Impact of Nutrition on Biomarkers of Cardiovascular Health

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Introduction

The role of nutrition and lifestyle as effective strategies to decrease diabetes and cardiovascular disease risk is becoming increasingly important as over one-third of Americans are prediabetic and more than 60% of Americans eat more than the daily recommended amount of sodium, added sugar, and saturated fats [1, 2]. Although a wide variety of diet and lifestyle treatment options are available to patients, clinical dietary counseling often fails to meet patient needs and provide sufficient guidance and feedback on progress [3]. One way to understand the impact of diet is through biomarkers, which serve as noninvasive, cost-effective, and diverse tools for physicians to quantify a patient's responses to nutritional therapy. While there are several methods of monitoring a patient's response to nutritional therapy, biomarkers are preferred due to their low cost, greater accessibility, and availability of rapid testing. The biomarkers discussed in this chapter were selected based on their clinical relevance and strength of literature available. This chapter will focus on how biomarkers can be used to assess the impact of diet and lifestyle changes on cardiovascular health (Fig. 2.1).

BMI/Body Composition

Obesity, defined by a BMI of greater than 30 kg/m^2, is a well-known risk factor for dyslipidemia, hypertension, diabetes, cardiometabolic syndrome, CVD, and cancer. However, extending beyond a pure weight-based assessment, new evidence sheds light on the importance of body fat distribution and body composition in overall health [4, 5]. Numerous tools are available to clinicians to quantify body composition. For example, dual energy absorptiometry (DEXA) scans are used to analyze body composition and are an important diagnostic tool for osteopenia and osteoporosis. Further, DEXA scans have been utilized to assess fat mass normalized by height squared (FMI), which is advantageous over BMI in that the value is independent of lean muscle mass, and FMI may be used as a predictor for cardiovascular health [6]. DEXA scans have been used in clinical research and in special populations such as athletes [7]. However, current guidelines suggest that the clinical utility of DEXA scans in metabolic syndrome evaluation requires further research [8].

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Fig. 2.1 The effects of each major food group on cardiovascular biomarkers reviewed in this chapter

An even less invasive measurement of body composition is the waist-to-hip ratio, measured simply by circumference. An increased waist-to-hip ratio shows a significant association with risk of myocardial infarction, as well as coronary artery disease, and T2D [9], [10]. In fact, waist-to-hip ratio shows both a graded and a significant association with myocardial infarction, especially in comparison to BMI, across ethnic groups [11]. The population-attributable risks of MI for waist-to-hip ratio in the top two quintiles of INTERHEART study participants was 24.3% compared with only 7.7% for the top two quintiles of BMI [9]. The importance of waist-to-hip ratio and waist circumference in predicting cardiometabolic risk has been increasingly recognized in the literature, and qualitative descriptors known as "pear" body shaped and "apple" body have been applied to describe patients with more weight around the hips and more weight around the waist, respectively [12, 13]. Furthermore, there is evidence to suggest that even in women with normal weight, central obesity is associated with increased risk of mortality, similar to mortality in women with elevated BMI with central obesity [14]. These findings underscore the importance of assessing not only BMI as a risk factor for future cardiovascular disease, but also central obesity.

Studies have shown when body composition is modified with modalities such as high intensity exercise and diet, there is a reduction in body fat, waist circumference and increase in muscle mass. For example, patients with a history of myocardial infarction (n = 90) who performed high intensity exercise lost 4 pounds more of body fat, gained 1.5 pounds more of muscle, and reduced their waist circumference by 2.54 cm more than those who solely performed moderate exercise [15]. In addition, the Mediterranean diet (MD) in particular can be useful in reducing weight circumference, as demonstrated in a meta-analysis by Kastorini et al. [16].

Blood Pressure

Numerous large-scale studies have provided strong and consistent evidence that both systolic (SBP) and diastolic (DBP) blood pressures are positively associated with cardiovascular disease outcomes [17]. These findings are consistent across genders, various age groups, racial and ethnic groups, and across different countries. Not only is elevated blood pressure an overall predictor for cardiovascular outcomes, systolic and diastolic values are helpful in differentiating risk for patients and may act as a marker to assess risk of cardiometabolic syndrome [17]. While hypertension significantly affects the heart, it has multi-organ effects and is a risk factor for kidney disease and stroke [18].

Vegetarians have been shown to have lower blood pressure than those who eat omnivorous diets. In a meta-analysis of 258 studies, vegetarian diets were found to reduce SBP ~5–7 mm Hg and DBP by ~2–5 mm Hg, which is equivalent to the effect of losing 2.5 lbs [19]. Mirroring these findings , the MD decreases both SBP (−2.35 mm Hg) and DBP (−1.58 mm Hg) blood pressure [16]. Conversely, salty foods increase risk of hypertension: increasing SBP by 4.58 mm Hg and DBP by 2.25 mm Hg per 1000 mg of sodium [20]. Alarmingly, the risk of hypertension for participants in the upper third and fourth quartile (>3819 mg/day) is more than 4x higher compared to those in the lower two quartiles ($P < 0.01$).

