuring fat consumption in individuals and populations is difficult because people usually consume several milk products in their meals differing in fat composition, and these usually constitute only a modest part of their diet. This makes justification for limiting or excluding fat as a single food nutrient surprising. The limitations on the intake of milkfat in the context of its influence on CVH becomes subsumed within the bigger controversy about how dietary fat and its saturation in general affects CVH and CHD. Currently, the advice of the American Heart Association is to follow the 2015 to 2020 Dietary Guidelines for Americans to consume less than 10% of daily calories from saturated (SAT) fat and 20 to 35% of calories from all types of fat [116]. Disagreements over the rationale for this advice have persisted over the past seven decades. Therefore, the history of how dietary fat became the focus of CHD hypothesis and influenced US and global dietary recommendations is briefly outlined in section 5.1. A summary of quantity and type of fat in different milk products is listed in section 5.2, and facts and uncertainties about the effects of milk fat on CVH, CVD, and CHD are in section 5.3. As a preamble to saturated-fat controversy, it may be useful to state that scientific knowledge relies on balanced examination of evidence formulated as hypotheses and on a disproof rather than proof of a hypothesis based on this evidence, a principle that was posited by two philosophers, Francis Bacon in 17th century, and Sir Karl Popper in 20th century [117]. This suggests caution against an inflexible view of how individual nutrients such as SAT fat, as opposed to their inclusion in a composite complex- food matrix, affects multifactorial health conditions such as atherosclerotic aspects of CHD.

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5.1. How Dietary Fat Became the Focus of CHD Hypothesis and Influenced US and Global Dietary Recommendations

Cardiovascular diseases have become the major cause of mortality and disability globally in developed societies for the past century [118]. As of 2023, there were 437 million disability-adjusted life years (DALYs) and deaths, a 1.5-fold increase from 320 million in 1990. In 2023, ischemic heart disease (IHD) and stroke, intracerebral hemorrhage, and hypertensive heart disease and heart failure (HF) were the leading global causes of cardiovascular DALYs. It is estimated that 80% of the CVD burden is attributable to modifiable risk factors such as high systolic blood pressure, dietary risks including consumption of SAT fat, high levels of low-density-lipoprotein cholesterol (LDLc), and air pollution. In the 1940s and 1950s, the efforts to modify the trajectory of CVD associated with vascular atherosclerosis, MIs, and stroke [119] considered dietary manipulation of fat consumption as a means of reducing plasma cholesterol's link to CVD risk. A key individual who influenced the Dietary fat-CHD (DF-CHD) idea was Ansel Benjamin Keys at the University of Minnesota. He made an inductive leap hypothesizing a link between fat and cholesterol content of human diet and development of atherosclerotic CHD plaques. To examine this association, Keys initially studied how different sources, types, and quantities of dietary fat influenced plasma cholesterol in small-scale studies with modest numbers (between 50 and 100) of institutionalized subjects. Keys varied quantities of fat (between 50 and 1450 mg) and types of fat, from animal SAT fats and cholesterol to monounsaturated (MUFA) and polyunsaturated (PUFA) plant oils [120–122]. His studies included calculations which specified the proportion of dietary saturated FAs (SFAs) or cholesterol relative to PUFA or plant oils that would allow a linear relationship between fat consumption and a proportional positive relationship to plasma cholesterol. These papers indicated that dietary SFAs, mostly from animal sources like meat, eggs, and dairy, increased plasma cholesterol concentration, while plant oils reduced it. The data supported the dietary fat-to-blood cholesterol portion of DF-CHD hypothesis and were supported by research of others [123], despite some contradictory evidence that SFAs intake did not invariably increase plasma cholesterol [124]. While the first presentation of DF-CHD hypothesis was in a low-circulation Dutch journal Voeding in 1953, an international presentation introduced it globally in 1955 at the World Health Organization in Geneva, Switzerland. Next, Keys conducted a prospective cohort study of middle-aged Minnesota professionals and businessmen funded by US Public Health Service to test the association between dietary cholesterol and CHD. Its results were interpreted as evidence that total serum cholesterol was the key predictor of CHD [125].

In 1948, US Public Health Service also initiated and funded an even larger epidemiological study, The Framingham Heart Study [126] which was not initiated by Keys but is relevant as a check of validity of the Keys DF-HD hypothesis. It is an ongoing cardiovascular cohort study of residents of the city of Framingham, Massachusetts, that has allowed exploration of hypertensive and atherosclerotic basis of CVD. It identified high blood pressure, high total and LDL-cholesterol (HDLc), smoking, and T2D, as major risk factors for CVD. It also established that physical activity and "good" HDLc are protective, that there is an association between atrial fibrillation and stroke, and that neurological and genetic factors also are related to heart disease. An analysis of the Framingham Heart study data [127] checked the significance of coefficients of correlation (CCs) between the five identified risk factors, smoking, hypertension, overweight, obesity, and plasma cholesterol on one hand, and incidence of all CVD, heart failure, stroke, and CHD in male and female study participants, on the other. Smoking was significantly correlated with all CVDs, with heart failure in women, and with CHD in men, but not with stroke in either gender, with this last result disproving dietary link with atherosclerotic CVD Hypertension CCs were significant across the board for all CVDs, HF, stroke, and CHD for both genders. Overweight CCs also were significant across the board except for stroke in men. Plasma cholesterol CCs, on the other hand, were not significant for stroke ([128], another disproof of Keys' hypothesis) and for HF in men but were significant for all CVDs and CHD in both genders.

To test his Diet fat-CHD hypothesis beyond epidemiological associations, Keys in 1958 recruited collaborators in seven countries (USA, Italy, Greece, Yugoslavia, the Netherlands, Finland, and

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Japan) to conduct the Seven Countries Study, the first cross-cultural comparison of dietary influence on MI and CHD risk in male populations engaged in traditional occupations and living in cultures differing in the consumption of quantities and types of dietary fat. The study was not a randomized controlled trial because country selection excluded France, recognized for its high fat consumption and low incidence of CHD [129]. In addition, dietary information in Greece was unrepresentative by being collected during Lent when the orthodox Greeks avoid eating meat, dairy, and eggs in favor of vegetables, legumes, fruits, and some seafood like shrimp, squid, and octopus. This flaw and exclusion of sugar as a possible risk of CHD was promptly noted and criticized [130]. The outcome of the study was interpreted as providing strong support for the contention that consumption of SFAs (which have all carbons bound to hydrogen) over 5 to 40 years increased total plasma cholesterol concentration and was strongly associated with incidence of CHD [131]. The study also suggested that the mortality rate after a CHD event or stroke was associated with level of hypertension, and that cholesterol and obesity correlated with increased mortality from cancer.

