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Review Article

Reducing Cardiovascular Disease Risk in Youth with Type 2 Diabetes Through Dietary Carbohydrate Restriction: Clinical and Molecular Perspectives

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Abstract

Every year, increasing numbers of children and adolescents are diagnosed with type 2 diabetes. Compared to older adults with type 2 diabetes, youth have more difficulty controlling their glycemia and are faced with a much greater risk for cardiovascular disease. Although pharmaceutical options for management of diabetes and its co-morbidities are limited for youth, studies in adults as well as adolescents indicate that a low-carbohydrate diet may be an alternative way to reduce body weight and control blood sugar levels. To assess whether low-carbohydrate diets can also reduce the cardiovascular disease risk that accompanies type 2 diabetes in youth, we discuss the impact of both ketogenic and non-ketogenic low-carbohydrate diets on indicators of cardiovascular disease. This discussion includes not only traditional cardiovascular disease risk markers such as dyslipidemia, but also other factors that are associated with obesity and type 2 diabetes in adolescents such as interleukin-6, C-reactive protein and endothelial dysfunction. We also explore the practicalities involved in adopting and adhering to a low-carbohydrate diet such as the ketogenic diet, discussing its effects on satiety, its cultural adaptability and the types of foods that it emphasizes. The proficiency with which low-carbohydrate diets can reduce excess body weight, improve lipid profiles and attenuate inflammation without restricting calories or decreasing satiety, suggests that they may be an effective tool to reduce cardiovascular disease risk in youth with type 2 diabetes.

Keywords: *Low-Carbohydrate; Ketogenic; Type 2 Diabetes; Adolescents*

Abbreviations

ALA: A-Linoleic Acid; BMI: Body Mass Index; CRP: C-Reactive Protein; CVD: Cardiovascular Disease; DHA: Docosahexaenoic Acid; EFA: Essential Fatty Acids; EPA: Eicosapentaenoic Acid; FMD: Flow-Mediated Dilation; FGF-21: Fibroblast Growth Factor-21; HbA1c: Hemoglobin A1c; HDL: High-Density Lipoprotein; ICAM: Intercellular Adhesion Molecule; IL-1β: Interleukin-1β; IL-6: Interleukin-6; LDL: Low-Density Lipoprotein; LPL: Lipoprotein Lipase; LPS: Lipopolysaccharide; MUFA: Monounsaturated Fatty Acids; PBMC: Peripheral Blood Mononuclear Cell; PUFA: Polyunsaturated Fatty Acids; REE: Resting Energy Expenditure; SFA: Saturated Fatty Acids

Introduction

Type 2 diabetes is becoming increasingly common among children and adolescents [1]. Not only do these youth face challenges controlling their blood sugar levels, but they have a much higher risk for cardiovascular disease (CVD) compared to both healthy youth and older adults with type 2 diabetes [2]. In addition to high blood sugar levels and excess body weight, studies in youth with, or at risk for type 2 diabetes suggest that other risk factors for CVD include dyslipidemia, interleukin-1β (IL-1β), tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), C-reactive protein (CRP), markers of endothelial dysfunction, fetuin-A, fibroblast growth factor-21 (FGF-21), adiponectin, cortisol and leptin (Figure 1) [3-17].

Pharmaceutical treatment options for youth-onset type 2 diabetes and its associated CVD risks are limited, but a few studies suggest that low-carbohydrate diets can help youth manage their diabetes by controlling glycemia and reducing body weight [8,18]. However, it is not clear if low-carbohydrate diets affect other CVD risk factors in this population. The purpose of this review is to discuss the impact of low-carbohydrate diets on dyslipidemia, mediators of inflammation and insulin signaling, and several hormones which regulate these processes, in order to provide more comprehensive insight into the efficacy of these diets as tools to help youth manage the CVD risk that accompanies their diabetes.

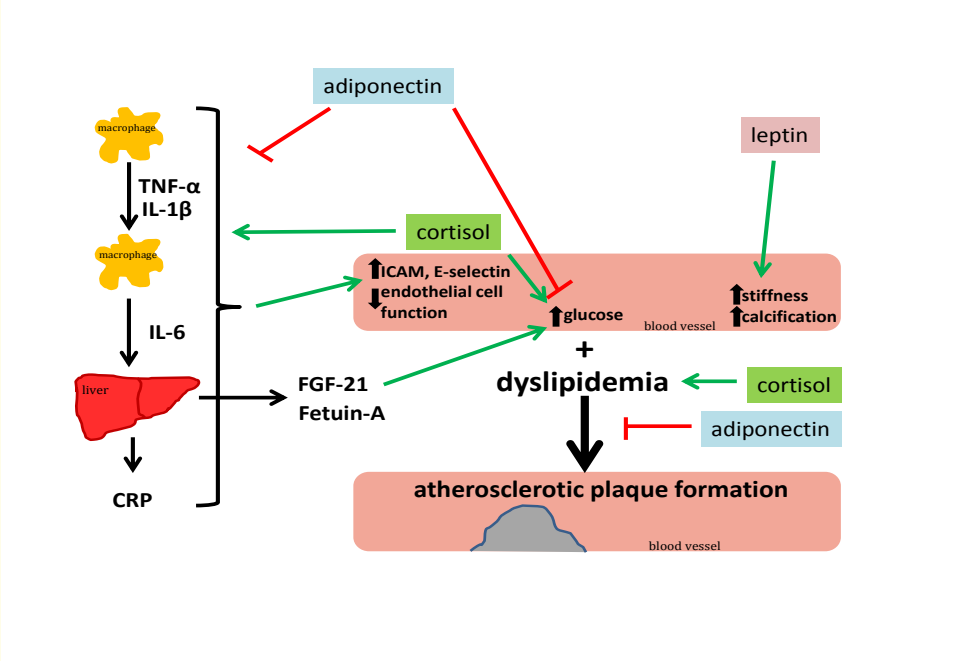


Figure 1: *Mediators of CVD risk in youth with type 2 diabetes. Following a stimulus, immune cells such as macrophages can initiate an inflammatory response by secreting IL-1β and TNF-α. These cytokines can stimulate other immune cells to release IL-6, which stimulates the liver to release CRP. Increased circulating levels of all of these cytokines (in addition to many others) are associated with decreased function of endothelial cells in the vasculature as well as increased expression of adhesion molecules such as ICAM and E-selectin. The liver also secretes FGF-21 (modulates glucose uptake and insulin secretion) and fetuin-A (inhibits insulin receptor signaling) whose high levels are associated with hyperglycemia. High levels of the hormone leptin are associated with increased vascular stiffness and calcification. All of these vascular changes, in combination with dyslipidemia, increase the susceptibility of the vessel to atherogenesis. Similar to leptin, high levels of cortisol increase CVD risk, but do so by augmenting the inflammatory response, increasing fasting glucose levels and contributing to dyslipidemia. Conversely, high levels of adiponectin reduce CVD risk by reducing inflammation and glucose levels and slowing the development of atherosclerotic lesions.*

The rising prevalence of youth-onset type 2 diabetes

"Adult-onset" is not only an antiquated descriptor of type 2 diabetes, but unfortunately incorrect as well. As demonstrated by data from the National Health and Nutrition Examination Survey, between 1999 and 2010, 119,224 adolescents in the U.S. suffered from type 2 diabetes, about one-third of whom were undiagnosed [19]. Concomitant with the 43% increase in new cases of diabetes in adults between 2001 and 2009 [20] (approximately 90% of these cases were type 2 [21,22]), the number of new cases of type 2 diabetes in youth (diagnosed before the age of 20) has increased by over 30% [1]. In fact, a more recent analysis suggests that the prevalence of type 2 diabetes in youth has been increasing by 4.8% per year, and slightly faster among those ethnicities more affected by this epidemic such as

American Indians and Hispanics [23,24]. Excessive diet, sedentary lifestyle and genetic predisposition have all been found to increase risk for type 2 diabetes among older adults as well as adolescents. Furthermore, it has been suggested that maternal obesity and gestational, type 1 or type 2 diabetes in pregnant women can increase the chances of youth-onset diabetes in their offspring by at least 3-fold [25,26].

Both youth and older adults are diagnosed with type 2 diabetes when any 1 of the following criteria are met: hemoglobin A1c (HbA1c) > 6.5%, fasting plasma glucose level ≥ 126 mg/dL, 2 hour plasma glucose level ≥ 200 mg/dL following an oral glucose challenge, or a random plasma glucose level of ≥ 200 mg/dL if the patient presents other symptoms of hyperglycemia such as polyuria, polydipsia and weight loss [27]. No matter the age of the patient, type 2 diabetes can broadly be characterized as a dysfunction of pancreatic β cells that ultimately leads to insulin resistance [28]. However, youth-onset type 2 diabetes distinguishes itself from its manifestation in older adults by the speed at which glycemic control is lost. In youth, β cells are estimated to be damaged at approximately twice the rate they are damaged in adults [29]. This rapid deterioration of the pancreas, combined with physiological insulin resistance that naturally occurs during puberty, may explain why adolescents with type 2 diabetes have more difficulty controlling their glycemia than do older adults [2,30,31].

Cardiovascular disease risk is higher in youth-onset than adult-onset type 2 diabetes

Another major difference between adult-onset and youth-onset type 2 diabetes is that the severity of risk for CVD in the macrovasculature (e.g. aorta, femoral artery, carotid artery) is tremendously greater in youth. In a study that compared risk for diabetic complications between adults who were diagnosed with diabetes before the age of 45 and those who were diagnosed after the age of 45, Hillier and colleagues found that risk for myocardial infarction was increased in type 2 diabetes by about 14-fold in the younger-onset group, whereas diabetes only increased risk by 4-fold in the older-onset group. Patients with younger-onset diabetes had a 30-fold greater risk for cerebrovascular disease compared to their healthy age-matched controls, while the older-onset group had a 3-fold greater risk compared to their age-matched controls [2]. Taking all of these risks into consideration, it is estimated that youth-onset type 2 diabetes can reduce life span by about 15 years, and severely impact quality of life by the time patients reach middle age [32].