Exercise also plays a crucial role in managing hypertension. Endurance training, dynamic resistance training and isometric training lower both SBP and DBP [21]. A systematic review and meta-analysis by Cornelissen and Smart in 2013 found that blood pressure reductions after low-intensity endurance exercise were smaller than blood pressure reductions after moderate- or high-intensity training [21]. (Low-intensity exercise training was defined by <55% of heart rate maximum or < 40% of heart rate reserve) [21]. Surprisingly, this same meta-analysis found that the groups exercising >210 min a week had the smallest reductions in blood pressure, possibly due to the fact that more exercise was performed at a lower intensity [21]. There are many different effective options for exercise to reduce blood pressure, but it may be worthwhile to consider prescribing a supervised facility-based exercise program for patients new to exercise, as this does yield the highest adherence [21].

Total Cholesterol

Total cholesterol, a commonly performed measure, is the sum of LDL cholesterol, VLDL cholesterol, HDL cholesterol, intermediate-density lipoprotein (IDL) cholesterol and cholesterol associated with lipoprotein(a) (Lp(a)). Cholesterol is a requirement for physiological function—it is an essential structural component of cell membranes and acts as a precursor for steroid hormones produced by the body. While the liver's synthesis of cholesterol is largely determined by genetic factors and feedback mechanisms, the remainder of cholesterol is obtained through dietary intake. Foods such as dairy products, eggs, meat, and poultry are significant sources of cholesterol in the diet. Though reducing such animal product intake seems intuitive to lower total cholesterol in patients with hyperlipidemia, dietary cholesterol has little effect on cardiovascular disease risk [22]. In fact, the relationship between dietary cholesterol and cardiovascular disease is different in a given individual; studies demonstrate that the fractional absorption rate of dietary cholesterol is variable, ranging from 20% to 80% [22].

According to US population studies, an optimal total cholesterol level in an adult is <150 mg/dL [23]. It is important to note, however, that there is a large difference in cardiovascular mortality rates for a given total cholesterol value [24]. Total serum cholesterol may be tracked longitudinally as a way to assess both risk for cardiovascular disease and nutrition status, alongside other clinically significant values, discussed below.

Low-Density Lipoproteins (LDL)

While total cholesterol is an important value to track over time and is a predictor of cardiovascular risk, LDL is colloquially termed "bad cholesterol" and is the main target of lipid lowering therapies such as statins. LDL is particularly utilized clinically as epidemiologic data demonstrates a positive and consistent relationship between LDL concentration and cardiovascular mortality and cardiovascular events. There is also substantial data to support the effort of lowering LDL, as reduction decreases patients' cardiovascular risk across a wide spectrum of patients, including those with known cardiovascular disease.

LDL is known to play a key role in the pathophysiology of atherosclerosis. Portions of blood vessels that are susceptible to atherosclerosis retain lipoproteins like LDL, and it is this retention that is an initial and key step in the formation of atherosclerotic plaques in the arteries. The mechanism of plaque formation is well understood, and the evidence for LDL's key role in atherosclerotic formation is corroborated by the understanding of Familial Hypercholesterolemia, an inherited disease associated with severely elevated LDL levels and premature atherosclerotic cardiovascular disease [25].

While LDL is the target of pharmacotherapy, diet plays a vital role in LDL reduction. The MD, which contains large amounts of plant sterols and nuts, lowers LDL, as compared to a low-fat controlled diet [26]. Meta-analyses of vegetarian diets corroborate that vegetarian diets not only lower total cholesterol, but LDL as well [27]. Further, nuts such as almonds, hazelnuts, and walnuts have been linked with a decrease in LDL and C-reactive protein, an acute phase reactant discussed later in this chapter. Additionally, viscous fiber has been shown to reduce LDL by trapping bile salts and preventing reuptake in the GI tract, as well as interfering with cholesterol being absorbed into cells [26].

Target LDL is based on multiple factors, but US population studies suggest that LDL <100 mg/dL manifests in low levels of atherosclerotic cardiovascular disease and patients with an LDL >190 mg/dL have a high risk of atherosclerotic cardiovascular disease [23]. LDL is an important value in the clinical assessment of risk for heart disease, and clinicians target therapies based on changes in LDL, which acts as a useful biomarker. Pharmacologic therapies used to lower LDL include statins, ezetimibe, bile acid sequestrants, and PCSK9 inhibitors [23].

High-Density Lipoproteins (HDL)

Opposite of LDL, HDL is often introduced to patients as the "good cholesterol." And unlike LDL, there is a known inverse relationship between HDL and the risk for cardiovascular events [28]. HDL is a scavenger of cholesterol—it assists in facilitating the return of cholesterol from the blood vessels back to the liver for eventual elimination. Furthermore, HDL prevents oxidation of LDL to limit LDL's role in the generation of atherosclerotic plaque and prevents secretion of the vasoconstrictor endothelin [29].