The Seven Countries Study was the precursor of the Mediterranean diet (MedD) concept developed on the premise that the diets eaten in Crete, Greece and southern Italy and France, account for the low incidence of CHD and high longevity in these populations. The instigators of MedD were Antonia Trichopoulou [132] representing Greece and Anna Ferro Luzzi representing Italy. The diet was publicized by Walter Willet of the Harward School of Public Health [133]. MedD generically describes an eating pattern that has been refined based on the results of multiple scientific studies and influenced by Keys DF-CHD hypothesis. It emphasizes plant-based foods, particularly unprocessed cereals, legumes, vegetables, and fruits; moderate consumption of fish and dairy products (mostly cheese and yogurt); and low amounts of red meat, refined grains, and sugar. Alcohol intake is limited to wine (typically the red variety) consumed in low to moderate amounts, usually with meals. Olive oil (consisting of 73 to 78% of MUFAs) is the principal source of fat and has been studied as a potential health factor for reducing all-cause mortality and the risk of chronic diseases. It should be noted that MedD follows the Keys' hypothesis of low total fat intake despite the fact that the diet of some MedD countries like Catalonia in southern France typically includes vastly more total fat calories in the form of olive oil and cheese [134] than the 20 to 35% total fat calories and a maximum of 10% of SAT fat recommended by the Dietary Guidelines for Americans [135]. Many Mediterranean countries also use sheep milk that contains higher percentage of saturated fat than cow milk [136].

Ancel Keys' DF-CHD hypothesis was popularized with his and his wife's books [137,138]. In 1961, Keys joined the nutrition committee of the American Heart Association (AHA) where he promulgated his DF-CHD hypothesis. That year the AHA became the first group anywhere in the world to advise cutting back on SAT and total fat as well as dietary cholesterol to prevent heart disease [139]. These recommendations have persisted ever since.

5.2. A Summary of Quantity and Type of Fat in Different Milk Products

In view of the core premise in the Diet fat-CHD hypothesis that both total dietary fat and particularly its SFAs cause CHD and CVD, it is useful to examine the quantity and type of fats in foods manufactured from milk. Milk products have, like other animal fats, a relatively high proportion of SAT fat in contrast to avocado and most MUFA and PUFA oils except for coconut and palm oil. There are large differences in fat content and type of fatty acids between butter, produced by churning cream separated from whole milk by gravitation, and skimmed milk products produced by fermentation. Cheeses present a wide range of fat content where fermentation alters both the amount and type of fat by lipases activated by bacteria [140]. By contrast, many PUFA plant oils are seen as suitable for prevention of CVD and CHD and in support of cardiovascular health (CVH) because they significantly reduce circulating total and LDLc concentrations [120–123,141]. Table 6 presents total fat content of dietary fats, dairy, and oils, a comparison that is relevant for selection of foods based on their content and composition of fat.

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Table 6. Quantity of fat and types of fatty acids in milk products and commonly used plant oils.

Source of fat	Total (g fat/100 g)	SFA (%)	MUFA (%)	PUFA (%)
Avocado	15.4	2.13	9.80	1.82
Beef tallow	100	47.6	50.1	2.3
Butter	83	57.4	38.7	4
Canola oil	100	7	63	30
Cheese- cheddar	34	56.5	21.9	3.5
Cheese- Swiss	31	58.7	23.4	3.7
Cheese- ricotta	11	63.4	23.3	3.5
Cheese- parmesan	28	24.9	22.9	4.3
Cocoa butter	100	60.7	7.3	2
Coconut oil	100	93	5.6	3.3
Corn oil	100	11.2	36	52.8
Cottonseed oil	100	25.9	25.1	47.2
Greek Yogurt- whole	4.4	54.3	21.8	2.5
Kefir	3.6	60.5	28.9	3.4
Lard	100	34.6	52	12.9
Margarine	81	64.5	27.5	8
Milk whole	3.8	60.5	28.9	3.4
Milk skim	0.2	45.0	30.0	5.0
Olive oil	100	10	80.3	9.5
Palm oil	100	48.8	41	8,9
Peanut oil	100	17.7	56.5	25.8
Safflower oil	100	9.5	14.1	76.3
Sardine oil	100	23	23	54
Sesame oil	100	11.9	45.2	42.9
Soybean oil	100	14	29	57
Sunflower seed oil	100	10	29	61
Yogurt-natural	2.6	57.7	27.7	5.0
Yogurt- skimmed	0.32	31.3	46.9	0.0

MUFA= monounsaturated fatty acids, PUFA= polyunsaturated fatty acids, SFA= saturated fatty acids. (Data from [142,143]).

5.3. Facts and Uncertainties About the Effects of Milkfat on CVH, CVD, and CHD.

One third of deaths worldwide are due to atherosclerotic vascular events [144] which encompass a range of morbidities [145]. These include coronary and carotid artery disease, ischemic heart disease (IHD), myocardial infarct (MI), stroke, and heart failure (HF). Main behavioral risk factors of these morbidities are smoking and the hypothesis regarding intake of SFAs, while physiological and metabolic ones include hyperlipidemia as a source of arterial atherosclerotic plaques, hypertension, hyperglycemia or pre-diabetes, and T2D. As the history of atherosclerotic CVD hypothesis outlined in section 5.1 indicates, a SAT-fat diet of animal origin such as red meat and dairy, has been implicated as its cause. Yet results from several RCTs, meta-analyses, and epidemiological cohort studies have not uniformly supported the association between dairy SFA intake with atherosclerotic vascular morbidities. To examine the current state of knowledge on the role of milkfat SFA intake in cardiovascular morbidities and try to resolve some uncertainties and controversies about this issue, it is useful to examine relevant facts from four perspectives. The first one, in section 5.3.1 presents current understanding of the effects of dairy SFA intake on cholesterol- and atherogenic-linked lipoproteins. The second one examines the effects of replacing dairy SFAs with unsaturated plant FAs in section 5.3.2. The third one compares the cardiovascular effects of fermented to full-fat milk

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products in section 5.3.3. The fourth one compares a SFA-centric to dairy food-matrix interpretation of CVH, CVD, and CHD outcomes in section 5.3.4.