Risk factors for CVD associated with youth-onset type 2 diabetes

Beginning work just four days after conception, and not ceasing until death, the heart is one of the most important organs in the human body. This super pump controls the whole circulatory system, from tiny branching capillaries that swaddle tissues in nutrient-exchanging vessels, to hardy arteries and veins that endlessly circulate streams of deoxygenated and oxygenated blood through the body. In type 2 diabetes, these carefully orchestrated networks can incur damage from pathophysiological conditions such as high amounts of sugar, triglycerides, and cholesterol, which are associated with atherogenesis: the accumulation and hardening of plaque in the crevices of blood vessels [33]. In addition to atherogenesis, there are a number of other CVD-related phenotypes that heighten cardiovascular risk in adolescents with type 2 diabetes compared to age-matched, healthy controls including hypertension, arterial stiffness, coronary artery calcification and impaired diastolic and systolic filling. Other CVD risk factors that are more prevalent in youth with, or at risk for, type 2 diabetes than in age-matched healthy controls include obesity, dyslipidemia (a composite of clinical markers in blood including increased levels of triglycerides, total cholesterol and low-density lipoprotein (LDL), and decreased levels of high-density lipoprotein (HDL)), higher levels of the hormones leptin and cortisol and lower levels of the anti-inflammatory hormone adiponectin [3-11]. In addition, youth-onset type 2 diabetes has been associated with higher levels of the pro-inflammatory molecules IL-1β, TNF-α, IL-6, CRP, fetuin-A and FGF-21 [4-9,11-17] (Figure 1).

Several of these CVD biomarkers have been found to be associated with body weight and/or glycemic control, whereas others are seemingly independent of both. West and colleagues compared 106 adolescents with type 2 diabetes with 189 age-matched healthy controls, and found that LDL levels were dependent on HbA1c, a long-term indicator of glycemic control, while levels of HDL and adiponectin were dependent upon body weight. Levels of triglycerides and CRP were dependent on both HbA1c and body weight, while IL-6 and leptin levels were independent of both HbA1c and body weight [7]. In a cohort of Asian Indian adolescents containing 50 lean youth with type 2 diabetes, 50 obese youth with type 2 diabetes, 50 obese youth without diabetes and 100 lean youth without diabetes, triglyceride

levels were associated with HbA1c, whereas both HbA1c and body weight affected levels of LDL, HDL, adiponectin, leptin, and TNF-α [11]. In a comparison of 74 obese adolescents with type 2 diabetes and 74 obese adolescents without type 2 diabetes, Reinehr and colleagues found that FGF-21 and fetuin-A were associated with HbA1c, yet were not affected by administration of metformin or insulin [14]. In 281 obese youth, Syrenicz and colleagues found that CRP, IL-6 and IL-1β levels were associated with insulin resistance, dyslipidemia, HbA1c and a family history of type 2 diabetes [16]. These analyses illustrate two points: 1) Excessive body weight is a strong CVD risk factor, both independently and via its contribution to type 2 diabetes. Reduction of excessive body weight may be an excellent way to reduce CVD risk in youth both with and without type 2 diabetes. 2) Glycemia and adiposity are important risk factors, but they are not responsible for all CVD risk. Therefore, approaches that may restore circulating inflammatory molecules such as IL-6 and fetuin-A to normal levels, even if they do not affect glycemia and/or body weight are worth considering as alternative therapies.

Consistent with the increased levels of pro-inflammatory molecules circulating in patients with type 2 diabetes, experiments using peripheral blood mononuclear cells (PBMC) isolated from both non-diabetic and type 2 diabetic youth showed that patients with type 2 diabetes elicit a more exaggerated inflammatory response. When stimulated with the endotoxin lipopolysaccharide (LPS) or the fatty acid palmitate, monocytes from youth with diabetes produced more TNF-α and IL-1β than monocytes from youth without diabetes. Surprisingly, the highest amount of IL-1β was not secreted from diabetic monocytes in response to the highest dose of LPS, but rather in response to an intermediary level of LPS. This suggests that diabetes may increase the sensitivity of immune cells to invading pathogens, thus only a weak stimulus is needed to elicit an inflammatory response [15].

High levels of circulating pro-inflammatory cytokines and other potential stimuli tend to inflict damage upon the very blood vessels that they travel in. Shortly after the endothelial cells that line the blood vessel succumb to the harm imposed by the circulating toxin(s), an inflammatory response will ensue in which additional cytokines such as intercellular adhesion molecule (ICAM) and E-selectin are released from the endothelial cells (Figure 1). These adhesion molecules increase the adherence and penetration of immune cells in the endothelium to hopefully resolve the initial insult. However, if the inflammatory response persists uncontrollably, not only can endothelial cells become impaired (in a process called endothelial dysfunction), but excess immune factors can accumulate and potentially develop into an atherosclerotic lesion. The lesion can damage the endothelium to which it is attached and impede blood flow through the vessel [34]. One aspect of endothelial function that is used in the clinic to assess cardiovascular health is maintenance of vascular tone, measured by flow-mediated dilation (FMD). FMD measures the extent to which blood flow increases through the brachial artery when a cuff is released on a patient's forearm [35]. The few studies in which endothelial dysfunction was measured in youth with or at risk for type 2 diabetes have mixed results, but overall suggest that ICAM and E-selectin may be associated with obesity and risk for type 2 diabetes [36-40]. Furthermore, FMD is impaired in severely obese adolescents and those with type 2 diabetes compared to healthy age-matched controls [41-43].

Pharmaceutical options and efficacy of exercise regimens may be limited for youth with type 2 diabetes

Metformin and insulin are the only approved medications for youth with type 2 diabetes. This dearth of treatment options available to youth further exacerbates the dangers associated with their predisposition to uncontrollable glycemia and high CVD risk. FDA approval of additional drugs can only follow clinical trials, and due to specific restrictions of these trials as well as limited access to participating medical facilities, it is estimated that only about 2% of all youth with type 2 diabetes are involved in clinical trials [31]. Currently, these trials are testing a number of different types of medications that can be taken alone or with metformin, such as sulfonylureas (Glimepiride), DPP4 inhibitors (Saxagliptin), GLP1 receptor agonists (Lixisenatide, Liraglutide) and SGLT2 inhibitors (Dapagliflozin) [44]. While these medications may offer youth better control of their diabetes, several studies with metformin highlight the difficulty in getting youth to adhere to any prescription schedule. In a 6-month trial of metformin use in adolescents with type 2 diabetes (MOCA study), 29% of participants in the placebo group and 26% in the metformin group were lost to follow-up [45]. This suggests that metformin's gastrointestinal side effects didn't play the only role in decreasing adherence, but that youth may have difficulty following any prescription schedule, potentially reducing the efficacy of any drug.

The TODAY study followed adolescents with type 2 diabetes over almost five years, as researchers compared subjects' ability to achieve glycemic control using either metformin alone, metformin + lifestyle intervention, or metformin + rosiglitazone (a thiazolidinedione). The lifestyle intervention included guidance on adhering to a calorie-restricting diet (to lose weight), as well as an emphasis on physical activity. During the first 6 months, when adherence was the highest, the best glycemic control was achieved in the metformin + rosiglitazone group, followed by the metformin + lifestyle intervention group, followed by the metformin only group (although there was no statistical difference between the metformin only group and the metformin + lifestyle intervention group). In the metformin only and metformin + rosiglitazone groups, adherence was high at the beginning of the study (both at 84% in month 8), but by month 60, they dropped to approximately 57% and 60%, respectively. Adherence in the metformin + lifestyle group ranged from 78% in year 1 to about 68% in year 5. As a result of this collective decrease in adherence, there was no difference between the groups in glycemic control after the first 6 months [46]. The metformin + rosiglitazone group did achieve the best glycemic control in the first half year, but these drugs did not improve other markers of CVD risk such as LDL, triglycerides and HDL levels. Only one biomarker, small dense LDL levels, decreased over the first 36 months of the study – the remaining lipid risk factors increased in all 3 treatment groups. While not statistically significant, the metformin + lifestyle intervention group was the only group to show a decrease in the inflammatory molecule CRP and had the smallest increase in triglycerides [5]. This suggests that alteration of dietary and exercise practices (perhaps aided by the improved long-term adherence in this treatment arm compared to others) may offer some cardiovascular benefit, at least comparable to that of a second medication in addition to metformin.

Lifestyle interventions have both exercise and dietary components. Several studies have reported a number of metabolic benefits of exercise in youth at risk for developing type 2 diabetes [47,48], but additional evidence suggests that this population may be especially resistant to such benefits. Perhaps related to Whalley's findings that heart function in adolescents with diabetes is abnormal [3], Nadeau and colleagues found that youth with type 2 diabetes have decreased cardiopulmonary fitness that could lead to less exercise capacity than their healthy age-matched counterparts [49]. Even after at-risk youth adhere to an exercise training program, work by Hatunic and colleagues suggests that their metabolism may not reap the expected benefits. They found that after a 3-month training program, there was no change in cardiovascular fitness or inflammatory markers among youth with type 2 diabetes. Among age-matched obese subjects with normal glucose metabolism, cardiovascular fitness increased, but there was no change in inflammatory markers [50]. Therefore, given the possible limitations of both pharmaceuticals and exercise regimens in attenuating the CVD risks associated with type 2 diabetes in youth, dietary interventions may be a more effective alternative for reducing disease risk in this population.

Low-carbohydrate and ketogenic diets

In contrast to established dietetic dogma, recent studies have suggested that diets that are low in carbohydrate content can help older adults with type 2 diabetes control glycemia and reduce risk for co-morbidities such as CVD [51-53]. There is also limited evidence suggesting that these diets may be effective in both controlling glucose and body weight in patients with youth-onset type 2 diabetes [8,18]. One type of low-carbohydrate diet that is particularly effective for these outcomes is known as the ketogenic diet.

To this day, the Inuit of the northern hemisphere, who are exposed to some of the harshest conditions known to man, maintain a low-carbohydrate diet composed of 80 - 85% fat and 15 - 20% protein [54]. While these Natives may not have had a particular choice in selecting their dietary regimen given the barren terrain they inhabit, it turns out that what may be thought of as an "unhealthy" high-fat diet offers them significant CVD protection [55,56], possibly due to the high levels of omega-3 polyunsaturated fatty acids (PUFA) present in their predominantly marine diets [57]. In addition to these fatty acids, the high-fat diet of the Inuit is also very low in carbohydrate content. High-fat, very low-carbohydrate diets, also known as ketogenic diets, are well-known for their use in treatments for epilepsy [58], but may possess therapeutic potential for type 2 diabetes [59] and CVD [60] as well.

With its ratio of 77-80% fat, 15% protein, and 5 - 8% carbohydrate [58], the ketogenic diet stimulates the metabolic effects of fasting by forcing the body to use fat, instead of carbohydrates, as a source of glucose. In this state of fasting or starvation, the body provides the central nervous system with glucose by breaking down muscle tissue into amino acid precursors to be used in gluconeogenesis [61]. Since

the body could not endure such deteriorating conditions for too long, it proceeds to utilize an alternative, fat-based, anti-catabolic fuel source – ketone bodies – to spare muscle protein of degradation [61]. The body metabolizes fat stores through lipolysis and β-oxidation into ketones such as acetoacetate, β-hydroxybutyrate, and acetone. These by-products of fatty acid metabolism can serve as precursors (instead of glucose) in the generation of adenosine triphosphate [58].