HDL values <40 mg/dL are considered an independent risk factor for cardiovascular disease [30]. Although low HDL is correlated with cardiovascular disease, raising HDL by pharmacologic interventions has not been consistently shown to have significant clinical benefit [31]. Some diets, such as the MD, have been shown to increase HDL levels, but the maximum threshold of improvement appears to be as low as 12%. Importantly, saturated fats and, to a lesser extent, unsaturated fatty acids have been shown to increase HDL [32]. Moderate alcohol consumption, specifically wine, is positively associated with higher levels of HDL [33, 34]. Conversely, diets high in carbohydrates and low in fats have been associated with low HDL [35]. There is preliminary evidence that aerobic exercise improves the anti-inflammatory and anti-oxidative properties of HDL, but the lack of consistent findings in this regard warrants more studies to determine the importance of exercise on HDL values and function [31].

Non-HDL Cholesterol

The sum of LDL and VLDL values is termed non-HDL cholesterol , which is more atherogenic than LDL or VLDL alone [23]. Therefore, non-HDL more accurately assesses atherogenic lipids and CV risk than LDL, especially in patients with hypertriglyceridemia. In patients with high triglycerides, such as patients with metabolic syndrome and Type II Diabetes, LDL is less accurately estimated by means of the Friedewald equation [23, 25]. Due to the limitations of the Friedewald equation, other ways of estimating LDL have been developed such as the Martin-Hopkins equation, which is a novel method to estimate LDL by using an adjustable factor of triglycerides to VLDL ratio [36]. Given that there are atherogenic lipids beyond LDL, some evidence suggests non-HDL cholesterol values could be more predictive of cardiovascular risk than LDL [37, 38]. In a recent 10-year risk cohort study, both LDL and non-HDL cholesterol values above 160 mg/dL were independently associated with a 50–80% increased relative risk of mortality [39].

In addition to underscoring the importance of non-HDL cholesterol as a marker of atherogenicity, the 2018 cholesterol management guidelines also underscore apolipoprotein B (apoB), the major apolipoprotein embedded in LDL and VLDL, as a stronger indicator of atherogenicity than LDL [23]. Another atherogenic biomarker similar in clinical utility and risk assessment to apoB is LDL particle number [40]. Both apoB and LDL particle number have been shown to be stronger cardiovascular disease risk factors than LDL cholesterol, but apoB has been the preferable particle for guideline adoption given lower cost, standardization, and scalability [40].

Triglycerides

Meta-analyses have demonstrated that both elevated fasting and non-fasting triglycerides are associated with increased risk of coronary artery disease [41]. The Women's Health Study further corroborated the strong association between raised triglycerides and coronary artery disease, as well as risk of myocardial infarction and all-cause mortality [42, 43]. In addition to cardiovascular risk, a triglyceride level > 150 mg/dL is a significant risk factor for metabolic syndrome, a cluster of pathological processes related to insulin resistance and elevated free fatty acids [44]. Additionally, elevated triglyceride concentrations (>885 mg/dL) are associated with risk of pancreatitis [45].

While these correlations between hypertriglyceridemia and risk for cardiovascular disease have been well studied, there is a need to further evaluate the clinical significance of lowering triglycerides by pharmacotherapy [45]. However, triglycerides are highly affected by diet and lifestyle. The MD, high in MUFA, PUFA and dietary fiber, can be particularly helpful in lowering triglycerides [44]. Many studies have shown that high intake of carbohydrates (greater than 60% of caloric intake) is associated with a rise in triglycerides [44]. In addition, high alcohol consumption is associated with elevated triglycerides, but low and moderate alcohol intake are associated with lower triglycerides; this is likely dependent on the type of alcohol consumed [46].

There are several classes of pharmacologic agents, such as fibrates, that reduce triglyceride levels, but both weight loss and moderate intensity exercise, such as brisk walking and social dancing, have been identified as key interventions to reduce triglyceride levels [47]. Additionally, dietary supplementation of ω-3 acid ethyl esters can be considered as an additional therapy for hypertriglyceridemia with a very minimal side effect profile [48]. Icospaent ethyl, a prescription highly purified eicosapentaenoic acid, has been shown to lower triglycerides and reduce the risk of ischemic cardiac events [49].

Lipoprotein(a)

Lp(a) is a well-known risk factor for coronary disease that is highly heritable; elevated levels are associated with atherosclerosis development

and incidence of cardiovascular events [50]. Specifically, elevated Lp(a) levels have been associated with both coronary disease and calcific aortic valve disease. Lp(a) is distinguished from LDL by the presence of apolipoprotein (a), which likely mediates proinflammatory and prothrombotic effects of the protein [51]. While Lp(a) is a modified LDL particle, Lp(a) levels are independent of LDL levels [25]. There is significant evidence to support the use of Lp(a) as a risk factor for CVD, and there are randomized trials ongoing that are targeting Lp(a) [52, 53]. It is important to note that treatment with niacin can reduce Lp(a) up to 20–30% but has not been associated with improved outcomes [25]. Interestingly, monoclonal antibodies to PCSK9 may lower Lp(a) by 30% and have been associated with improved outcomes in large clinical trials such as FOURIER and ODESSEY [25, 54, 55]. Additionally, there are new pharmacologic approaches in phase III clinical trials that target Lp(a) lowering and it will be important to assess if lowering Lp (a) translates to decreased CV events [53]. There are little data available to support the influence of dietary choices on lowering Lp(a), but several studies suggest that low-fat diets may result in an increase in Lp(a) [56].