5.3.1. Effects of Dairy SFA Intake on Cholesterol-Linked Lipoproteins

SFA consumption increases circulating LDLc concentrations, but there is disagreement about atherogenicity of this effect [146]. SFA consumption is accompanied by a shift toward larger and lighter subspecies of LDLc lipoproteins which are believed to be less atherogenic than small, dense lipoproteins. The health effects cannot be predicted by their individual components without considering the overall macronutrient distribution of the particular food [147]. Small, dense LDL particles are associated with more than a three-fold increase in the risk of CHD compared to larger and lighter lipoproteins, but are frequently contingent on additional metabolic disorders such as insulin resistance, hypertriglyceridemia, reduced HDL cholesterol levels, abdominal obesity, and increased susceptibility to thrombosis [148]. But in contrast to association of dairy SFA intake with less atherogenic larger and lighter LDLc particles, intake of SFAs also is associated with increases in plasma apolipoprotein B (ApoB) concentrations reflecting the total of LDL atherogenic lipoproteins. These lipoproteins reduce liver LDL-receptor activity, slow their clearance from arterial plaques, and may increase plasma cholesterol [149]. This indicates that high consumption of dairy SFAs can elicit both anti- and pro-atherogenic blood-cholesterol raising responses. To add to uncertainties about the health risks of SFAs, much evidence from both RCTs and and epidemiological cohort studies shows a lower circulating concentration of LDLc is associated with reduced risk of of major cardiovascular events [150,151]. A very recent review [152] suggests that novel ultimate risk factors for CHD transcend total cholesterol, and even LDLc, and involve density and distribution of blood lipoproteins in the context of individual nutrient levels of a dairy product matrix,. Therefore a thorough analysis of the connection between consumption of dairy SFAs and their influence on atherogenicity of lipoprotein subtypes will be an important goal of future research which has not been fully explored to date. Among the few studies exploring that issue, an RCT [153] examined the role of consumption of high-fat cheese affected the plasma LDLc characteristics. The comparison involved 12 weeks of consumption of regular-fat (REG), reduced-fat cheese (RED), and an iso-energetic carbohydrate-based food (CHO). REG and RED groups consumed 80 g cheese/d per 10 MJ, whereas subjects in the CHO consumed bread and jam corresponding to 90 g/d and 25 g/d per 10 MJ, respectively. Two aspects of the results were unexpected: higher-fat REG diet decreased total medium-sized LDL particle number (- 223.2 nmol/l) compared to the lower-fat RED diet, but the effect was manifested only in men. In women, the REG diet increased the concentration of small HDL cholesterol particles relative to the CHO diet (2.9 ± 1.0 mg/dl) suggesting a need to repeat the study and clarify the gender-associated differences in lipoprotein outcomes.

Another 6 week-long RCT [154] compared the effects of a low-fat (24% fat) and a high-fat (46% fat) diet on LDLc in 103 men who were randomly assigned to diets in a crossover design. The low-fat diet contained 24% of energy as fat (6% SAT, 12% MUFA, and 4% PUFA) and 59% as carbohydrate (CHO). The high-fat diet contained 46% of energy as fat (18% SAT, 13% MUFA, and 12% PUFA) and 39% as CHP. Palmitic FA (16:0) was the primary dietary SAT FA in both diets. Changes in myristic acid, palmitic acid, stearic acid, oleic (18:1) and linoleic (18:2) FAs were also measured. Changes in intake (ie, high fat minus low fat) of total SFAs, as well as myristic (14:0) and palmitic (16:0) acids, were positively correlated ($P < 0.01$) with increases in mass of large LDL particles and with LDL peak particle diameter and flotation rate but not with changes in LDLc concentration. Changes in total SFAs as well as myristic and palmitic acids were also inversely associated with changes in hepatic lipase activity. With the high-fat diet only, variation in total dietary SFA intake was inversely correlated with concentrations of small, dense LDL of Sf 0-5. This correlation was significant specifically for myristic acid ($P < 0.001$). Stearic acid (18:0). MUFAs and PUFAs showed no significant associations with lipoprotein concentrations. These data indicate that a high- SAT fat intake (especially 14:0 and 16:0 carbon FAs) is associated with increased concentrations of larger, cholesterol-enriched LDL particles.

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In addition to the evidence that dairy fat can alter the size and distribution of lipoprotein particles with an effect on the risk of CVD, there also is evidence that HDL proteome, the collection of nearly 100 different proteins within the HDLc particles, can be modulated by diets in their role of stimulating the efflux of cholesterol from macrophages in atherosclerotic lesions to the liver [155]. Poor understanding of atherogenicity of milkfat consumption may be a consequence of emphasis on concentrations of traditional individual markers of CHD and CVD risks such as fat saturation or cholesterol. With this demonstration of the effects of dairy fat consumption on lipoprotein particle size and distribution, more research is needed to link both dairy food matrices and their particular SFA composition to lipoprotein atherogenicity.

5.3.2. Effects of Replacing Milk-Product SFAs with Unsaturated FAs on CVH, CVD, and CHD

The strongest argument supporting lower dietary SFA intake for reduction of atherosclerotic cardiovascular accidents are studies where higher fat dairy is replaced with products containing lower FA saturation PUFA, MUFA or unsaturated FAs. Much evidence from both RCTs and epidemiological cohort studies shows a reduced risk of major cardiovascular events after fat replacements associated with a lower circulating concentration of LDLc [150,151]. The aim of a systematic review [156] was to assess the effect of exchanging SFA intake with cis-MUFA, cis-PUFA or carbohydrates on serum lipid and lipoprotein levels in the context of affecting the risk of CHD and CVD. Analysis included 84 RCTs with 1538 men and 801 women. For each 1% of dietary energy as SFA replaced with an equivalent amount of cis–PUFA, there was a significant decrease in total cholesterol (TC) of 0.064 mmol/L, in LDLc of 0.055 mmol/L, in HDLc of 0.005 mmol/L, in triglyceride (TG) of 0.010 mmol/L, in the TC-to-HDLc ratio of 0.034, in the LDLc-to-HDLc ratio of 0.034, in the TG-to-HDLc ratio of 0.005, in ApoA-I of 4.9 mg/dL and in ApoB of 10.2 mg/dL For each 1% of dietary energy as SFA replaced with an equivalent amount of cis-MUFA, there was a: significant decrease1 in TC of 0.046 mmol/L, in LDLc of 0.042 mmol/L, in HDLc of 0.002 mmol/L, in TG of 0.004 mmol/L, in the TC-to-HDLc ratio of 0.027, in the LDLc-to-HDLc ratio of 0.029 and in ApoB of 7.8 mg/dL. For each 1% of dietary energy as SFA replaced with an equivalent amount of carbohydrates (CHOs), there was a: significant decrease in TC of 0.041 mmol/L, in LDLc of 0.033 mmol/L, in HDLc of 0.010 mmol/L, in the LDLc-to HDLc-ratio of 0.007, in ApoA-I of 7.0 mg/dL, and in ApoB of 3.6 mg/dL, and a significant increase1 in TG of 0.011 mmol/L, and the TG-to HDLc-ratio of 0.014.