Studies show that the capacity of ketone bodies to serve as an alternative fuel source is crucial in preventing epilepsy and other neurological disorders, since ketones can transverse the blood-brain barrier [62] and power the brain, which cannot utilize fatty acids for energy when glucose levels fall below homeostatic concentrations [63]. In fact, ketone metabolism has even been shown to have advantages over glucose metabolism, namely by improving metabolic efficiency, reducing the production of reactive oxygen species, increasing antioxidant capacity [64], and supplying up to 60% of the brain's energy needs [65,66]. A large part of this capacity has to do with the speed with which ketones are metabolized. Unlike glucose, which goes through an 11-step metabolic pathway that includes glycolysis, ketone bodies are processed in fewer steps. This allows them to enter the tricarboxylic acid cycle much faster than glucose and be converted into adenosine triphosphate almost instantly [64].

As a result of low glucose levels, insulin, which increases lipogenesis and decreases lipolysis, remains very low during nutritional ketosis [54,67,68]. This facilitates a mobilization of fat stores that can contribute to a decrease in body weight [61]. Other changes induced by nutritional ketosis include improved insulin sensitivity and signaling and increased energy expenditure [69-74], which are associated with significant drops in HbA1c, body fat mass, body weight and triglycerides [69-72,75]. Data in experimental models also suggests that ketogenic diets exert anti-inflammatory effects possibly through the actions of adenosine, peroxisome proliferator-activated receptor–α and reactive oxygen species [76-83]. The decreases in body weight and body fat (without negatively affecting muscle strength) and inflammation that can be achieved through the ketogenic diet suggest that this may be an invaluable tool for overweight adolescents, who – pressured by various sociological factors – may otherwise revert to more dangerous weight loss methods (e.g. extremely low-calorie diets, deliberate dehydration, saunas etc.) that can potentially impair heath, physiological function, water balance, electrolytes, glycogen and lean body mass [61].

Low-carbohydrate diets decrease the use of diabetes medications in adults

A number of studies in adults with type 2 diabetes indicate that low-carbohydrate diets can often reduce body weight and improve glycemia and lipid profiles more quickly and effectively than observed with low-fat diets. One indicator of such efficacy is a patient's reduced need for diabetes medications. Although there aren't any clinical studies in youth addressing this question, in adults, low-carbohydrate diets have been found to reduce patients' reliance on diabetes medications [84-88]. In a study comparing the effects of a low-carbohydrate (20% carbohydrate (C), 50% fat (F), 30% protein (P)) and a high-carbohydrate diet (50 - 60% C, 25 - 30% F, 15% P) for a total of 44 months, it was found that in as little as 6 months, 23 obese patients with type 2 diabetes who maintained a low-carbohydrate regimen could reduce their body weight and blood glucose levels. Furthermore, 5 of the patients who were originally taking sulfonylurea for their diabetes were able to completely discontinue their medication and 3 of the 11 patients that were taking insulin (60 ± 33 IU/d) were also able to discontinue medication. Among those patients who still required insulin, they were able to lower their medication requirements to 18 ± 11 IU/d after only 6 months on the low-carbohydrate diet. Interestingly, 2 patients who were able to discontinue insulin treatment on the low-carbohydrate diet resumed after increasing their carbohydrate intake again, suggesting that carbohydrate intake had a strong influence on glycemia [89]. In a study involving 21 overweight males with type 2 diabetes, Yancy and colleagues found that 16 weeks on a ketogenic diet (< 20 g/d carbohydrate) decreased HbA1c, body weight and fasting serum triglyceride levels. As a result, diabetes medications were discontinued in 7 participants and reduced in 10 participants, a notable achievement considering that participants were buying and preparing their own meals and not restricting calories [59]. A 24-week study of 363 obese adults (28% of whom had type 2 diabetes) showed that a low calorie ketogenic diet (LCKD) (intake of < 20g/d carbohydrate) improved body weight and glycemia and reduced antidiabetic medications to about half of initial quantities. Among patients with type 2 diabetes in the study, the LCKD reduced triglyceride levels, and among patients with and without diabetes, the LCKD reduced total cholesterol levels and increased HDL levels

compared to the standard low-calorie diet. The LCKD did not affect LDL levels [90]. These studies in adults indicate that in addition to reducing dyslipidemia, low-carbohydrate diets often concurrently reduce body weight and control glycemia. All together, these changes reduce the need for diabetic medications, suggesting that low-carbohydrate diets may be an effective treatment alternative for populations that don't respond well or have access to pharmacological treatments, such as youth.

Low-carbohydrate and ketogenic diets in the reduction of type 2 diabetes-associated CVD risk in youth

As previously discussed, there are a number of factors that contribute to an increased risk for CVD among youth with type 2 diabetes, in addition to glycemia and body weight. These important CVD risk factors not only include the "established" risk factors such as high levels of triglycerides and low levels of HDL, but also include factors such as LDL particle size, IL-1β, TNF-α, IL-6, CRP, markers of endothelial dysfunction, fetuin-A, FGF-21, adiponectin, cortisol and leptin (Figure 1). This review's systematic evaluation of the impact of low-carbohydrate diets on each of these parameters will address the therapeutic potential of these diets and perhaps validate their use for the reduction of CVD risk in youth with type 2 diabetes. We will also discuss satiety, as it plays a large role in whether subjects can reap the potential benefits of a diet and is a crucial factor in determining the feasibility of a dietary recommendation. (While it is not clear exactly why adherence to the lifestyle intervention in the TODAY study was not high [46], it may have been due to the low satiety of its calorie-restricted low-fat diet.) We will discuss both non-ketogenic and ketogenic low-carbohydrate diets. While "ketogenic" diets are typically comprised of approximately 80% fat, 15% protein and 5% carbohydrate with a ratio of fat:carbohydrate+protein in the range of 2:1 – 4:1 [58], this term has been applied to diets with slightly higher carbohydrate contributions as well. Similarly, "low-carbohydrate" has often been used as a relative term by researchers and can refer to diets in which anywhere from 10 - 50% of energy comes from carbohydrate. Therefore, in our discussion, the macronutrient composition (% energy) will be specified for each study to help elucidate the contribution (or lack thereof) of carbohydrate to the study outcome. For each biomarker, we will focus on available clinical or observational data in studies of obese or diabetic youth, but when these studies are not available, we will also include some studies of patients with, or at risk for, adult-onset diabetes and studies using experimental animals that highlight relevant diet-biomarker relationships. We will conclude with a discussion about the feasibility and food choices that are involved in following such diets.

Dyslipidemia

Dyslipidemia is a state of abnormal blood lipid levels that increases risk for CVD. It is characterized by elevated levels of total cholesterol (> 240 mg/dL), LDL (> 160 mg/dL) and triglycerides (> 150 mg/dL) and reduced levels of HDL (< 40 mg/dL) in circulation. Unlike LDL and triglycerides that increase risk for CVD by directly contributing to the formation of atherogenic plaque, HDL is inversely associated with CVD risk because of its actions in removing excess LDL from arteries [91]. More recently, the term "dyslipidemia" has come to also include an increase in the numbers of small, dense LDL particles that are more atherogenic than larger, often heavier particles [92-97].

In a study involving 120 obese adolescents, Ibarra-Reynoso and colleagues found that those consuming a low-carbohydrate diet (50% C, 30% F, 20% P) for 2 months experienced a decrease in body mass index (BMI) and triglycerides, while levels of total cholesterol, LDL and HDL cholesterol increased. Adolescents that consumed a low-fat diet (60% C, 25% F, 15% P) exhibited similar changes in BMI and HDL but showed a decrease in LDL levels [98]. Such an effect of a low-carbohydrate diet on triglyceride levels was confirmed in a smaller study by Casazza and colleagues. Twenty-six overweight or obese African American girls were either randomized to a eucaloric low-carbohydrate diet (42% C, 40% F, 18% P) for 5 weeks, followed by a calorie-restricted diet with the same macronutrient profile for 11 weeks, or randomized to a low-fat diet (55% C, 27% F, 18% P) for 16 weeks. Both groups lost the same amount of weight, but only the low-carbohydrate group experienced a significant drop in triglyceride levels [99]. The effects of low-carbohydrate diets on HDL and triglycerides that are described in these interventions are consistent with several cross-sectional studies in children. In an analysis of 1234 white children, among 13 - 19 year old boys, sucrose intake was inversely related to HDL and the ratio of HDL/total cholesterol. In the same study, among girls aged 6 - 12 years, sucrose intake was positively associated with triglyceride levels [100]. Similar findings regarding sucrose intake were reported in another study of 949 children (ages 6 - 19), where it was inversely related to HDL levels and positively associated with triglycerides [101].

The potential cardiovascular benefits of a low-carbohydrate ketogenic diet (LCKD) (8% C, 59.6% F, 32.4% P) was studied by Sondike and colleagues over a 12-week period in 16 overweight adolescents. The study showed that compared to a low-fat diet (56.1% C, 12.3% F, 31.6% P), overweight adolescents on the LCKD experienced more weight loss, despite consuming more calories. This demonstrates that adolescents on a LCKD can lose considerable amounts of weight without specific calorie, fat, or cholesterol restrictions, possibly making the diet easier to follow. In fact, even though it may seem counter-intuitive, the authors suggested that the increased caloric content of the LCKD actually contributed to that group's weight loss because of increased palatability and better maintenance of metabolic rate [102]. (Supporting this relationship between ketogenic diets and metabolic rate, a study by Srivastava and colleagues showed that a eucaloric ketogenic diet significantly increased brown adipose tissue mass in mice. Within the brown adipose tissue, the ketogenic diet also induced the formation of larger mitochondria and higher levels of uncoupling protein 1. These changes may explain why the mice fed the ketogenic diet *ad libitum* did not gain weight like their chow-fed counterparts, since brown adipose tissue expends energy in the form of heat due to uncoupling of UCP1 within the mitochondria [103]). Sondike and colleagues also found that the LCKD was associated with greater improvements in non-HDL cholesterol levels, a greater increase in HDL levels and greater reductions in triglyceride levels than the low-fat diet. Meanwhile, adolescents on the low-fat diet experienced a decrease in total cholesterol and LDL levels (LDL did not change on the LCKD) [102].