Hs-CRP

C-reactive protein (CRP), produced by the liver, is a marker of systemic inflammation [57]. High-sensitivity C-reactive protein (hs-CRP) is a higher sensitivity test that can detect lower grades of inflammation than a standard CRP test [57]. While numerous pathologic processes ranging from infection to autoimmune disease can elevate hs-CRP levels, it can also be used as a global assessment of cardiovascular risk. Given that many processes can lead to systemic inflammation, hs-CRP elevations may be transient in response to infection and should be repeated when these confounding processes are quiescent. Meta-analysis conducted by Li et al. suggests hs-CRP can stratify cardiovascular risk and all-cause mortality risk in the general population [57]. Further, data from the Women's Health Study suggests hs-CRP predicts cardiovascular events even in groups that have no other apparent markers of cardiovascular disease [58]. An hs-CRP <2.0 mg/L is often considered the threshold for low risk and a value of >2.0 mg/L is considered the threshold for higher risk [59].

Provided that inflammation plays a key role in the pathophysiology of atherosclerotic formation, the correlation between hs-CRP and cardiovascular disease is not surprising. Even in patients with low levels of atherogenic biomarkers such as non-HDL cholesterol and apoB, a discordantly elevated hs-CRP level resulted in a 30–60% greater relative risk of developing ASCVD compared to patients with low hs-CRP [59]. While many cardiovascular risk factors such as smoking, diabetes, and hypertension can increase the inflammatory response and, thereby, hs-CRP, an anti-inflammatory diet may be helpful in reducing systemic inflammation and could help improve cardiovascular outcomes. Anti-inflammatory diets are the subject of many studies currently, but it has been well established that ω-3 fatty acids are anti-inflammatory, and ω-6 fatty acids tend to be pro-inflammatory. ω-3 fatty acids may be found in walnuts, canola oil, and soybean oil, and fish such as salmon, halibut, and mackerel. Conversely, ω-6 acids are found in corn and sunflower oils. It is generally recommended that protein in an anti-inflammatory diet be plant-based with small amounts of fish and lean meats. Further, the phytonutrients found in soy-based proteins have been demonstrated to have anti-inflammatory properties [60]. While a comprehensive anti-inflammatory diet is beyond the scope of this text, the Mediterranean and other plant-based diets have been identified as general guidelines with anti-inflammatory properties.

TMAO and the Gut Microbiome (Fig. 2.2)

Trimethylamine N-oxide is a gut microbiota-dependent biomarker derived from L-carnitine, choline, and betaine. TMAO levels reflect a pro-atherogenic milieu in the gut microbi-

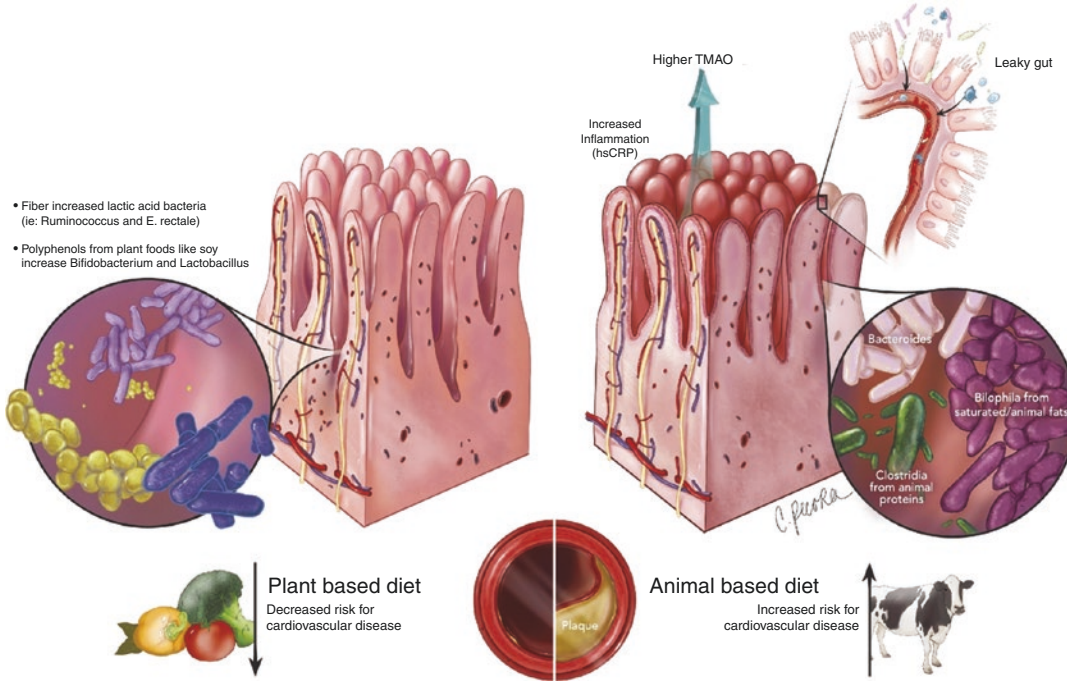

Fig. 2.2 The impacts of a plant-based diet vs. animal-based diet on TMAO levels, the gut microbiome, and the risk of coronary arterial plaque buildup. (Printed with permission from ©*Christina Pecora*)