Between 2016 and 2025, additional surveys of the role that SAT fat replacement has on CHD were presented in the form of meta-analyses, systematic reviews, or prospective cohort studies. In a 2016 survey [157] that included cohort data from the Health Professionals Follow-Up Study (1986-2010) with 43,652 men, Nurses' Health Study (1980-2012) with 87,907 women, and Nurses' Health Study II (1991-2011) with 90,675 women, dairy fat and other fat intakes were assessed every 4 years with a FQ. During 5,158,337 person-years of follow-up, there were 14,815 incident CVD cases including 8974 CHD cases (nonfatal MI or fatal coronary disease) and 5841 strokes. Compared to an equivalent amount of energy from CHOs (excluding fruit and vegetables), dairy fat intake was not significantly related to risk of total CVD. The relative risk (RR) for total CVD was 1.02, for stroke was 0.99, and CHD was 1.03. However, replacement of 5% of energy intake from dairy fat with equivalent amount of PUFA or vegetable fat was associated with 24% lower risk of CVD (RR=0.76) and 10% lower risk of CVD, respectively. On the other hand, a 5% energy intake substitution of other animal fat with dairy fat, was associated with 6% increased CVD risk (RR=1.06). The conclusion was that replacement of animal fats, including dairy fat, with vegetable sources of fats and PUFAs may reduce risk of CVD. Whether the food matrix may modify the effect of dairy fat on health outcomes warrants further investigation.

A Cochrane base systematic review [158] used 15 RCTs including 59,000 adults with mean intervention durations of 24 months and examined the consequences of reducing dietary SFAs. Long-term trials suggested that reducing dietary saturated fat reduced the risk of combined cardiovascular events by 21% (RR=0.79). Reducing saturated fat had little effect on all-cause mortality (RR=0.96) in 11 trials with 55,858 participants or cardiovascular mortality (RR 0.95) in 53,421 participants. There

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was little or no effect of reducing saturated fats on non-fatal MI (RR=0.97) or CHD mortality (RR=0.97). The conclusion was that reducing saturated fat intake for at least two years causes a potentially important reduction in combined cardiovascular events. Replacing the energy from saturated fat with PUFA fat or CHO appears to be a useful strategy, while effects of replacement with MUFA fat are less clear.

Another review of RCTs in the Cochrane Central Register of Controlled Trials [159] assessed the effect of reduction and/or modification of dietary fats on mortality, cardiovascular mortality, cardiovascular morbidity, and individual outcomes including myocardial infarction, stroke and cancer diagnoses in 65,508 participants of whom 7% had a prior cardiovascular event. Reducing and/or modifying dietary fat lowered the risk of cardiovascular events by 14% (RR=0.86) in 24 comparisons. Replacing the saturated fat with CHO (creating a low-fat diet) was not clearly protective despite modest improvements in weight, body mass index, TC and LDLc. There were no clear effects of dietary fat changes on total mortality (RR=0.98) in 71,790 participants) or CVD mortality (RR=0.94) in 65,978 participants. Conclusion was that the findings are suggestive of a small but potentially important reduction in cardiovascular risk after modification of dietary fat, but not reduction of total fat, in longer trials.

A meta-analysis of RCTs [160] compared the effects of higher one-year intake of PUFA SFA to lower CHD events (MI or cardiac death). Higher PUFA consumption averaged 14.9% of energy as compared to 5% in controls. Eight trials involved 13,614 participants who experienced 1,042 CHD events. For each 5% increase of PUFA calories, the overall risk reduction was 19% (RR=0.81) corresponding to 19% reduced CHD risk (RR=0.90). These findings provide evidence that consuming PUFA in place of SFA reduces CHD events in RCTs. This suggests that a shift toward greater population PUFA consumption rather than trying to lower SFA consumption, would significantly reduce rates of CHD.

A similar approach to the previous meta-analysis was used in another one [161] asking whether a difference in the consumption of a specific SFA affects risk of CHD. The SFA of choice was the essential linoleic PUFA (with 18:2 structure), the most common SFA in Western diet. When the highest consumption category was compared with the lowest category, dietary LA was associated with a 15% lower risk of CHD events (pooled RR=0.85) and with a 21% lower risk of CHD deaths (pooled RR=0.79). A 5% energy increment in LA intake replacing energy from SAT fat intake was associated with a 9% lower risk of CHD events (RR=0.91) and a 13% lower risk of CHD deaths (RR=0.87).

The effects on CVD of replacing dairy SFAs with specific MUFA and PUFA FAs, or with CHO, and protein was also discussed in a systematic review [162]. The review first stated that different chain length of SFAs affect the risk of CHD. No significant increase in coronary heart disease (CHD) risk was associated with consuming SCFAs to medium chain SFAs (4 to 10 carbon lengths) that are common in coconut oil, palm oil, and dairy products. Longer chain SFAs (12:0–18:0 carbons) increased CHD risk and of those, 12-carbon lauric acid increased LDL cholesterol to the greatest extent. Replacing dairy SFA with MUFA (the main source of which is red meat) has produced inconsistent results. Replacing dairy SFAs with mixed (n-3 and n-6) PUFA reduced total cholesterol, LDL, HDLc, and triglycerides. In one study, for every 5% of energy from SFA exchanged for PUFA in short-term feeding trials, LDL cholesterol was reduced by 10 mg/dL, and the ratio of total cholesterol to HDL cholesterol decreased 0.16 mg/dL. This led in another study to a 17% to 27% reduction of CVD risk but no effect on risk of death. When 5% of energy from SFA was replaced with the n-6 PUFA linoleic acid, risk of coronary events was reduced by 9% and risk of death by 13%. Less information is available about the effects on n-3 PUFA because they are found in dairy products in small amounts, although one study indicates that a 3% replacement reduced all-cause mortality by 5%. Replacement of dairy SFAs with carbohydrates produced mixed results, which sometimes included increases in plasma triglycerides. Any substitution with animal protein increased some categories of CVD risk.

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It is possible that at least a portion of the benefit of reducing SFA intake is attributable to increased intake of replacement nutrients. An expert panel of American Heart Association [146] concluded from a review of RCTs that omega-6 PUFA replacement of SFAs causes a significant 25% greater reduction in CHD than replacement with MUFA (-15%) or whole-grain CHOs (-9%), while refined starches were ineffective, and trans-fat was detrimental [163].