Overall, these studies are consistent with those in both healthy adults and adults with type 2 diabetes, showing that consumption of a low-carbohydrate diet reduces serum triglyceride levels and increases HDL levels – 2 changes that could significantly reduce CVD risk in adolescents. The effect of low-carbohydrate diets on levels of LDL is less consistent among at-risk adolescents and adults with type 2 diabetes. Some studies indicate that low-fat diets are more effective in lowering LDL levels and low-carbohydrate diets may actually increase LDL levels in the blood [98,102]. While increased concentrations of LDL levels in the blood may be considered to be pro-atherogenic, it is actually the size of the LDL particle that is more closely associated with CVD risk, rather than the concentration. Small, dense LDL particles confer more of a risk because they are more likely to get lodged in the vessel wall and contribute to an atherosclerotic lesion. Larger LDL particles have less atherogenic risk, even though these particles may contribute to an increased LDL concentration [93,95-97]. There is very little information available about the effects of low-carbohydrate diets on LDL particle size in youth, but an analysis of 50 children of parents who had small LDL particles indicated that consumption of a low-fat diet (75% C, 10% F, 15% P) slightly increased CVD risk by increasing the relative percentage of small, dense LDL in their blood [104].

In adults, several studies have shown that low-carbohydrate diets are associated with increased LDL particle size (and therefore decreased CVD risk). Stoernell and colleagues followed 28 hypertriglyceridemic men and women for 8 weeks on either a low-carbohydrate (15% C, 55-65% F, 20-30% P) or low-fat (50-60% C, 30% F, 15% P) diet. The concentration of small, dense LDL decreased in those on the low-carbohydrate diet, while it increased in those on the low-fat diet [105]. In another study of patients with hypertriglyceridemia, 19 men followed a high-fat, reduced-carbohydrate diet (43% C, 39% F, 18% P) for 3 weeks. They were then switched to a low-fat diet for three weeks (54% C, 28% F, 18% P). While on the reduced-carbohydrate diet, LDL particle size increased and total LDL concentration decreased. On the low-fat diet, there was no change in LDL particle size [106]. In a third study, 39 adults were randomized to either 12 weeks of a restricted-carbohydrate diet (20 - 25% C, 45 - 50% F, 15 - 20% P) or 6 weeks of this diet followed by 6 weeks of a low-fat diet (55% C, 30% F, 15 - 20% P). Those that stayed on the restricted-carbohydrate diet for 12 weeks had a higher LDL concentration than those that switched to the low-fat diet, but they had fewer medium and small, dense LDL particles, suggesting that they were at a reduced risk for CVD [107].

The effect of the ketogenic diet (8% C, 61% F, 30% P) on lipoprotein particle size was evaluated by Sharman and colleagues in a 6-week study on 20 healthy men. After 6 weeks, LDL and oxidized LDL levels remained unaffected by the ketogenic diet, but LDL particle size increased. The ketogenic diet also increased HDL levels and decreased both fasting and postprandial levels of triglycerides compared to subjects on a standard diet (59% C, 25% F, 15% P) [60]. This ability of the ketogenic diet, specifically one rich in monounsaturated fatty acids (MUFA) and PUFA, to increase the rate of triglyceride clearance may be due to its induction of plasma lipoprotein lipase (LPL) activ-

ity [108]. (LPL helps reduce the accumulation of triglycerides in the blood vessels by hydrolyzing triglyceride molecules into free fatty acids (FFA) and monacylglcyerol, which can then be utilized by the body in a tissue-specific manner [109]).

All together, the data suggest that in youth at risk for type 2 diabetes, consumption of a low-carbohydrate diet may reduce CVD risk by attenuating dyslipidemia. In as little as 3 weeks, subjects may reduce the number of small, dense LDL particles in their blood. Furthermore, adhering to a low-carbohydrate diet for at least 2 months will also likely reduce body weight and total cholesterol levels and increase levels of HDL. Overall, these benefits were observed in diets whose carbohydrate content ranged from 8-50%, but the lower the carbohydrate amount, the sooner the lipid profile may improve.

Interleukin-1β

When an invading pathogen binds to an immune cell such as a macrophage, a protein complex called the inflammasome converts the cytokine interleukin-1β (IL-1β) into its active form [110]. IL-1β can then stimulate the release of additional pro-inflammatory cytokines, such as interleukin-6, from other types of immune cells to mount an inflammatory response against the pathogen [111]. In obesity, macrophage-derived IL-1β production in insulin-sensitive organs has been found to lead to the progression of insulin resistance, and in type 2 diabetes, IL-1β induces the destruction of insulin-producing β cells within pancreatic islets [112]. Due to its pro-inflammatory actions, IL-1β has been associated with atherosclerosis in both experimental animal models and in humans [113]. In obese adolescents, serum IL-1β has been shown to correlate with insulin resistance and a family history of type 2 diabetes [16], and a comparison of PBMC from youth with and without type 2 diabetes revealed that PBMC from diabetics produce more IL-1β in response to LPS [15].

IL-1β has not been analyzed in dietary interventions in youth, but in adults with type 2 diabetes, consumption of a low-carbohydrate diet (< 20g/d carbohydrate) decreased expression of the IL-1β receptor [114], suggesting the reduction of carbohydrates may reduce circulating IL-1β levels. Ketogenic diet interventions remain to be performed, but in an *in vitro* study with human monocytes, β-hydroxybutyrate and acetoacetate, ketone bodies produced in subjects following a ketogenic diet, inhibited inflammasome activation, effectively down-regulating inflammation by reducing IL-1β secretion [115]. In a study of rats with gout (chronic inflammatory arthritis), Goldberg and colleagues found that feeding them a high-fat, low-carbohydrate ketogenic diet for 1 week increased levels of β-hydroxybutyrate which inhibited the inflammasome and reduced gout flares. These investigators also found that β-hydroxybutyrate blocked inflammasome activation and IL-1β secretion in neutrophils of mice as well as humans [116]. Dupuis and colleagues likewise found that a ketogenic diet (1.6% C, 90% F, 8.4% P) for 2 weeks was associated with lower IL-1β levels in blood and mRNA levels in the brain following an LPS injection than rats fed a standard diet (65.4% C, 11.1% F, 23.5% P). Lowering of IL-1β, along with other anti-inflammatory properties of the ketogenic diet also helped reduce the fever induced by LPS in these rats [117]. These preliminary studies are a promising foundation for future investigations into whether low-carbohydrate diets can limit IL-1β secretion in youth with type 2 diabetes.

Tumor necrosis factor-α

Tumor necrosis factor-α (TNF-α) is a pro-inflammatory cytokine synthesized by immune cells that, similar to IL-1β, helps mount an inflammatory response by stimulating the secretion of other pro-inflammatory cytokines. TNF-α is secreted in higher amounts in patients with type 2 diabetes, and contributes to endothelial cell damage that precedes atherogenesis [118]. In a comparison of Asian Indian adolescents who were either lean, lean with type 2 diabetes, obese or obese and type 2 diabetes, TNF-α levels were increased in both groups of diabetics, regardless of body weight [11]. When Rempel and colleagues isolated PBMC from healthy youth and those with type 2 diabetes, they found that when stimulated with LPS, the PBMC from the youth with diabetes produced more TNF-α [15].

Although there are no intervention data for youth regarding the effects of low-carbohydrate diets on TNF-α levels, several analyses have been conducted in adults and experimental animals. After 12 weeks on a low-carbohydrate diet (13% C, 60% F, 27% P), Wood and colleagues found that the BMI and TNF-α levels of 29 overweight men decreased [119]. Data from a crossover study in overweight or obese adult men found a similar relationship between TNF-α and BMI since both factors decreased after both a 6-week intervention on

a ketogenic diet (10% C, 60% F, 30% P) and a low-fat diet (55% C, 25% F, 20% P) [120]. However, when 10 healthy women followed these same diets for only 4 weeks each, TNF-α levels did not change [121]. TNF-α receptor levels were measured in a 6-month study of 30 adults with type 2 diabetes who followed either a low-carbohydrate diet (25% C, 49% F, 23% P) or a low-fat diet (49% C, 29% F, 20% P). Both diets reduced body weight and did not affect levels of the TNF-α receptor 1. However, there was a slight increase in expression of TNF-α receptor 2 ($p = 0.06$) after 6 months of the low-fat diet [114]. In a rat study, Dupuis and colleagues found that a ketogenic diet (1.6% C, 90% F, 8.4% P) for 2 weeks decreased TNF-α levels following an LPS injection more so than rats fed a standard diet (65.4% C, 11.1% F, 23.5% P) [117]. While the results are mixed, it appears that longer-term low-carbohydrate diet interventions, which may be more effective in reducing body weight, may be required to affect TNF-α levels in youth who are at risk for diabetes.

Interleukin-6

Interleukin-6 (IL-6) is a cytokine produced by immune and vascular cells that participates in both pro-inflammatory and anti-inflammatory signaling. Often stimulated by other cytokines such as IL-1β and TNF-α, its pro-inflammatory actions in endothelial cells, adipose tissue, liver, skeletal muscle and pancreas have been implicated in CVD [122]. Consistent with findings in adults with type 2 diabetes, Syrenicz and colleagues found that in obese adolescents, IL-6 levels were associated with insulin resistance and family history of type 2 diabetes [16]. Although there haven't been any studies in youth that analyzed IL-6 levels in response to a low-carbohydrate diet, a study by Goletzke and colleagues indicates that increased carbohydrate consumption during puberty (ages 9 - 14) is associated with increased IL-6 levels in these same individuals once they become adults (ages 18 - 36) [123]. Consistent with this relationship between IL-6 and carbohydrates, evidence from adult interventions suggests that excess IL-6 levels may be attenuated by a low-carbohydrate diet that decreases body weight.

Bladbjerg found that in obese adults, those following a low-carbohydrate diet (40 - 50% C, 35 - 45% F, 5 - 25% P) for 6 months experienced a decrease in body weight as well as IL-6 levels [124]. Similarly, a crossover study in overweight or obese adult men found that IL-6 levels and body weight decreased after 6 weeks of both a ketogenic diet (10% C, 60% F, 30% P) and a low-fat diet (55% C, 25% F, 20% P) [120].