ome and is associated with poor CV outcomes [61]. The normal range for serum TMAO is 0.5–5 μmol/L. TMAO is felt to play a role in cardiovascular disease and enhancing CV risk. A study on adults undergoing elective diagnostic cardiac catheterization found that participants who had a major cardiac event ≤3 years of catheterization had higher baseline TMAO levels compared to those who did not experience a cardiac event (5.0 μM vs. 3.5 μM; $P < 0.001$). Furthermore, elevated levels of TMAO were associated with a significant risk of mortality (hazard ratio (HR): 3.37; $P < 0.001$) and non-fatal myocardial infarction/stroke (HR: 2.135; $P < 0.001$) [61].

Foods rich in phosphatidylcholine (beef, eggs, and pork) get converted into trimethylamine and then TMAO. Increased choline levels induce greater gut microbial activity and, subsequently higher levels of TMAO. The KarMeN study, which monitored plasma TMAO levels in healthy adults after eating red meat, found a positive correlation ($r = 0.25$) between red meat consumption and choline levels. Additionally, participants with TMAO levels >3.98 μmol/L ate more than the daily recommended amount of red meat per day [62].

Conversely, plant-based diets can decrease TMAO levels by promoting more diverse and stable microbiota. This is due to greater intake of fiber, polyphenols, and beneficial bacteria. For example, Klimenko et al. found plant-based diets greatly improve microbiome diversity [63]. Long-term fruit and vegetable consumption also improved local microbial diversity ($p < 0.05$). Moreover, reduced meat and greater fruit/vegetable consumption can be cardioprotective and inhibit TMAO production. In Koeth et al.'s study on L-carnitine metabolism, omnivores produced >20× more plasma TMAO than vegans despite consuming the same amount of L-carnitine ($p = 0.001$) [64].

The MD has also shown to promote gut diversity and reduce TMAO levels. De Filippis et al. examined the relationship between MD adherence and gut microbiota, observing significantly lower urinary TMAO levels in plant-based eaters vs. omnivores ($p < 0.0001$) and MD adherence hav-

ing a negative correlation with TMAO levels [65]. Also, 25% of plasma metabolites are different between vegetarians and omnivores, further showing how diet can change the gut microbiome [66].

Although advertised as anti-inflammatory, the paleo diet may adversely interact with our gut microbiota and increase TMAO levels. Genoni et al. found serum TMAO levels were significantly higher in strict paleo diet eaters (<1 daily serving of grains/dairy) compared to those who eat a healthy balanced diet (9.53 μmol/L vs. 3.93 μmol/L, $P < 0.01$). This is possibly due to the lack of fiber in paleo diets [67]. In comparing the Atkins diet and Ornish diet after 4 weeks, Park et al. found the Atkins diet had higher TMAO levels compared to the Ornish diet: 3.3 vs. 1.8 μmol/L, $p = 0.01$ [68].

Additionally, Verdam et al. showed microbiome diversity is linked to inflammation in individuals who are obese. Compared to non-obese participants, participants who are obese exhibited lower *Bacteroidetes*:*Firmicutes* ratios ($p = 0.007$) and higher levels of *Proteobacteria*, inflammatory bacteria positively associated with BMI and CRP ($p = 0.0005$). Klimenko et al. also showed an inverse relationship between gut diversity and BMI ($p < 0.05$) [63]. This suggests obesity-induced loss of microbiota diversity results in greater inflammation [69]. Other studies, however, show an opposite relationship between obesity and *Bacteroidetes*:*Firmicutes* ratios, indicating further research is needed on the specific interactions between our gut microbiota and lifestyle [70, 71].

Albumin and Prealbumin

Albumin and prealbumin give important information into a patient's protein and calorie intake. Albumin, the most abundant serum protein, is a moderate indicator of malnutrition, with the normal range being 3.5–5.2 g/dL. As a negative acute-phase protein, its serum concentration and production is downregulated during inflammation [72]. Although prealbumin is also a negative acute-phase protein, its shorter half-life (~2 days) makes it a more sensitive indicator of acute malnutrition and protein-calorie consumption compared to albumin. Prealbumin's reference range is 15–35 mg/dL [73]. As negative acute-phase proteins, prealbumin and albumin have high sensitivities to inflammation and additional steps are required to determine if reduced levels are malnutrition- or inflammation-induced.

Prealbumin and malnutrition risk are inversely related, where hypoalbuminemia (<3.5 g/dL) and/or hypoprealbuminemia (<15 mg/dL) indicate higher malnutrition risk. This is because visceral protein synthesis is not prioritized by the liver and is only made in sufficiently nourished states. Consequently, inadequate nutritional intake inhibits synthesis of albumin and prealbumin, and subsequently lowers each protein's levels. Additionally, Saka et al. ($n = 97$, 55 malnourished) observed prealbumin levels increased by 20% and risk of malnutrition decreased by 12% after 1 week of nutritional support, highlighting prealbumin's sensitivity to dietary changes [74].