5.3.3. Effects of Fermented, Compared to Full-Fat, Milk Products on CVH, CVD, and CHD

Several meta-analyses and systematic reviews state that general milk products as a component of healthy diet and lifestyle are either neutral or protective against cardiovascular diseases. An example is an overview of systematic reviews and meta-analyses [164]. Pooled risk ratio from 17 cohort studies estimated the association between the consumption of different dairy products at different dose-responses and cardiovascular outcomes (CVD, CHD, and stroke). It showed a statistically significant inverse association with RR values <1, or did not find evidence of significant positive association. The overview of 12 meta-analyses involving RCTs likewise did not find significant changes in total cholesterol and LDLc. The conclusion was that the consumption of total dairy products, with either regular- or low-fat content, does not adversely affect the risk of CVD.

In view of substantial differences in SFA content between whole milk, skim fermented milk products, cheese, and butter (Table 6), many studies have compared their associations with the risk of CVH CHD, and stroke. Here is where the facts and interpretations widely diverge. Considering first comparisons between the cardiovascular risks of full-fat and reduced fat milk, a 2017 review [165] reported total intakes of nonfermented milk (or by fat content) in 103,256 adult participants from Northern Sweden who were followed up for about 14 years. High consumers of nonfermented milk (≥2.5 times/d) had a 32% increase in hazard (HR=1.32) for all-cause mortality. All nonfermented milk-fat types were independently associated with increased HRs but compared with full-fat milk, HRs were lower in consumers of medium- and low-fat milk. A meta-analysis [112] examined the associations between consumption of total (high-fat/low-fat) dairy and milk and CHD and CVD and associated mortality in 29 cohort studies with 938,465 participants. No associations were found for total (high-fat/low-fat) dairy, and milk with the health outcomes of mortality (93,158 cases), CHD (28,419 cases) or CVD (25,416). The conclusion was that the associations between dairy products and the risks of cardiovascular and all-cause mortality are neutral. An investigation [114] of the association between skimmed, reduced-fat, and regular milk consumption and stroke was conducted between 1997 and 2009 in 79,618 Swedish women and men 61.3 years old. Risk of total stroke, cerebral infarction, and hemorrhagic stroke was reported during about 18 years of follow up. There were 9735 total strokes, of which 7573 were cerebral infarctions, 1470 hemorrhagic strokes, and 692 unspecified strokes. Compared with an intake of 100 g/day of milk, there was a dose-dependent increase in the hemorrhagic stroke with increased milk consumption. Hazard ratios were 0.98 for 0 g/day, 1.02 for 200 g/day, 1.07 for 400 g/day, 1.13 for 600 g/day, and 1.19 for 800 g/day. No associations were observed between milk consumption and total stroke or any other stroke outcomes.

A study of 4365 Dutch patients 60-80 years old from the Alpha Omega Cohort prospective analysis who previously had a MI [166], examined the association of fat content of milk products they consumed on their mortality risk. Median intakes were 39 g/d for plain yogurt, 88 g/d for total nonfermented milk, and 17 g/d for hard cheeses. Of the cohort, 10% consumed full-fat milk. During ~12 y of follow-up involving 48,473 person-years, 2035 deaths occurred, including 903 from CVD, 558 from IHD, and 170 from stroke. Yogurt was linearly inversely associated with CVD mortality (HR=0.96) per 25 g/d and nonlinearly inversely associated with all-cause mortality. Milk was not associated with any of the outcomes (HRs=~1.0) per 100 g/d, except for a higher mortality risk in full-fat milk consumers (HR: 1.30). Other dairy groups were not associated with mortality risk. The conclusion was that yogurt consumption was inversely associated with CVD mortality and all-cause mortality while associations for milk and other dairy products were neutral or inconsistent. Finally, a prospective study of 409 885 men and women in the pan-European EPIC cohort from 9 European countries, [167] examined the association between meat, fish, dairy products, and eggs on one hand,

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and risk for ischemic heart disease (IHD), on the other. During 12.6 years of follow-up, 7198 participants had a myocardial infarction or died of IHD. HR for IHD was 1.19 for a 100-g/d increment in intake of red and processed meat. Risk was inversely associated with intakes of yogurt (HR=0.93) per 100-g/d increment, cheese (HR=0.92) per 30-g/d increment, and eggs (HR, 0.93) per 20-g/d increment. Risk was not significantly associated with intakes of poultry, fish, or milk. Replacement of 100 kcal/d from red and processed meat with 100 kcal/d from fatty fish, yogurt, cheese, or eggs was associated with 20% lower risk of IHD. Consumption of red and processed meat was positively associated with serum non-HDLc concentration and systolic blood pressure, and of cheese, was inversely associated with serum non-HDLc. The conclusion was that risk for IHD was positively associated with consumption of red and processed meat and inversely associated with consumption of yogurt, cheese, and eggs.

Intake of nonfermented milk was contrasted to fermented milk in several studies. In a meta-analysis of prospective cohort studies [112], the goal was to examine dose-response associations between total (high-fat/low-fat) dairy, milk, fermented dairy, cheese and yogurt health outcomes of mortality, CHD or CVD. A total of 29 cohort studies were examined. including with 938,465 participants of whom 93,158 died, and 28,419 and 25,416 experienced CHD and CVD, respectively. No associations were found between total (full-fat/low-fat) dairy, and milk and mortality, CHD or CVD. However, beneficial inverse associations were found between total fermented dairy (included were sour milk products, cheese or yogurt; per 20 g/day) with mortality (RR=0.98), and CVD risk (RR=0.98).

Association between non-fermented and fermented dairy intake and coronary artery calcification (CAC), a marker of subclinical coronary atherosclerosis was followed in 3110 young participants for 25 years in CARDIA study [168]. During follow-up, 904 participants were observed to have CAC. Highest intake of full-fat dairy compared with the lowest quartile was inversely associated with risk of future development of CAC (HR=0.76). Associations with CAC of total and low-fat dairy, as well as individual dairy products and fermented dairy, were not statistically significant. Conclusion was that these results do not support dietary guidelines that emphasize low-fat dairy intake for the prevention of coronary artery disease.

An interpretation for the greater health benefit of fermented relative to non-fermented milk products is provided in a hypothesis by Melnik [115]. It posits that consumption of pasteurized and refrigerated non-fermented milk, introduced during the Industrial Revolution, shortens human lifespan and increases all-cause mortality. The aging effect is mediated by high bioavailability of insulinotropic BCAAs, abundance of lactose, and of bioactive exosomal microRNAs (miRs) in non-fermented milk all of which enhance mTORC1 signaling and activity. Milk not chilled or heated allows fermenting LAB bacteria to degrade milk's BCAAs, galactose and bioactive miRs and slow down biological aging and all-cause mortality.