However, a number of studies have also reported no effects of a low-carbohydrate diet on IL-6 levels. In a 6-month study of adults with type 2 diabetes, both a low-carbohydrate diet (25% C, 49% F, 23% P) and a low-fat diet (49% C, 29% F, 20% P) resulted in a similar amount of weight lost. IL-6 levels increased in those on the low-fat diet, while it decreased slightly (albeit non-significantly) in those on the low-carbohydrate diet [114]. IL-6 was not affected by either a low-fat diet (60% C, 20% F, 20% P) or a moderate carbohydrate diet (45% C, 37% F, 18% P) when 30 women consumed each for a period of 6 weeks [125] Similarly, a crossover study comprised of 4 weeks of a low-carbohydrate diet (10% C, 60% F, 30% P) followed by 4 weeks of a low-fat diet (55% C, 25% F, 20% P) in 10 healthy women did not alter IL-6 levels [121]. Nor did IL-6 levels change in a 6-week intervention of healthy adults who were given either a low-carbohydrate diet (46% C, 36% F, 18% P) or a low-fat diet (64% C, 18% F, 18% P) [126]. A 12-week study of 40 overweight individuals with atherogenic dyslipidemia found that IL-6 levels did not change on either a low-carbohydrate (12.4% C, 58.9% F, 28.1% P) or low-fat (23.8% C, 55.8% F, 19.6% P) diet [127]. An absence of an effect of low-carbohydrate diets on IL-6 levels in these studies is not surprising since the low-carbohydrate diets did not significantly affect body weight. However, there was a 3-month low-carbohydrate intervention (13% C, 60% F, 27% P) in 29 overweight men that did result in a decrease in body weight without affecting IL-6 levels [119].

A few studies have also reported that low-carbohydrate diets increase IL-6 levels. In a study of 40 overweight adults with high triglycerides, half consumed a hypocaloric (1500 calories) low-carbohydrate diet (12% C, 59% F, 28% P) while the other half consumed a hypocaloric low-fat diet (56% C, 24% F, 20% P). IL-6 levels increased in both diet groups after 3 months, but there was less of a change in the low-carbohydrate group, the group that lost more weight [128]. Finally, a 4-week study involving 29 overweight women found that those on either a low-carbohydrate diet (24% C, 59% F, 18% P) or a low-fat diet (58% C, 12% F, 30% P) increased their IL-6 levels, despite losing weight [129]. It is not entirely clear why IL-6 levels increased in these studies, and if it was in fact related to the diet intervention. Comparison of those studies in which there was no change in IL-6 levels versus those in which IL-6 levels decreased suggests that further research that focuses on the effects of low-carbohydrate diets on IL-6 should plan interventions for at least 6 months.

C-reactive protein

C-reactive protein (CRP) is a member of the innate immune system that is made in the liver in response to the secretion of other pro-inflammatory cytokines such as IL-6. CRP is positively associated with visceral adiposity and inflammation since adipocyte-mediated cytokines drive its synthesis [130]. High levels of CRP have also been associated with insulin resistance and CVD in adults as well as adolescents [9,16,131]. There haven't been any studies in adolescents with obesity or type 2 diabetes that analyzed the effects of a diet intervention on CRP levels, but there have been a number of these studies in adults that provide a wide variety of results.

Four studies in overweight and obese adults suggest that CRP levels can be effectively diminished by low-carbohydrate diets in which weight loss occurs. Noakes and colleagues found that 8 weeks of a very low-carbohydrate diet (4% C, 61% F, 35% P) decreased weight as well as CRP in obese individuals [68], and Wood and colleagues found that 29 overweight men on a low-carbohydrate diet (13% C, 60% F, 27% P) for 12 weeks decreased their body weights and CRP levels [119]. In a study done by Seshadri and colleagues, it was found that among 48 severely obese subjects with a high-risk baseline level of CRP (> 3 mg/dL) those on a low-carbohydrate diet (32% C, 43% F, 25% P) experienced a greater decrease in CRP levels than those on a conventional diet (50% C, 33% F, 16% P) over a 6-month period, even after adjusting for weight loss [132]. A similar effect was observed by Ruth and colleagues, who found that while body weight and composition were similarly affect by a low-fat, high-carbohydrate diet (55.7% C, 25% F, 22% P) and a high-fat, low-carbohydrate diet (9.6% C, 56% F, 33.5% P) for 3 months, only the low-carbohydrate diet group experienced decreases in CRP levels [133].

Several studies have also suggested that changes in CRP levels are not dependent on carbohydrate content of diet. Tay and colleagues found that changes in CRP are not specific to low-carbohydrate diets. After 24 weeks on calorie-reducing diets that were either low-fat (46% C, 30% F, 25% P) or very low-carbohydrate (4% C, 61% F, 35% P), both groups experienced weight loss as well as a reduction in CRP levels [134]. In the POUNDS trial, overweight and obese adults were randomized to one of 4 diets: (65% C, 20% F, 15% P); (55% C, 20% F, 25% P); (45% C, 40% F, 15% P); and (35% C, 40% F, 25% P). At both 6 months and 24 months after beginning the diets, all groups decreased body weight and CRP. Interestingly, even during periods of weight regain, CRP levels remained low [135]. Similar observations were made in the DIRECT study, in which CRP levels decreased steadily in all diet groups (low-fat, low-carbohydrate, Mediterranean) even as all diet groups experienced long periods of weight loss and short periods of weight regain. Compared to the low-fat diet, the low-carbohydrate diet group did experience a larger decrease in both body weight and CRP levels [136]. O'Brien and colleagues found that among 41 obese women following either a low-carbohydrate (15% C, 57% F, 28% P) or a low-fat diet (54% C, 28% F, 18 % P) for 3 months, there was no significant difference in changes in CRP level between the diet groups, but CRP levels were associated with changes in body weight [137]. A similar relationship between CRP and body weight was found in a crossover study using a ketogenic diet. Volek and Sharman followed 15 overweight or obese men who consumed a low-fat diet (55% C, 25% F, 20% P) for 6 weeks, followed by a ketogenic diet (10% C, 60% F, 30% P) for another 6 weeks. Body weight as well as CRP levels decreased significantly during both diet periods [120].

All of these studies suggest that changes in CRP may be dependent on body weight, yet in a 6-month dietary intervention with 131 obese individuals, Bladbjerg and colleagues found that a lower carbohydrate diet that emphasized MUFA (40 - 50% C, 35 - 45% F, 5 - 25% P), a low-fat diet (57.6% C, 23.6% F, 15.8% P), and a control diet (49.8% C, 32.1% F, 15.9% P) all caused increases in BMI and decreases in CRP. Contrary to the above-mentioned studies, in this 6-month study, participants could eat *ad libitum*, which may explain why there was no weight loss and was even a slight weight gain. The decrease in CRP among all diet groups suggests that all of the diets offered some metabolic benefit that decreased the patients' inflammatory profiles [124].

There are also a number of studies in adults indicating that low-carbohydrate diets have negligible effects on CRP levels. A 6-month intervention using a low-carbohydrate diet (20% C, 50% F, 30% P) in patients with type 2 diabetes induced a small decrease in CRP levels, but these changes were statistically insignificant [114]. A 12-week study of 40 overweight individuals with atherogenic dyslipidemia found that CRP levels did not change on either a low carbohydrate (12.4% C, 58.9% F, 28.1% P) or low-fat (23.8% C, 55.8% F, 19.6% P) diet [127]. Many of the short-term diet interventions also showed no effect on CRP levels. Two crossover studies in women showed no effect

of either a low-fat or low-carbohydrate diet on CRP levels. The first involved 30 overweight women who followed a low-fat diet (60% C, 20% F, 20% P) for 6 weeks, then switched to a moderate-carbohydrate diet (45% C, 37% F, 18% P) for an additional 6 weeks [125]. The second consisted of 10 healthy women who followed a low-carbohydrate diet for 4 weeks (10% C, 60% F, 30% P) followed by 4 weeks of a low-fat diet (55% C, 25% F, 20% P) [121]. Neither of these crossover studies resulted in significant changes in body weight. Similarly, in an 8-week low-carbohydrate diet (15% C, 55-65% F, 20 - 30%P) or low-fat diet (55% C, 30% F, 15% P) intervention in adults with hypertriglyceridemia, differences between the diet groups in weight change or CRP level change were not significant. However, the CRP level decreased marginally in the low-carbohydrate group whereas it increased marginally in the low-fat group, consistent with the slightly greater decrease in body weight in the low-carbohydrate group [105]. In a 6-week intervention of healthy adults, there was no difference in body weight or CRP levels between those randomized to a low-carbohydrate diet (46% C, 36% F, 18% P) and those on a low-fat diet (64% C, 18% F, 18% P) [126]. Following a 6-month intervention of either a low-carbohydrate diet (34% C, 43% F, 23%P) or a low-fat diet (48% C, 31% F, 21% P) in adults with type 2 diabetes, Davis and colleagues found that both groups lost the same amount of weight, but only the low-fat group showed a decrease in CRP levels [138].

Overall, these studies suggest that weight loss is the primary driver of decreases in CRP levels in patients with obesity and diabetes. Many studies show that this can be achieved with a low-carbohydrate diet, particularly one with < 20% energy from carbohydrates that has a relatively long duration (>6 months). These types of diets are most likely to result in the most dramatic weight loss. However, some studies have shown similar effects using low-fat diets that also achieved significant weight loss. Many diet interventions that did not alter CRP levels also did not alter body weight, and the inclusion of relatively insulin-sensitive individuals in some of these studies could also mask changes in CRP levels because CRP does not necessarily change when insulin-sensitive individuals lose weight [139].

Markers of endothelial dysfunction

Adhesion molecules such as ICAM and E-selectin exacerbate the inflammatory response by recruiting circulating immune cells and cholesterol particles to the endothelium where they may not only damage endothelial cells but may become incorporated into an atherosclerotic plaque. Increased secretion of adhesion molecules and deterioration of endothelial function are more commonly observed in patients with type 2 diabetes [140]. Youth at risk for type 2 diabetes have been shown to have elevated levels of the adhesion molecules ICAM and E-selectin and also have impaired endothelial function, as assessed by FMD, compared to healthy age-matched controls [36-43].