Maintaining healthy nutritional intake is also integral in predicting morbidity and mortality. A study on admitted patients with acute coronary syndrome and lower prealbumin levels showed their risk of a major in-hospital cardiac event was more than 3× the risk of patients with normal prealbumin levels: 20.8 vs 6.1% [75]. Also, Lourenço et al. found the risk of heart failure death doubled in patients with discharge prealbumin levels ≤15 mg/dL, citing an imbalance protein-energy demands [76].

There are concerns, however, on albumin's reliability in monitoring nutritional status. For example, Lee et al. showed patients did not exhibit abnormal albumin levels until they reached extreme starvation: <12 BMI or > 6 weeks of starvation [77]. And while a meta-analysis found the risk ratio for a CVD event per 1 g/dL decrease in plasma albumin was 1.96 (95% CI, 1.43–2.68), this was likely due to inflammation and not malnutrition [78]. Additionally, another study ($n = 262$) showed 80% of geriatric patients had low albumin levels despite receiving adequate nutrition [79]. Additional steps beyond albumin testing should

therefore be taken to accurately determine a patient's nutritional status.

Magnesium

Magnesium plays a dual role as a marker of nutritional status and cardiovascular health due to its interactions with CRP and serum plasma. Hypomagnesemia (<1.4 mg/dL) is linked to such conditions as hypertension, arrhythmia, diabetes, and CHD [80]. Magnesium deficiency is so prevalent, in fact, that over 10% of hospitalized patients exhibit hypomagnesaemia [81]. Also, thiazide and loop diuretics have been shown to induce moderate reductions in magnesium concentration, but usually at or close to the normal range [82]. The normal range of serum magnesium is 1.46–2.68 mg/dL and 4.2–6.8 mg/dL for RBC magnesium [80, 83].

Magnesium is often acquired through green vegetables, meat, and dietary supplements. Global trends in diet have contributed to declining magnesium intakes through increased consumption of soda and processed foods, which increase bodily phosphorus levels and thus the required daily magnesium intake. Additionally, the Framingham Heart Study ($n = 2695$) showed hypomagnesemia can increase the risk of connective tissue inflammation and aortic calcification due to a surplus of intracellular calcium. It was found that a 50-mg/day magnesium intake (by diet and supplements) was linked to 22% lower coronary artery calcification (CAC) ($p < 0.001$) and 12% lower abdominal aortic calcification (AAC) ($p = 0.07$). Further, the risk of having CAC was 58% lower ($p < 0.001$) and any AAC was 34% lower ($p = 0.01$) in those with the highest magnesium intake compared to those with the lowest magnesium intake [84]. This is because the deficiency of magnesium allows calcium ions to dominate the binding sites of cardiac and smooth muscle cells, resulting in intracellular calcium buildup. Salaminia et al. showed magnesium supplementation plays a role in cardiac arrhythmia risk, with magnesium supplements decreasing ventricular and supraventricular arrhythmias compared to placebo (OR = 0.32; $p < 0.001$ and OR = 0.42; $p < 0.001$, respectively) [85]. Moreover, each 100 mg/day increase of dietary magnesium has been linked to a 22% reduction in HF risk [86].

Magnesium intake is often higher in those eating a plant-based diet, as indicated by Koebnick et al.'s prospective study of 108 pregnant women. Women eating a plant-based diet (ovo-lacto vegetarian or low meat) had significantly higher magnesium intakes compared to women on the Western (control) diet: 508 ± 14 mg/day for ovo-lacto vegetarians ($P < 0.001$) 504 ± 11 mg/day for low-meat eaters ($P < 0.001$) vs. 412 ± 9 mg/day for the control diet. While serum magnesium levels were similar across groups, RBC magnesium levels were higher in the low-meat group than the control group ($P = 0.058$) [87]. The MD has also exhibited moderate success in ensuring sufficient magnesium intake, with 66.9% of participants in the MEAL study ($n = 1838$) meeting the daily recommended intake (~200–522 mg/day) [88].

Numerous magnesium diagnostic tests are currently available. Although using RBC magnesium is sometimes preferable given RBC's higher magnesium content, its utility and reliability has yet to be established [83, 89]. A 24-h urine analysis has also shown to be unreliable due to variability of renal magnesium reabsorption and excretion [90, 91]. Additionally, current serum magnesium guidelines have come under scrutiny for being insufficient in ascertaining a patient's status [92]. As such, the combined use of 24 h urine, serum, and dietary magnesium tests is suggested to gain the most complete picture of a patient's magnesium status.

HbA_{1c} and Fasting Glucose

Normal range for fasting blood glucose is 70–99 mg/dL, with hyperglycemia resulting in risk of diabetes and hypoglycemia leading to acute neurological changes. $HbA1_C$ is a quantitative measure of average blood glucose of the past 2–3 months and is critical for diagnosing and monitoring diabetes and determining cardiovascular mortality. The ideal range for non-

diabetics is <5.7% and ≤ 7.0% for patients with T2D [93, 94].