Substantial attention has been given to comparisons between the fermented product cheese and non-fermented butter which do not differ much in their fat saturation (Table 6) as they do in their food matrix, FA and AA content and bioactivities, and enzymatic changes during cheese fermentation and aging. A meta-analysis considering cheese alone [72] has recalculated the risks between cheese and different cardiovascular mortalities and incidence of CVD and CHD. Hazard ratios for these outcomes by highest versus lowest cheese consumption were 0.95 for all-cause mortality and 0.93 for cardiovascular one. HRs for incidence of CVD CHD were both 0.92, and for stroke was 0.93, demonstrating the consistent health benefit from this fermented milk product. This consistent inverse relationship between cheese consumption and beneficial cardiovascular health outcomes is likely to explain the "French paradox" [129]. Low rates of cardiovascular mortality have existed in France for decades despite high saturated fat consumption. It is hypothesized that the good health of cheese consumption reflects beneficial lipoprotein turnover and plasma lipid profile, haemorheological parameters, and inflammatory status. Enzymatic transformation of the cheese core controlled by microbiota during the ripening process forms biologically active substances reducing major pro-inflammatory markers and cytokines (C-reactive protein, interleukin 6, tumor necrosis

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factor alpha). Molded cheeses, including Roquefort, may be even more favorable to cardiovascular health due to the presence of secondary metabolites produced by *Penicillium roqueforti* and other fungi. Among them are andrastins A-D and roquefortine, whose ability to inhibit cholesterol biosynthesis and bacterial growth may be a key mechanism in the prevention of cardiovascular disease.

The effect of SFA content in cheese on plasma lipoproteins was examined in a RCT of 12-week duration [153] in which comparison was made between daily consumption of 80 g of regular-fat cheese (REG) or reduced-fat cheese (RED) per 10 MJ or a no-cheese/carbohydrate (CHO) which involved consumption of bread and jam corresponding to 90 g/d and 25 g/d per 10 MJ. REG and RED consumption did not impact lipoprotein particle number and size, but REG diet decreased total medium-sized LDL particle number (LDL-P, - 223.2 nmol/l in men, while it increased the concentration of small high-density HDLc particles compared with the CHO diet (2.9 mg/dl) in women.

Three studies compared the effects of cheese and butter after having their SFAs matched. In the first one [169], 92 subjects with abdominal obesity and relatively low HDLc concentrations were exposed for 4 weeks to different diets with a 4-week washout period between them. The diets were 2 diets rich in SFAs (12.4-12.6% of calories) from either cheese or butter; a MUFA-rich diet (SFAs: 5.8%, MUFAs: 19.6%); a PUFA-rich diet (SFAs: 5.8%, PUFAs: 11.5%); and a low-fat, high-CHO diet (fat: 25%, SFAs: 5.8%). Serum HDLc- concentrations were similar after the cheese and butter diets but were significantly higher than after the CHO diet (+3.8% and +4.7%, respectively). LDLc concentrations after the cheese diet were lower than after the butter diet (-3.3%) but were higher than after the carbohydrate (+2.6%), MUFA (+5.3%), and PUFA (+12.3%) diets). LDLc concentrations after the butter diet also increased significantly (from +6.1% to +16.2%) compared with the CHO, MUFA, and PUFA diets. The second study was a meta-analysis of 12 RCTs [170] that compared intake of hard cheese and butter (weight mean difference 145.0 g/day), both of which were matched for PUFA and SFA (P/S) ratios. Compared with butter intake, cheese intake reduced LDLc by 6.5% (-0.22 mmol/l) and HDLc by 3.9% (-0.05 mmol/l) but had no effect on triglycerides. Compared with tofu or fat-modified cheese intake, cheese intake increased total cholesterol or LDLc, as was expected since the P/S ratio of the diets. Conclusion was that despite the similar P/S ratios of hard cheese and butter, consumption of hard cheese lowers LDLc and HDLc when compared with consumption of butter. Explanation of these findings in terms of differences in calcium, specific types of SFAs, or the food matrix requires further research. The third study was a RCT [171] that examined whether daily consumption of 40 g of fat as cheese had a different effect on plasma lipids than when consumed as butter. The 6-week study engaged 164 volunteers who were randomly provided one of 4 diets: (A) 120 g full-fat Irish cheddar cheese (FFCC) (n = 46); (B) 120 g reduced-fat Irish cheddar cheese + butter (21 g) (RFC + B) (n = 45); (C) butter (49 g), calcium caseinate powder (30 g), and Ca supplement (CaCO3) (500 mg) (BCC) (n = 42); or (D) 120 g FFCC, for 6 wk (as per A) (n = 31). Since all fat was contained within the cheese matrix (Group A), compared with Group C where it was not, total cholesterol (TC) and LDLc, were significantly lower post-intervention, TC by 5.57 mmol/L and LDLc by 3.43 mmol/L. The conclusion was that dairy fat, eaten in the form of cheese, appears to differently affect blood lipids compared with the same constituents eaten in different matrices. Significantly lower TC is observed when all nutrients are consumed within a cheese matrix.

The expectation of a larger detrimental effect of butter on cardiovasular health yielded different results in 3 studies. In the already mentioned one [165], a cohort of 103,256 adult participants from Northern Sweden were followed for about 14 years to determine associations between all-cause mortality and reported intakes of nonfermented milk (total or by fat content), fermented milk, cheese, and butter. Consumption of butter and other dairy products was scored from reported intakes such as 1) never or, 1 time/week, 2) 1 time/week to 1 time/day, 3) 1 to 2.5 times/day, and 4) 2.5 times/day. High consumers of butter had 11% higher risk of mortality. (HR=1.11.