Low-carbohydrate diet interventions in youth have yet to analyze markers of endothelial dysfunction, but in adults with type 2 diabetes, the available research suggests that these diets do in fact improve certain markers of endothelial cell health. In adults with type 2 diabetes, Davis and colleagues found that 6 months of a low-carbohydrate diet (5% C, 75% F, 20% P) decreased levels of ICAM and E-selectin [138]. ICAM levels also decreased following 6 weeks of a ketogenic diet (10% C, 60% F, 30% P), as they did following 6 weeks of a low-fat diet (55% C, 25% F, 20% P) in overweight or obese adult men [120]. ICAM and E-selectin levels decreased after only 6 weeks of a low-carbohydrate diet (11% C, 58% F, 28% P) among 21 overweight adults taking statins. In this same study, Ballard and colleagues found that FMD didn't increase in those on the low-carbohydrate diet, but peak forearm blood flow, another indicator of endothelial health, was improved [141]. FMD has been observed to increase following 12 weeks of a hypocaloric low-carbohydrate diet (12% C, 59% F, 28% P) in overweight hypertriglyceridemic men and women. Those in this study that followed a hypocaloric low-fat diet (56% C, 24% F, 20% P) for 12 weeks experienced a decrease in FMD [128]. In a 6-week study with 30 overweight or obese women, both a high-carbohydrate diet (60% C, 20% F, 18% P) and a moderate-carbohydrate diet (45% C, 37% F, 18% P) decreased E-selectin levels [125]. Amidst these studies showing varying amounts of endothelium improvement with low-carbohydrate diets, there is a study with 40 overweight individuals with atherogenic dyslipidemia in which levels of ICAM and E-selectin did not change following 12 weeks of either a low-carbohydrate (12.4% C, 58.9% F, 28.1% P) or low-fat (23.8% C, 55.8% F, 19.6% P) diet [127]. Despite this null result, the majority of the studies suggest the low-carbohydrate diets may offer significant improvements in endothelial function, which would be worth investigating in youth with type 2 diabetes.

Fetuin-A

Fetuin-A (alpha-2-HS-glycoprotein) is a multifunctional glycoprotein that is secreted from hepatocytes [142]. Other than being an indicator of hepatic lipid accumulation [143], studies have found that fetuin-A can induce inflammation and impair insulin sensitivity by inhibiting insulin receptor tyrosine kinase activity [144-147]. Since regulation of blood glucose and insulin levels requires efficient signaling of the insulin receptor, it is not surprising that in humans, increased levels of fetuin-A have been associated with type 2 diabetes in adults as well as adolescents [14,148]. Although a cross-sectional study of 558 healthy adults found no clear association between fetuin-A levels and a single specific macronutrient [143], fetuin-A has been shown to be associated with increased weight, blood pressure and insulin resistance and decreased HDL levels, suggesting that it may also be a marker for CVD [149,150].

Only 2 low-carbohydrate intervention studies have analyzed fetuin-A levels, but they both suggest that a composite of factors influence fetuin-A. In a 1-year intervention for obese youth at risk for metabolic syndrome, participants followed either a high-carbohydrate diet (55% C, 30% F, 15% P) in conjunction with an exercise program and counseling session or a standard, lower-carbohydrate diet (49% C, 38% F, 13% P). At baseline, there was no difference in fetuin-A levels between lean controls and obese subjects, but at the conclusion of the study, there was a decrease in fetuin-A levels among those that lost weight, regardless of which diet they followed. Interestingly, fetuin-A levels did not change upon administration of metformin or insulin, suggesting that glycemia alone doesn't alter fetuin-A levels [151]. In the DIRECT study, obese adults with and without diabetes all lost a significant amount of weight during the first 6 months of the study, regardless of whether they were following a low-fat (50% C, 31% F, 19% P), Mediterranean (50% C, 32% F, 18% P) or low-carbohydrate (41% C, 38% F, 21% P) diet. All three groups then experienced a slight regain in weight, which was still significantly lower than their baseline. Despite this fluctuation, fetuin-A levels in all three groups decreased steadily over the course of the entire 2-year study. Coincident with the initial decline in body weight was a rise in HDL levels that continued even after some weight was re-gained, suggesting that rising HDL levels may have influenced the steady decline of fetuin-A levels [152]. Additional diet intervention studies in youth with type 2 diabetes are needed, but these two studies suggest that while any diet intervention that reduces weight may also improve fetuin-A levels, low-carbohydrate diets may be particularly suited for this task given their role in not only reducing excess body weight but increasing levels of HDL.

Fibroblast Growth Factor-21

Fibroblast growth factor-21 (FGF-21) is a protein secreted by the liver, skeletal muscle and adipose tissue that modulates glucose uptake in these same tissues. It is also secreted by pancreatic β-cells where it regulates insulin secretion. During ketosis, FGF-21 plays a critical role in metabolic fuel homeostasis, working in concert with peroxisome proliferator-activated receptor-α to oxidize fatty acids into ketones [153,154]. Impairment of FGF-21 production during ketosis can result in a decrease in hepatic ketone output, lipemia (the presence of high levels of emulsified fat in the blood) and possibly fatty liver [153]. Consistent with this need for FGF-21 during nutritional ketosis, Badman and colleagues found that FGF-21 was markedly induced in livers of mice fed a very low-carbohydrate ketogenic diet (0.76% C, 78.9% F, 9.5% P) for 30 days compared to mice fed standard chow (38.2% C, 6.5% F, 23.5% P) [153]. Pharmacological doses of FGF-21 in experimental animal models of obesity and diabetes result in improvements in insulin sensitivity and blood lipid profiles, suggesting that obese humans may be FGF-21 resistant [155,156]. Nevertheless, FGF-21 levels remain positively associated with body weight and type 2 diabetes in adults as well as adolescents [14,157].

In a 2-month diet intervention, Ibarra-Reynoso and colleagues found that both a low-fat diet (60% C, 25% F, 15% P) and a low-carbohydrate diet (50% C, 30% F, 20% P) reduced body weight and FGF-21 levels in obese adolescents, although the magnitude of change in FGF-21 was non-significantly greater in those on the low-carbohydrate diet [98]. It is difficult to draw a general conclusion about FGF-21 in youth from this 1 study, especially since the difference in carbohydrate content between these two diets is small. However, the reduction of both weight and FGF-21 levels is consistent with other studies that have suggested a relationship between these two factors [14,157].

Further insight into how low-carbohydrate diets may affect FGF-21 is found in several animal studies. The KKAy mouse is an animal model of diabetes that resembles youth-onset type 2 diabetes in that these mice develop diabetes early in life (by 6 weeks of age). Handa and colleagues found that KKAy mice fed a low-carbohydrate diet (38% C, 37% F, 25% P) exhibited slightly lower (albeit non-significant) levels of serum FGF-21 than mice fed standard chow (63% C, 22% F, 15% P). However, mice fed a very low-carbohydrate diet (18% C, 37% F, 45% P) had significantly lower levels of FGF-21. Importantly, there was no difference in body weight between these groups [158]. Similar findings were reported in a study using C57BL/6 mice (an all-purpose mouse model that is not diabetic) in which FGF-21 levels were highest in mice fed a high-carbohydrate diet (84% C, 10.5% F, 5.2% P) followed by a moderate-carbohydrate diet (75% C, 10.5% F, 14.5% P), followed by a low-carbohydrate diet (34.9% C, 10.5% F, 54.6% P) for 3 weeks. Similar to the KKAy mouse study, there was no difference in body weight between the diet groups. Additional studies in rat hepatocytes suggested that the low FGF-21 levels observed in the low-carbohydrate diet group were due to the low levels of glucose, rather than the high levels of protein [159]. This trend of decreasing carbohydrate intake with decreasing FGF-21 levels from these animal studies suggests that FGF-21 levels are not dependent on body weight, but rather are closely related to carbohydrate content. Such compelling data certainly warrant an investigation into this relationship in youth with type 2 diabetes.

Adiponectin

Adiponectin is a hormone produced by adipose tissue and typically secreted into circulation in amounts proportional to lean mass. Low adiponectin levels are associated with increased risk for CVD, due in part to its positive associations with insulin sensitivity, lipolysis and endothelial function, and negative associations with oxidative stress, inflammation and atherosclerotic plaque development [160-163].

Only two studies have looked at adiponectin levels in obese adolescents in the context of a low-carbohydrate diet. Neither of these studies suggests a strong relationship between this diet and adiponectin levels, likely because the carbohydrate content of the "low-carbohydrate" diets was actually quite high and did not substantially alter body weight, a key determinant in adiponectin levels. In a 1-year dietary intervention, 16 normal weight and 37 obese adolescents were randomized to either a low-fat diet (55% C, 30% F, 15% P) or a low-carbohydrate diet (49% C, 38% F, 13% P). Among those subjects that lost substantial weight (BMI changed from 26.0 to 22.7), adiponectin levels increased significantly. However, among the subjects that did not lose weight, adiponectin levels didn't change. Interestingly, the macronutrient intake profile of those who lost weight more closely resembled the low-fat diet, rather than the low-carbohydrate diet [164]. In another study of 120 obese adolescents that were randomized to either a low-fat diet (60% C, 25% F, 15% P) or low-carbohydrate diet (50% C, 30% F, 20% P) for 2 months, those on the low-carbohydrate diet experienced a slight, but statistically insignificant rise in adiponectin levels [98]. This is not surprising because in the low-carbohydrate group, BMI only decreased slightly (27.62 to 26.41) and percent body fat did not change. A cursory look at these two studies might suggest that low-carbohydrate diets have negligible effect on adiponectin levels. However, in each of these studies, "low-carbohydrate" was defined as approximately 50% energy intake, which is on the high-end of a "low-carbohydrate" diet. While additional studies are needed to determine the effect of low-carbohydrate diets on adiponectin in youth, evidence from studies in adults suggests that diets composed of < 50% carbohydrate can effectively increase adiponectin levels.

DIRECT was a 2-year diet intervention which consisted of moderately obese men (ages 40 - 65), about 12% of whom had type 2 diabetes. After 2 years on a low-carbohydrate (41% C, 38% F, 21% P) diet, the adiponectin levels of these men rose dramatically. Compared to those randomized to a low-fat (50% C, 31% F, 19% P) or Mediterranean diet (50% C, 32% F, 18% P), those on the low-carbohydrate diet experienced the largest increase in adiponectin and also the most weight lost (approximately 5 kg) [152]. A shorter study in obese subjects that were randomized to either a low-carbohydrate diet (9.6% C, 56% F, 33.5% P) or a low-fat diet (60% C, 25% F, 15% P) (both of which were hypocaloric by 500 calories/d) showed that after 3 months, only the low-carbohydrate group increased their adiponectin levels, even though both groups lost the same amount of weight [133]. Finally, in a 6-week intervention of healthy adults, those randomized to a low-carbohydrate diet (46% C, 36% F, 18% P) and those on a low-fat diet (64% C, 18% F, 18% P) experienced similar decreases

in both body weight and adiponectin over the course of the study [126]. Together, these studies suggest that reduction of dietary carbohydrate (i.e. either a slight reduction to 41% over 2 years, or a sharp reduction to 9.6% over 3 months) may increase adiponectin levels. Most of the available data suggests that a large part of this effect is due to weight loss and may be more readily observable in those who are overweight or obese, such as youth with type 2 diabetes.