Plant-based diets have shown to be successful in regulating blood glucose levels and reducing insulin resistance [95]. A meta-analysis found that T2D patients eating a plant-based diet reduced their HbA_{1c} levels by 3.9% ($P = 0.001$) but had a nonsignificant 6.49 mg/dL decrease ($P = 0.301$) in fasting blood glucose levels [96]. Further, a randomized, 10-week study on eight men with untreated T2D showed diets composed of high-protein and low-carbohydrate foods can potentially improve blood glucose and HbA_{1c} levels, exhibiting an average glucose of 126 mg/dL and 7.6 ± 0.3 HbA_{1c} in the diet group vs. 198 mg/dL glucose and 9.8 ± 0.5 HbA_{1c} in the control group [97]. The MD has also shown potential, reducing blood glucose levels by 3.89 mg/dL in a meta-analysis ($n = 534{,}906$) [16]. Intermittent fasting (500–600 cal/day for 2 nonconsecutive days/week), an increasingly popular eating pattern, can also slightly decrease HbA_{1c} levels in patients with T2D. In a 12-month randomized noninferiority trial, HbA_{1c} lowered by 0.3% but did not show as much of an improvement compared to the continuous restriction diet group (1200–1500 cal/day), which showed a 0.5% reduction [98].

Vitamin D

Vitamin D is a prohormone produced by the kidneys to regulate serum calcium concentration levels and immunological processes. As an essential vitamin it must be acquired externally. The greatest natural source of vitamin D, besides sunlight, is animal products such as dairy, fatty fish (salmon, tuna, etc), and some red meat and cruciferous vegetables. As we transition into a more indoors-oriented society, with 62% of respondents in the Indoor Generation Report ($n = 16{,}000$) spending 15–24 h indoors per day, vitamin D supplementation is becoming increasingly important [99]. The most clinically relevant form of serum vitamin D is 25(OH)D and the reference range is 50 nmol/L to 125 nmol/L.

Since the majority of vitamin D rich foods are derived from animal sources and vitamin D fortified foods are not common, vegans and vegetarians may be at a greater risk of vitamin D deficiency. In fact, the EPIC Oxford Study ($n = 226$ omnivores, 231 vegetarians, 232 vegans) found male vegans, vegetarians, and omnivores ate 0.88 μg/day, 1.56 μg/day, and 3.39 μg/day, respectively. Women had similar results: 0.88 μg/day, 1.51 μg/day, and 3.32 μg/day [100].

In terms of supplementation, Barger-Lux et al. ($n = 116$) found supplementing with the recommended vitamin D3 intake of 10 μg/day (400 IU/day), the equivalent of 10 large eggs or 3 oz. of salmon, raises 25(OH)D by 11 nmol/L [101]. The issue therefore becomes the efficacy and sustainability of acquiring vitamin D from food sources, a concern also brought up in the Adventist Health-2 study, since salmon is expensive and eating ten eggs a day introduces numerous other health risks, namely hypercholesterolemia. Also, as the EPIC Oxford study showed, neither omnivores nor vegetarians/vegans are meeting their recommended daily vitamin D intake, meaning all eating groups have to augment their diet with vitamin D3 supplements to fulfill the recommended dietary intake.

Additionally, vitamin D may possess anti-inflammatory effects against cancer and diabetes, with several meta-analyses indicating vitamin D supplementation lowers cancer mortality rates [102]. In fact, a double-blinded randomized study on vitamin D supplementation and prostate cancer risk ($n = 250$) found 58% of patients in the supplement group vs 49% in the placebo group had a ≥ 50% reduction of prostate-specific antigens (PSA) and a HR of 0.67 ($P = 0.04$) [103]. Also, Mousa et al. ($n = 1270$) found patients with T2D and taking vitamin D supplements had lower levels of CRP (standardized mean difference (SMD) −0.23; $P = 0.002$), tumor necrosis factor α (SMD −0.49; $P = 0.005$), erythrocyte sedimentation rate (SMD −0.47; $P = 0.03$), and higher levels of leptin (SMD 0.42; $P = 0.03$) compared to control groups, highlighting how vitamin D supplementation can mediate chronic inflammation in T2D patients [104]. Further, the Health Professionals Follow-up Study ($n = 18{,}225$ men)

found those with 25(OH)D deficiencies were 2× more likely to develop myocardial infarction than those who had healthy 25(OH)D concentrations (relative risk, 2.42; $P < 0.001$) [105].

The nationwide, randomized, placebo-controlled VITAL Study ($n = 25{,}871$) shows further research is required, however, with vitamin D3 supplementation showing no significant effects on cardiovascular health and cancer risk. For example, the HR between the vitamin D supplementation and placebo group was 0.96 ($P = 0.47$) and incidence of a major cardiovascular event had a hazard ratio of 0.97 ($P = 0.69$) [102]. Michos et al. parallel these findings, suggesting diet and sunlight should be prioritized over supplements for optimizing vitamin D levels [106]. The authors also found calcium supplements can increase one's risk of myocardial infarction and stroke, indicating dietary calcium and physical activity are safer methods of calcium intake.