In contrast to this cohort study documenting detrimental cardiovascular outcome of milk consumption, two others claim relatively small or neutral overall association between butter with

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mortality or CVH, CVD, or CHD. The first of these is a meta-analysis [172] based on 9 publications including 15 country-specific cohorts, reporting on 636,151 participants with 6.5 million person-years of follow-up and including 28,271 total deaths and 9,783 cases of incidence of CVD. Butter consumption was weakly associated with all-cause mortality per 14g (1 tablespoon) intake per day (RR= 1.01). It was not significantly associated with CVD (RR = 1.00), CHD (RR = 0.99), or stroke (RR = 1.01). This suggests relatively small or neutral overall associations of butter with mortality, CVD, and CHD and does not support a need for major emphasis in dietary guidelines on either increasing or decreasing butter consumption. The second study is a comprehensive review [173] which also reports a similar conclusion based on two meta-analyses. The first one [174] reported on absence of a significant association between butter intake and stroke using data from 15 prospective cohort studies with 28,138 stroke events among 764,635 participants. It reported that the relative risks of total dairy (RR=0.88), low-fat dairy (RR=0.92), fermented milk (RR=0.80), and cheese (RR=0.94) were significantly associated with reduced risk of stroke, but that full-fat dairy, nonfermented milk, butter and cream were not. The conclusion was that dairy foods might be inversely associated with the risk of stroke. The second review [175] examined the effects of dairy consumption on the risk of CVD in 22 observational studies published between 1997 and 2013. The dairy products were high- and low-fat dairy, milk, yogurt, cheese and butter, and the evaluation compared highest to lowest consumption. The endpoint was fatal and/or non-fatal CVD event, individual stroke, and CHD. A total of 91,057 participants with 7,641 cases were included in the CVD meta-analysis, and dairy consumption was associated with a significantly decreased CVD risk (RR=0.88). In stroke analysis, 504,803 participants were included with 21,801 cases. Dairy consumption was also associated with a significantly decreased risk of stroke (RR=0.87). A total of 253,260 participants with 8,792 cases were included in the CHD meta-analysis. Dairy consumption failed to show association with CHD risk (RR=0.94). A separate analysis examined the association between the consumption of high-fat dairy, low-fat dairy, yogurt, cheese, and butter and the risks of stroke and CHD. For CHD risk, a significantly decreased risk was observed in cheese consumption (RR=0.84), but not for low-fat dairy consumption (RR=1.02) High-fat dairy consumption showed a borderline increase in the CHD risk (RR=1.08). A possible explanation for more beneficial risk response to consumption of cheese than butter is that the high calcium content of cheese may increase fecal fat excretion. Alternatively, high protein content of cheese or its fermentation by-products, including microbial cultures, prebiotic substrates, a bioactive form of vitamin K, and bioactive peptides may account for different outcomes. In conclusion, dairy consumption shows a significant ability to reduce the risk of stroke, and cheese shows a significant beneficial association to reduce the incidence of stroke and CVD but also a considerable level of disagreement in study outcomes.

5.3.4. Comparison of SFA-Centric to Dairy Food-Matrix Interpretation of Dairy Intake Effects on CVH, CVD, and CHD Outcomes

Categorizing a single nutrient component, like dairy SFAs, as a health risk factor fails to consider that this component is usually a constituent of a complex food group. The concept of nutrient matrix [176] posits that foods are not simply a delivery vehicle for nutrients but represent a mix of nutrient and non-nutrient compounds that interact and induce physiological effects that differ from any specific isolated component [152,177]. The beneficial biological effects of other components in a food matrix may override any influence of a single studied component. Cheese and butter provide a good contrast for a distinction between a focus on single nutrient like its SFA composition and their different food matrices and structures. Butter is a oil-in-water emulsion, while cheese is a fermented product with fat incorporated within milkfat globules within a solid matrix [152,177].

The first study considered the difference in health outcomes of two dairy products from the perspective of a similar single component, but a different matrix [169]. This RCT compared the impact of consuming equal amounts of SFAs from cheese and butter on cardiometabolic risk factors. Ninety-two men and women with abdominal obesity were assigned to sequences of 5 predetermined isoenergetic diets for 4 weeks, each separated by 4-week washouts. Two diets were rich in SFAs (12.4-

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12.6% of calories) from either cheese or butter; the second option was a MUFA-rich diet (SFAs: 5.8%, MUFAs: 19.6%); the third one, a PUFA-rich diet (SFAs: 5.8%, PUFAs: 11.5%); and the last one, a low-fat, high-CHO diet (fat: 25%, SFAs: 5.8%. After the cheese diet LDLc concentrations were lower than after the butter diet (-3.3%) but were higher than after the CHO (+2.6%), MUFA (+5.3%), and PUFA (+12.3%) diets. LDL concentrations after the butter diet also increased significantly (from +6.1% to +16.2%, $P < 0.05$) compared with the CHO, MUFA, and PUFA diets. Serum HDLc concentrations were similar after the cheese and butter diets but were significantly higher than after the CHO diet (+3.8% and +4.7%). The conclusion was that consumption of SFAs from cheese and butter, two dairy products with different matrices, differentially modifies LDLc concentrations compared with the effects of CHO, MUFAs, and PUFAs, but has similar effects on HDL.

The second study was a meta-analysis of 12 RCTs and a meta-analysis of 5 RCTs [170]. It compared cheese and butter of equal SFA composition and equal ratio between PUFAs and SFAs, for their effects on cholesterol, When compared with butter of a similar P/S ratio, intake of hard cheese (weighted mean difference 145 g/d) consistently lowered total cholesterol by approximately 5% (0.28mmol/L), LDLc by approximately 6.5%, (0.22mmol/L), and HDLc by approximately 4% (0.05mmol/L) without affecting triglycerides. Butter, on the other hand, increased both the TC by 9% and LDLc by 15%. The interpretation is that phospholipids, present in milk fat globule membranes (MFGMs), which affect blood lipids and inhibit cholesterol intestinal uptake, may be responsible for different outcomes as butter does not have MFGMs. The outcome difference on plasma lipids suggests that the association between these two dairy foods and lipoproteins was driven primarily by food type or food matrix (cheese, yogurt, milk, butter) rather than by the type of constituent fat.

An RCT [171] compared how 40 g of dairy fat eaten daily over 6 weeks in 4 different food matrices affected plasma cholesterol. Volunteers received ~40 g of dairy fat/d, in 1 of 4 treatments: (A) 120 g full-fat Irish cheddar cheese (FFCC) (n = 46); (B) 120 g reduced-fat Irish cheddar cheese + butter (21 g) (RFC + B) (n = 45); (C) butter (49 g), calcium caseinate powder (30 g), and Ca supplement (CaCO3) (500 mg) (BCC) (n = 42); or (D) 120 g FFCC, for 6 wk (as per A) (n = 31). Significantly lower TC (mean ± SD) (5.23 ± 0.88 mmol/L) and LDLc (2.97 ± 0.67 mmol/L) was obtained when all of the fat was contained within the cheese matrix (Group A), compared with Group C when it was not (TC: 5.57 ± 0.86 mmol/L; LDL cholesterol: 3.43 ± 0.78 mmol/L). Dairy fat, eaten in the form of cheese, appears to differently affect blood lipids compared with the same constituents eaten in different matrices, with significantly lower total cholesterol observed when all nutrients are consumed within a cheese matrix.