Cortisol

Cortisol is a hormone that helps regulate glucose metabolism and inflammation in the body's response to stress. It has been shown that altered cortisol regulation patterns are strongly linked to the metabolic syndrome, a cluster of symptoms including increased waist circumference, hypertension, dyslipidemia and high fasting glucose levels. In general, studies have found that in the metabolic syndrome, the enzyme 11β-HSD, which converts cortisone (inactive) to cortisol (active), is underactive in liver tissue and overactive in fat tissue, thereby resulting in high rates of cortisol clearance and a low rate of regeneration, which can lead to a number of negative health effects [165-170]. For example, in overweight Latino youth, low cortisol levels have been associated with increased fasting glucose levels, pancreatic β cell dysfunction and insulin resistance [17,171].

There have not been any intervention studies in youth, but studies in adults suggest that a very low-carbohydrate diet may be effective in increasing serum cortisol levels. In a crossover study of 6 healthy young men (avg. age of 25), there was no change in cortisol levels following either 2 weeks on a low-fat diet (62% C, 31% F, 7% P) or 2 weeks on a low-carbohydrate diet (46.5% C, 46.5% F, 7% P) [172]. Although the carbohydrate content of this "low-carbohydrate" diet is relatively high, another crossover study using a much lower carbohydrate diet (10% C, 60% F, 30%P) in 8 obese young adults for 4 weeks also found no change in cortisol levels [173]. Similarly, Volek and colleagues followed 8 healthy men on a ketogenic diet with an even lower carbohydrate content (8% C, 61% F, 30% P) for 6 weeks, and found that cortisol levels did not change [174].

However, when the carbohydrate content was lowered even further with another ketogenic diet (4% C, 66% F, 30% P), cortisol levels increased. In a study conducted by Stimson and colleagues, obese men were placed on either a ketogenic diet (4% C, 66% F, 30% P) or on a moderate-fat, moderate-carbohydrate diet (35% C, 35% F, 30%P). Both diets allowed subjects to feed *ad libitum*. In the ketogenic diet group, metabolic syndrome patterns were significantly reversed, with blood cortisol levels increasing, clearance decreasing, and regeneration increasing. These observations were due to an increase of 11β-HSD1 activity in liver tissue on the ketogenic diet, an outcome that did not occur in the moderate-fat, moderate-carbohydrate diet group, despite similar weight loss effects between groups [175]. Together, these data suggest that if youth respond similar to adults, low-carbohydrate diets with approximately 4% carbohydrate may increase cortisol levels.

Leptin

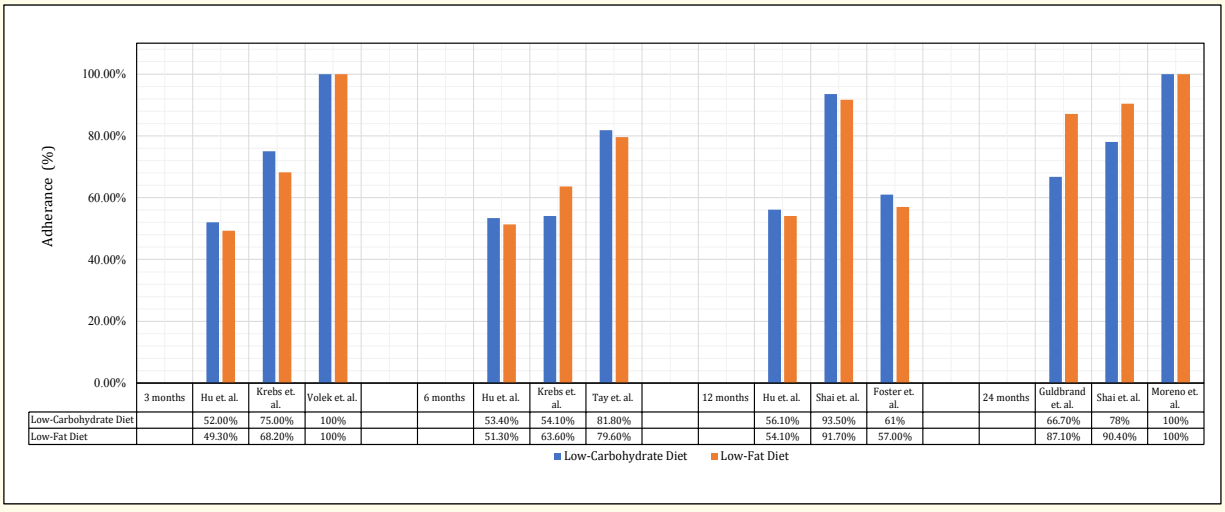
Leptin is a hormone produced by adipose tissue that targets the hypothalamus for the purpose of regulating appetite. In healthy individuals, higher levels of leptin are typically associated with higher fat mass and induce satiety to decrease appetite. A complete absence of leptin has been shown to lead to obesity in both humans and animal models [176,177]. Studies in mice have suggested that while weight loss is often accompanied by a decrease in leptin (due to a decrease in fat mass), successful maintenance of the lower weight may be associated with a subsequent increase in leptin levels (to curb appetite) [178,179]. For the most part though, studies in humans have shown that obese subjects usually have higher leptin levels than lean controls, suggesting that some individuals experience leptin resistance – a condition in which they have the same amount of leptin as lean individuals, but do not experience its satiating effects [180]. While the molecular mechanism by which leptin resistance occurs is under investigation, evidence suggests that independent of its effects on body weight, high levels of leptin are still an independent risk factor for CVD in healthy adults, adults with type 2 diabetes, and youth with type 2 diabetes [11,14,181-183] in part due to their association with calcification of vascular cells [184] and arterial stiffness [185].

Two studies in obese adolescents have analyzed changes in leptin levels in the context of different diets, and they support the notion that leptin levels are chiefly regulated by fat mass. Ibarra-Reynoso and colleagues found that among 120 adolescents, 2 months of a low-

carbohydrate diet (50% C, 30% F, 20% P) or low-fat diet (60%C, 25% F, 15% P) resulted in a decrease in BMI as well as leptin [98]. In a long-term intervention, Reinehr and colleagues randomized 16 normal weight and 37 obese adolescents to either a low-fat diet (55% C, 30% F, 15% P) or a low-carbohydrate diet (49% C, 38% F, 13% P). After 1 year, leptin levels decreased only in those who lost weight, regardless of the diet they were following [164]. Thus, a diet that decreases weight, regardless of its macronutrient distribution is likely to decrease leptin levels in adolescents.

Factors that affect adherence to low-carbohydrate diets

The potential benefits of low-carbohydrate diets on the various CVD biomarkers discussed thus far can only be manifest if subjects adhere to the diet. While low-carbohydrate diets may be restrictive, especially considering the popularity of high-carbohydrate foods, this diet may be more appealing than other diets because its metabolic benefits can be realized without caloric restriction [102]. Compilation of data from long-term interventions (3 - 24 months) with low-carbohydrate diets reveals that up to about 12 months, low-carbohydrate diets have approximately the same or slightly better adherence levels than low-fat diets, whereas at the 2-year time-point, low-fat diets have better adherence (Figure 2).



	3 months	Hu et. al.	Krebs et. al.	Volek et. al.		6 months	Hu et. al.	Krebs et. al.	Tay et. al.		12 months	Hu et. al.	Shai et. al.	Foster et. al.		24 months	Guldbrand et. al.	Shai et. al.	Moreno et. al.
Low-Carbohydrate Diet		52.00%	75.00%	100%			53.40%	54.10%	81.80%			56.10%	93.50%	61%			66.70%	78%	100%
Low-Fat Diet		49.30%	68.20%	100%			51.30%	63.60%	79.60%			54.10%	91.70%	57.00%			87.10%	90.40%	100%

Figure 2: *Adherence levels for low-carbohydrate and low-fat diets over a 2-year period. Comparison of adherence levels between low-carbohydrate and low-fat diets used in long-term intervention studies [134,152,223-228] indicates that low-carbohydrate diets do not have lower adherence levels than low-fat diets. In fact, low-carbohydrate diets may have slightly greater adherence during the first 12 months, while at the 24-month time-point, low-fat diets may have better adherence levels. The studies depicted in this graph include both adolescent and adult populations. Blue bars represent low-carbohydrate diets and orange bars represent low-fat diets.*

The increased adherence observed on the low-carbohydrate diets at these early time-points is likely due to several physiological factors, one of which is satiety, or the degree of fullness someone feels after consuming a food or meal. There is only 1 long-term low-carbohydrate intervention in which satiety was measured in subjects at risk for CVD. Among 28 hypertriglyceridemic adults randomized to either a low-fat (55% C, 30% F, 15% P) or a low-carbohydrate (15% C, 50 -60% F, 25 - 35% P) diet for 8 weeks, there was no difference in satiety or weight loss between the diet groups [105]. Needless to say, additional long-term studies are needed to verify this finding. However, there are an abundance of studies exploring the effects of macronutrients on short-term appetite regulation, some of which have been conducted in youth – the population that is the focus of this review.

Zandstra and colleagues studied the effect of macronutrient composition on satiety among three different age groups: children (ages 4 - 6), young adults (ages 18 - 26) and elderly adults (ages 61 - 86). Subjects were given either a hypocaloric (157 calories) control yogurt (54% C, 2% F, 45% P), high-fat yogurt (17% C, 71% F, 12% P) of 457 calories, high-carbohydrate yogurt (87% C, 1% F, 14% P) of 447 calories or moderate-fat moderate-carbohydrate yogurt (53% C, 42% F, 7% P) of 729 calories. In all 3 age groups, only after consumption of the moderate-fat, moderate-carbohydrate yogurt did subsequent energy intake decrease (suggesting satiety), which is not surprising given its increased caloric density. Following the high-carbohydrate yogurt, children actually ate more at subsequent meals, while the elderly ate less. Interestingly, young adults reported a significant reduction in appetite following consumption of each yogurt, compared to those who didn't consume any yogurt. However, in the elderly, there was very little difference in appetite between those who didn't consume any yogurt and those who consumed any of the test yogurts, indicating that regulation of appetite changes upon aging [186]. These data suggest that a satiating diet that can offer metabolic benefits (potentially a low-carbohydrate diet) may be more effective (due to its effects on appetite) when consumed by young adults rather than older adults.