Vitamin B12 and Folate

While the plant-based diet dramatically improves cardiovascular health, there are limitations of implementing this diet–primarily risks of essential vitamin deficiencies. Vitamin B12, the largest and most complex essential vitamin, is primarily sourced from animal products and is a critical enzyme cofactor involved in the oxidation of odd-numbered fatty acid chains. Additionally, it is neuroprotective and converts homocysteine into nontoxic molecules. B12 deficiency not only leads to neurological damage but also a buildup of homocysteine, which promotes arterial plaque buildup, increasing the risk of atherothrombosis [107, 108]. Folate is another essential vitamin and is critical for the biosynthesis of nucleotide bases involved in amino acid synthesis and metabolism. Folate in its natural form is commonly found and consumed in spinach, nuts, beans, and other leafy green vegetables. In its synthetic form, folic acid, folate can be found in fortified foods such as bread and cereals.

While meats are rich in B12, folate is primarily found in plant-based foods. It therefore comes as no surprise that vegetarians and vegans may have B12 deficiencies since their diets lack the only natural source of B12: meat. Rauma et al.'s analysis of serum B12 concentrations and dietary intakes of living food diet vegans, who follow a strict raw food diet, and omnivores, it was found vegans have significantly ($P < 0.001$) lower average B12 serum concentrations (193 pmol/L) as opposed to omnivores (311 pmol/L). Additionally, the serum concentrations in participants who supplemented their diets with B12 rich foods, such as seaweed, had levels twice as high compared to those who did not supplement, having an average B12 concentration of 221 pmol/L vs 105 pmol/L ($P = 0.025$). It should be noted, however, that this study population is part of a very strict subset of vegans and their B12 levels could be drastically different from average vegans due to dietary differences [109]. In a study on B12 supplementation in 50 vegetarians with B12 deficiency (<150 pmol/L), supplementation was shown to be crucial for vegans in keeping healthy B12 concentrations. By supplementing with 500 μg/day, participants exhibited significant improvements in B12 serum concentration (from 134 ± 125.6 to 379 ± 206.2 pmol/L, $p < 0.0001$) and reductions in plasma homocysteine levels (from 16.7 ± 11 to 11.3 ± 6 μmol/L, $p < 0.01$) [110].

In the EPIC-Oxford study ($n = 689$: 226 omnivores, 231 vegetarians, and 232 vegans), 52% of vegans, 7% of vegetarians, and 1 omnivore were B12 deficient (<118 pmol/L). Consequently, average serum B12 concentrations in vegans were the lowest (122 pmol/L), with vegetarians coming second (182 pmol/L), and omnivores with the average highest concentration (281 pmol/L) ($P < 0.001$). This is, of course, due to plant-based diets lacking natural sources of B12. Also, of the vegans and vegetarians that were not using B12 supplements, 95% and 31% of vegans and vegetarians were failing to meet the recommended daily intake (1.5 μg/day), mirroring the trends found in Rauma et al. Conversely, folate concentrations were highest in vegans (37.5 nmol/L) and lowest in omnivores (20.0 nmol/L), indicating an inverse relationship between folate and B12 ($P < 0.001$) [111]. Schupbach et al.'s study ($n = 206$, 100 omnivores, 53 vegans, 53 vegetar-

ians) corroborates these findings, with 58% of omnivores being deficient in folate (<15 nmol/L, $p < 0.05$) [112].

The MD, a diet rich in fruits, vegetables, and lean meats (fish, poultry, etc.), can mediate B12 deficiency in vegans. In the KIDMED study (n = 3166, 6- to 24-yr-olds), none of the participants were found to have B12 deficiencies however 14.3% of 6- to 14-yr-olds (P = 0.021) and 25.5% of 15- to 24-yr-olds (P = 0.002) were deficient in folate [113]. This is likely due to a lack of MD adherence and greater average consumption of sweet drinks and processed foods found in younger adults [114]. Fortified foods rich in folate and other essential vitamins such as ready-to-eat cereals, however, have been shown to decrease folate deficiency risk ($p < 0.001$) in MD eaters (n = 3534) [115]. Also, Planells et al. (n = 384) showed the MD provided enough B12 (89.1% had acceptable levels) but was moderately successful in mediating folate deficiency (57.6% acceptable) [116]. In a study on the MD and pregnant women (n = 72), however, 70.8% were B12 deficient and none were folate deficient, indicating pregnant women may be a vulnerable population to B12 deficiency [117].

Conclusion

The role and use of biomarkers and lifestyle changes to monitor and treat cardiovascular health and nutrition are of increasing interest among health providers and patients. In this chapter, we reviewed the potential of biomarkers to monitor the impact of lifestyle changes. We also presented data on how plant-based diets and minimal red meat consumption can have positive effects on biomarkers like triglycerides, TMAO, and cholesterol.

While the biomarkers reviewed in this chapter are the most clinically relevant and useful measures for detecting the impact of diets and nutritional therapy, the list of possible biomarkers that could contribute to clinical nutrition is continually evolving.

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