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Comprehensive review of the impact of dairy foods and dairy fat on cardiometabolic risk

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This section provides a detailed description of the cardiometabolic risk factors discussed in this review and evidence supporting their association with cardiovascular disease (CVD).

**HDL-cholesterol**

Several epidemiological studies have shown that elevated HDL-cholesterol (C) is associated with a lower risk of coronary heart disease (CHD) (1, 2). HDL comprises a wide array of particles that differ greatly from metabolic and functional perspectives and the cholesterol content of HDL may not fully reflect these various functions (3, 4). HDL is considered to be anti-atherogenic primarily through its role in promoting reverse cholesterol transport. Cholesterol efflux capacity - a key step in this process - has been inversely correlated with carotid intima-media thickness and with coronary disease status even after adjustment for HDL-C concentrations (5), although this has not been a systematic finding (6). More studies are warranted to verify the extent to which enhanced cholesterol efflux per se reduces the risk of CHD. Other mechanisms involving anti-inflammatory, anti-oxidant, anti-thrombotic and anti-apoptotic properties as well as nitric oxide and insulin-secretory effects of HDL have recently been characterized (3). Thus, the focus on HDL from a cardiovascular health perspective is now being shifted towards enhancing HDL’s many functions, as opposed to just raising its cholesterol concentration. HDL functionality is considered an emerging risk factor and its assessment cannot be used in clinical practice to determine and manage CHD risk.
**Triglycerides**

The extent to which increased plasma triglyceride (TG) is an independent risk factor for CVD remains controversial. A meta-analysis of data from 68 prospective studies mostly in Europe and in North America comprising more than 300,000 healthy men and women without initial vascular disease has shown that the risk of vascular disease increases proportionally to fasting plasma TG concentrations (7). Multivariate adjustment for other cardiovascular risk factors including age, systolic blood pressure (BP), smoking status, history of type 2 diabetes (T2D) and body mass index (BMI) did not completely eliminate the association between plasma TG and the risk of CHD. However, further adjustment for HDL-C and non HDL-C levels completely eliminated this association. Increased plasma TG were also not associated with an increased risk of ischemic stroke in this meta-analysis (7).

The fact that atherosclerosis is a postprandial phenomenon was highlighted more than 30 years ago. Although several studies have suggested that non-fasting TG levels are more strongly associated with CHD/CVD risk than fasting TG levels, this remains controversial (8). Also, the mechanisms by which a disproportionate postprandial TG response perturbs the vascular wall are just beginning to be unravelled. Evidence suggest that chylomicron remnants, which are generated by the intravascular hydrolysis of intestinally-derived chylomicrons, can directly infiltrate the arterial wall (9, 10). Increased fasting as well as postprandial TG levels are associated with metabolic features suggestive of an increased risk of CVD,
including the formation of small dense (sd) LDL (see below), as well as with reduced plasma HDL-C concentrations. An exaggerated postprandial response has also been associated with pro-inflammatory and pro-thrombotic states that are predictive of an increased risk of CVD (see below). Finally, several studies have shown that vascular function is impaired 2–8 h following ingestion of moderate to high-fat meals (11). In 2011, an expert panel reviewed the evidence relating non-fasting and postprandial plasma TG levels to CVD risk (8). Their conclusions were that a single measurement of plasma TG levels 4 hours after a fat load is a valid surrogate of the overall postprandial TG response, that plasma TG concentration < 2.5 mmol/L at any time after a fat load should be considered a normal postprandial response and finally that an exaggerated postprandial TG response can and should be treated aggressively by lifestyle modifications as well as pharmaceutical treatment (8). Postprandial TG levels are currently not being used in clinic to determine CVD risk.

**LDL particle size**

LDL particles are heterogeneous in size and density. LDL particle size and distribution can be measured by a number of methods that correlate quite well with each other (12). Several studies have shown that individuals with small dense LDL particles are at higher risk for CHD compared with individuals having larger LDL particles (12). This inverse association between LDL particle size and the risk of CHD has been shown to be fully independent of concurrent variations in LDL-C concentration; this is not unexpected considering that there is no correlation
between these two variables (13). On the other hand, sdLDL tend to co-segregate with reduced HDL-C and increased TG concentrations, to form the so-called atherogenic dyslipidemia (14). sdLDL are also most frequently found along insulin resistance and abdominal obesity as part of the metabolic syndrome (MetS) (12). Studies have shown that sdLDL compared to large LDL may be pro-atherogenic by being more prone to oxidation, a process contributing to foam cell formation and subsequent atherosclerosis (12). sdLDL are also cleared less rapidly from circulation than larger particles because of a reduced affinity to the LDL receptor (12). It remains unclear if sdLDL is an independent risk factor for CHD. Thus, assessing the sdLDL phenotype to predict and manage CHD risk is currently not recommended. Nevertheless, the presence of sdLDL is indicative of a perturbed lipid/lipoprotein profile that is in itself highly atherogenic. In general, pharmacological treatments that reduce plasma TG concentrations tend to increase the size of LDL particles (12). This is also true for diet (15). It is fairly well accepted that consumption of diets that are high in fat and in saturated fatty acids (SFA) increase the size of LDL particle compared with diets that are low in fat (15).

**Apolipoprotein B**

Apolipoprotein B-100 (apo B) is the main protein of LDL particles. Because LDL particles contain only one apo B, and because LDL represent 80-90% of all apo B in plasma, assessing apo B levels approximates the number of LDL particles in the circulation. Apo B as a measure of particle number and LDL-C concentrations provide complementary information on the risk of CHD (16, 17). In fact, there is
now convincing evidence that a high apo B level predicts an increased risk of CHD, independent of LDL-C (18, 19). Relative risk reduction across 7 major placebo-controlled statin trials has been shown to be more closely related to reductions in apo B than to reductions in either non HDL-C or LDL-C (20). Assessing the impact of diet on plasma apo B concentrations in that context is therefore highly relevant.