Difference in focus between a reduced-fat diet focus and a matrix approach that considered three versions of Mediterranean diet (MedD) produced substantial differences in the efficacy of reducing cumulative risk of CHD. Womens Health Initiative RCT [178] on one hand and two versions of MedD [179]. In the RCT [178], a total of 48,835 PM women, 50 to 79 years old, were assigned either to an intervention (19,541) or to a comparison group (29,294) between 1993 and 1998 with a mean follow-up of 8.1 years. Intervention entailed fat intake reduction to 20% of calories and increased intakes of vegetables/fruits to 5 servings/day and grains to at least 6 servings/day. Outcomes were CHD, CVD, and stroke, both fatal and nonfatal. Over the 8.1 years, total fat intake decreased by 8.2%, saturated fat by 2.9%, intakes of vegetables, fruits, and grains increased by 1.1 servings/day and grains by 0.5 servings/day. LDLc, decreased by 3.55 mg/dL, but the risk of CHD, stroke, or CVD did not significantly change. So, this was an intervention based on modification of a single dietary component that did not work in a large cohort studied for over 8 years.

In contrast to the poor outcome of this RCT focused on a single nutrient component, dietary fat, a multicenter trial in Spain [179], assigned 7447 participants, 55 to 80 y old, to one of three diets with a focus on food matrix: a Mediterranean diet supplemented with extra-virgin olive oil, a Mediterranean diet supplemented with mixed nuts, or a control diet involving advice to reduce dietary fat, and followed them for 4.8 years. The primary end point was a major cardiovascular event (myocardial infarction, stroke, or death from cardiovascular causes). In 288 participants; there were 96 events in the group assigned to a Mediterranean diet with extra-virgin olive oil (3.8%), 83 in the

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group assigned to a Mediterranean diet with nuts (3.4%), and 109 in the control group (4.4%). The hazard ratio was 0.69 for a Mediterranean diet with extra-virgin olive oil and 0.72 for a Mediterranean diet with nuts, as compared with the control diet. In this study that manipulated the food matrix of two mediterranean diets, the cardiovascular outcomes were significantly improved.

Consideration of the dairy product factors that may influence CVH, CVD, or CHD, needs to also include the effect of carbon chain length of constituent SFAs. FAs with chains between 12 and 18 carbons were associated with increased risk for CVD while SCFAs (C2 to C6), and medium-chain FAs (C4 to C10) were associated with neutral or favorable effects [180]. Molecular structure of fat therefore presents yet another variable for considering the characteristics of SFAs that may individually influence cardiovascular health.

An additional discovery was that, in addition to food matrix, food sources can make a difference in CHD risk. In a Multiethnic study of Atherosclerosis [181]. a 5% higher intake of dairy SAT fat was associated with lower CVD risk (RR=0.62), while a 5% higher intake of meat SFAs increased it (HR=1.48). Conclusion was that associations of SF with health may depend on food-specific fatty acids or other nutrient constituents in foods that contain SF, in addition to SF.

6. Summary and Conclusion

Milk was designed by natural selection to provide ideal nutrition for rapid growth and development of mammalian young up to the age of weaning when transition to other sources of nutrition becomes available. Abundant milk supply from lactating females assures attainment of optimal growth rate and adult body stature, while inadequate early milk supply stunts growth and can lead to obesity and metabolic disorders in adulthood. After humans domesticated dairy animals about ten and a half thousand years ago, they also adopted their milk for adult consumption and separated its components into milk proteins useful for muscle growth, fermented products beneficial to the gut microbiome and a source of fermented foods, and milkfat for churning butter prized for its taste. Unlike milk provided to newborn mammals for a limited period, milk products from dairy animals became available to humans for the duration of their adult life which raises the question of health consequences of this extended consumption in view of their substantial saturated fat content. This concern is justified in the context of the known connection between intake of saturated fat, atherogenic plasma lipids, and the global burden of high thrombogenic cardiovascular morbidity The first of three review themes examined the involvement of milk proteins whey and casein on skeletal muscle hypertrophy (MHT). The major contribution of resistance training (RET) to MHT is contrasted to the lesser, but still important, contribution of protein supplementation (PS). Controversies arose about the efficacy of the milk relative to plant proteins, and dose, training status, and timing of PS relative to RET in producing MHT. With their exceptionally rich concentration of essential and branched-chain amino acids, whey protein and casein are highly effective but not essential for MHT, which can also be achieved with higher quality plant PS and is not critically dependent on either the timing of PS, or the training status, or the age of users. The second theme examined the importance of milk fermentation in production of full-fat and low-fat yogurt, kefir, and cheese in terms of bacteria involved, their metabolism in the gut, and their beneficial influence on the gut microbiome and on overall, as well as cardiovascular, health. In the last section of the review, the influence of milkfat on cardiovascular health was discussed both from the perspective of its influence on blood lipids and cardiovascular physiology, but also from the perspective of its actions when evaluated as a component of a complex nutrient matrix. When viewed as part of a rich nutrient matrix, benefits of milk products to cardiovascular health were documented in numerous epidemiological studies with large cohorts of participants studied over several decades. These benefits are attributed to biologically active proteins, amino acids, and fatty acids, in full-fat milk products which can exert anti-inflammatory, anti-carcinogenic, antioxidative, and other beneficial actions, despite their high fat content and level of fat saturation. Fermentation usually lowers CVD and CHD risks of full-fat milk and its products, but some atherogenicity of milk products is revealed as lower cardiovascular risk factors when the saturation and content of milkfat is reduced. Specific types of dairy products may

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be differentially associated with atherosclerotic cardiovascular disease (CVD). Butter does not benefit from the biological activities of the milk proteins and is not fermented, so when consumed in large quantities. the balance of cardiovascular benefits shifts toward CVD and CHD risk. In addition, saturated fat in red and processed meat is more detrimental than the same amount and type of fat in dairy. Cheese is less atherogenic than butter even when the two are equalized for saturated fat because of the biological properties of complex cheese matrix. These facts disprove the hypothesis that all saturated fat is detrimental. Three knowledge gaps need to be corrected with additional research for a better understanding of health benefits of consumption of milk products. Individual nutrient components in dairy food matrices need to be measured as they may exert specific effects and influence health over long-term consumption. Their presence and actions need to be linked to a better understanding of how they influence atherogenic lipoproteins and protein synthesis. And maximal consumption limits need to be defined for full-fat milk products in the context of total human nutrition to assure the benefits their biologically active components offer, but also to reduce any detrimental effects on cardiovascular risk factors. Overall, as a food category, milk products justify acceptance as a healthy natural source of nutrition that was evolutionarily designed to support early growth and development of mammalian young, but need to be prudently implemented for their lifelong consumption in adulthood.

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