**Non HDL-C**

Non HDL-C has been advocated by many to overcome some of the limitations associated with the use of LDL-C as a risk factor for CHD. Indeed, unlike non HDL-C, which is obtained from the direct subtraction of HDL-C from total cholesterol concentrations, LDL-C levels are in most instances calculated from a formula that relies on variations in plasma TG concentrations. LDL-C also does not account for the atherogenic lipoproteins that are carried within VLDL. Plasma concentrations of non HDL-C appear to be a more powerful predictor of CHD risk as well as of the cardiovascular benefit of statin therapy than LDL-C levels in certain groups of the population (21). Although they correlate quite tightly, non HDL-C and apo B do not provide entirely concordant information about CHD risk (16, 17).

**Cholesterol ratios**

Lipid ratios reflect the balance between various lipoprotein fractions carrying cholesterol in the circulation. The total-C/HDL-C ratio is considered one of the most powerful lipid risk factor for CHD (19). Analysis of a large population of US adults who underwent advanced lipid testing has shown that the total-C/HDL-C ratio may
offer potential additional information to LDL-C and non HDL-C regarding CHD risk (22). Very few studies have assessed the impact of dairy consumption on the total-C/HDL-C or the LDL-C/HDL-C ratios.

**Inflammation**

Low-grade systemic inflammation is now considered a key etiological factor in the development and progression of several multifactorial disorders including atherosclerosis (23), metabolic syndrome (MetS) (24), T2D (25), and CHD (26). Prospective studies have indeed suggested that elevated levels of C-reactive protein (CRP), an acute-phase reactant, predict the risk of future cardiovascular events independently of sex (27, 28), plasma cholesterol concentrations (29) and history of CHD (30). The pro-inflammatory cytokines Tumor Necrosis Factor-α (TNF-α) and Interleukin (IL)-6 have also been associated with an increased risk of CHD (31, 32). Adiponectin is an adipokine that acts as an anti-inflammatory mediator. Among other mechanisms, adiponectin inhibits the production of TNF-α (33) as well as induce the production of the anti-inflammatory cytokines IL-10 and IL-1 receptor antagonist (34). The value of plasma adiponectin levels predicting CVD risk, independent of other cardiometabolic risk factors, remains unclear (35).

**Insulin resistance**

Insulin resistance plays a pivotal role in the metabolism of lipids and lipoproteins, in addition to regulating plasma glucose levels (36). Insulin resistance has been suggested to be the cornerstone, along with abdominal obesity, of the MetS (37).
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Studies published in the 1980s and 1990s have suggested that elevated plasma insulin levels, a surrogate marker of insulin resistance, may be an important risk factor not only for T2D but also for CHD (38). A recent meta-analysis of existing data from 22 studies comprising 36,617 individuals and 4,491 cases has shown that high plasma insulin levels were associated with an increased risk of hypertension and CHD, but showed no association with the risk of stroke (39). Whether elevated plasma insulin plays a key etiological role in the progression towards CHD through its impact on vascular function as well as on adipose tissue-related inflammation, or is simply a marker of numerous other metabolic complications, remains to be established.

Vascular function

Endothelial cells form the inner layer of blood vessels and play a central role in vascular homeostasis. These cells respond to hemodynamic changes or blood-borne signals by releasing vasoactive substances (40). A dysfunctional endothelium implies incapacity of blood vessels to adapt to various challenges and hemodynamic changes. It is involved in the atherosclerotic process through many mechanisms including up-regulation of adhesion molecules, increased chemokine secretion and leukocyte adherence, increased cell permeability, enhanced LDL oxidation, platelet activation, cytokine elaboration, and vascular smooth muscle cell proliferation and migration (40). Flow-mediated dilation (FMD) is an accepted technique to quantify endothelial function/dysfunction and has been shown to have prognostic value for CVD. The association between FMD and risk of CVD has been
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meta-analyzed based on data from 23 studies comprising 14,753 subjects (41). Data indicated that each 1% increase in FMD was associated with a significant 8% lower risk of overall CVD. This inverse association between FMD and CVD risk was stronger among populations with various forms of CVD than among healthy populations (41). Other measures of endothelial dysfunction include peripheral arterial tone (PAT) signal as well as surrogate measures of vascular activity as described below. The 15% increase in the risk of CHD for each 0.1 unit decrease in reactive hyperaemia index derived from PAT has also been shown to be significant based on meta-analysis of available studies (42). In general, FMD and PAT measures of vascular activity, both of which are non-invasive and hence applicable to large epidemiological studies, do not seem to correlate and therefore are considered to provide different information on the vascular function (43). The measure of FMD is challenging from a methodological perspective, being highly dependent on the assessor training and competence, hence affecting validity, reproducibility and interpretation (44).

Vascular function can also be assessed using surrogate markers in the blood such as the soluble adhesion molecules (e.g., intercellular adhesion molecule-1 [sICAM-1], vascular cell adhesion molecule-1 [sVCAM-1] and E-selectin). E-selectin is expressed on activated endothelial cells, where, along with P-selectin, it facilitates leukocyte adhesion to activated endothelial cells, a process facilitated by sICAM-1 and sVCAM-1. There is subsequent transmigration across the endothelial barrier to a site of injury or inflammation (45). The extent to which these
adhesion molecules predict the risk of CVD independent of other known risk factors remains debated (45).
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