To further explore the effects of macronutrients on satiety, Misra and colleagues studied the effects of a high-carbohydrate (60 - 65% C, 15 - 20% F, 15 - 20% P) high-fat (15 - 20% C, 60 - 65% F, 15 - 20% P) and high-protein (15 - 20% C, 15 - 20% F, 60 - 65% P) breakfast on satiety hormones in lean and obese adolescent girls (ages 12 - 18). Ghrelin is a hormone secreted by the stomach that is highest just before a meal and thought to contribute to meal initiation through its appetite-increasing effects. In adults, ghrelin levels decrease following consumption of carbohydrate and fat and increase following consumption of protein. PYY is a hormone secreted by the gut upon digestion of food and triggers signaling in the hypothalamus that increases satiety. Unlike adults, Misra found that in obese adolescents, ghrelin levels increased following a high-carbohydrate meal. In lean girls, PYY levels increased as expected following consumption of the high-fat meal, but no increase was observed in obese girls [187]. Together, these data indicate that obesity alters appetite hormone regulation in adolescents. In obese girls, minimal satiety followed carbohydrate-rich meals (since both ghrelin and PYY were dysregulated), while a more moderate level of satiety (albeit reduced compared to lean girls) was found following a fat-rich meal.

In a study of healthy lean men, those that consumed a low-carbohydrate meal (15% C, 55% F, 30% P) reported more satiety (based on scores of hunger and fullness) than those that consumed a high-protein meal (30% C, 25% F, 45% P), a high-carbohydrate meal (60% C, 30% F, 10% P) or an adequate-protein meal (40% C, 30% F, 30% P). However, obese men that consumed the same meals found that the high-protein diet induced the most satiety. In lean participants, the smallest amount of energy consumed at a subsequent meal was in the high-protein meal < low-carbohydrate meal < high-carbohydrate meal < adequate-protein meal. In obese participants, the least energy was consumed following the high-protein meal < adequate-protein meal<low-carbohydrate meal < high-carbohydrate meal [188]. Similar to these lean men, in another study of 16 healthy lean men, consumption of a snack high in protein (44% C, 24% F, 29% P) or fat (42% C, 40% F, 14% P) reduced energy intake at subsequent meals, whereas consumption of a high-carbohydrate (60% C, 24% F, 14% P) snack had no effect [189]. Among 12 healthy young women (ages 20 - 37), consumption of a low-carbohydrate, high-protein liquid meal (9.5% C, 19.2% F, 71.5% P) resulted in reduced subsequent energy intake compared to high-carbohydrate (55.1% C, 9.6% F, 35.7% P) and very-high carbohydrate (99% C) liquid meals [190]. Another way to measure appetite is through oral habituation, or the progressive decline in food consumption as the same item is repeatedly presented. Myers and colleagues found in 40 healthy women, that consumption of a high-fat, low-carbohydrate yogurt (25% C, 61% F, 14% P) resulted in quicker habituation, and perhaps more satiety than consumption of a low-fat, high-carbohydrate yogurt (65% C, 14% F, 21% P) [191].

Supporting this notion of higher fat content leading to more satiety are data that show that fullness-associated activation of the amygdala (the reward center of the brain) was greater in healthy adults that consumed a meal with a higher fat content [192]. The intricacies of how the rewarding nature of certain foods may influence intake was explored further in a study focusing on the emotions that surround eating habits. Lemmens and colleagues found that post-meal energy intake did not differ overall between healthy young adults (avg. age = 25, avg. BMI = 25) who consumed a low-carbohydrate (5% C, 30% F, 65% P) and a high-carbohydrate lunch (64% C, 30% F, 6% P). However, when separating the participants based on their degree of food disinhibition, it was found that among those with

high disinhibition (those more susceptible to "mindless" eating) post-meal energy intake was lower following the low-carbohydrate diet [193]. Since disinhibited eaters are more likely to be overweight and obese, these data suggest that low-carbohydrate diets may be an effective strategy to curb hyperphagia. Overall, these short-term studies suggest that compared to low-fat diets, low-carbohydrate diets induce more satiety, especially in obese subjects. Youth, more so than older adults, may be more sensitive to this response, enabling them to maintain such diets. Thus, low-carbohydrate diet regimens in youth with type 2 diabetes may be more successful in reducing CVD risk than when implemented in older adults.

Other lifestyle improvements, such as regulation of stress, exercise, and sleep, will likely only further enhance the already positive effects rendered by low-carbohydrate diets alone. In a non-conventional study done by Saslow and colleagues, it was found that individuals with type 2 diabetes improved their glycemic control and lost more weight after being randomized to a very low-carbohydrate ketogenic diet (VLCKD) (20 - 50 g/d carbohydrate) and a lifestyle online program (involved increasing mindfulness, setting attainable goals, moderation in eating, increased and regular physical activity, and sleep) for 32 weeks instead of the low-fat American Diabetes Association "Create Your Plate" diet online program. Since the intervention was conducted online, without any stringent supervision, participants had de facto more leniency in adhering to the diet as they pleased, making the effectiveness of the VLCKD in lowering HbA1c, body weight, and triglyceride levels without any in-person monitoring even more noteworthy. While a few participants had trouble adhering to the VLCKD, by the end of the trial, 92% of participants remained adherent to the intervention, perhaps because they were seeing such rapid results. In the control group, many of the 54% of participants resigned because of perceived inefficacy of the diet. In fact, Saslow and colleagues found that participants on the VLCKD diet rated themselves as less likely to cheat on their assigned diet, compared to participants in the control group. The investigators suggested that the intervention group's high rating was not only due to the VLCKD being easier to adhere to (more palatable), but also because extra supports were included in the intervention program [194].

Alleviating concerns about the use of ketogenic diets in youth

A possible concern regarding the ketogenic diet as a diabetes treatment in adolescents is safety, specifically regarding adequate nutrition intake. In a novel study conducted by Tagliabue and colleagues which focused on evaluating the nutritional status, resting energy expenditure (REE), and substrate oxidation of a ketogenic diet (4:1 ratio of fat:protein+carbohydrate) in 18 children with refractory epilepsy over a 6-month period, the study found that besides improving seizure control, the ketogenic diet increased fat oxidation, decreased the respiratory quotient, and did not change REE. The investigators found that height, weight, and BMI z-scores after 6 months on the ketogenic remained roughly constant and at healthy levels compared to baseline values, a reassuring fact considering that one of the major concerns of the ketogenic diet is whether or not the diet can provide adequate nutrition for proper development in children and adolescents [195]. Vining and colleagues also show in their study that the ketogenic diet generally provides sufficient nutrition to children older than 3 years to maintain growth within normal parameters over a defined period [196]; however, both studies suggest that careful monitoring is required for very young children on the ketogenic diet, since they are more susceptible to growth complications than older children and adolescents [197].

Another safety concern over using the ketogenic diet to treat youth-onset diabetes is the potentially higher protein content of the diet. However, since the ketogenic diet is actually a moderate-protein diet, with daily amounts ranging at about 1.2 - 1.5 grams of protein per kg of body weight [61,198], studies find that it does not contribute to harmful renal effects associated with nitrogen excretion and protein metabolism [199]. In fact, studies in mice even show that a ketogenic diet can cause regression of diabetic nephropathy due to reduced glucose metabolism and increases in ketone body production [200].

Finally, nutritional ketosis caused by the ketogenic diet results in a mild release of fatty acids and ketones which are not enough to cause adverse acidosis reactions. In fact, the concentration of ketone bodies never rises above 8 mmol/L on the ketogenic diet [201], which is much lower than the > 25 mmol/L levels observed in diabetic ketoacidosis [53]. Nevertheless, careful monitoring for diabetic patients on a ketogenic diet is required.

Cultural adaptability of low-carbohydrate diets

The ability of low-carbohydrate diets, including the ketogenic diet, to render beneficial effects without caloric restriction makes this diet an especially promising remedy for physiological abnormalities such as obesity and its associated metabolic disorders (e.g. metabolic syndrome and type 2 diabetes) that are becoming increasingly common. These conditions have become serious burdens on the health-care systems of various countries, who may not always be able to finance costly medicines or treatments for afflicted citizens [84]. The worldwide use of the ketogenic diet attests to the notion that despite its limit on carbohydrates, this diet can be incorporated palatably into a large variety of cultural cuisines. In a study done by Kossoff and McGrogan, it was found that 73 academic centers in 41 countries outside of the United States – from Europe to Africa to Asia – are implementing the ketogenic diet, with 16 countries offering the diet in more than one center. The mean duration of the diet was found to be 8 years, with 71.6 patients enrolled (per country) in the diet to date (2005), and 5.4 new patients enrolled annually. While difficulties with the diet included avoiding carbohydrates (particularly rice, in Asian countries), and adopting a higher fat-to-protein and carbohydrate ratio (4:1), overall, cultural and religious issues were not limiting, and patients, especially those in underprivileged countries, were highly motivated to try and maintain the diet [202].

When adapting to the ketogenic diet, families and individuals from around the world have taken various approaches to incorporate the ketogenic diet into their specific religious and cultural practices. For instance, in spite of carbohydrates (such as pasta and bread) being a major staple in their diet, Italian patients did not have trouble eliminating such foods. Likewise, with appropriate guidance, French patients did not have trouble excluding high-carbohydrate foods, such as pastries, from their diet. In Turkey, the only restriction to eating the ketogenic diet was a religious abstinence from pork, while in Israel, many families and patients were willing to try the ketogenic diet because of a traditional reserve towards medicine usage; however, their only issue with consuming a ketogenic diet were religious prohibitions from mixing meat and milk [203]. Furthermore, after parents of epileptic children in Saudi Arabia experienced the benefits of a ketogenic diet firsthand, instead of reverting back to traditional practices such as going to the Sheikh to read the Quran, or consuming black onion seeds, they developed a positive attitude towards the scientific ways of treating epilepsy [204]. Ultimately, Asian countries found it most difficult to comply with the ketogenic diet, as they are naturally pre-disposed to eating high-carbohydrate foods such as rice, dumplings, and noodles. In order to mitigate the restrictiveness of a ketogenic diet, most Asian countries implement lower ratios of fats to carbohydrate (2:1), or use an Atkins diet, which seems to work just as successfully [202,205].

Overall, the study showed that with communal support and encouragement, the ketogenic diet can be made into an effective and even tasteful diet. For instance, in Taiwan, parents, relatives, and friends of epileptic patients have come together to create a ketogenic diet cookbook containing traditional recipes and local foods, including sushi. Furthermore, Kossoff and McGrogan found great similarities among ketogenic diets worldwide, which may encourage the creation of an international cookbook. In fact, many epilepsy centers implementing the ketogenic diet are already coalescing into a tight-knit global community, providing one another with advice, ideas, and support as to how the ketogenic diet can be made successful, and in the meantime, delectable, for their patients [202].

Ketogenic foods

Familiarization with a low-carbohydrate diet, personal motivation and community support are all key factors in helping adolescents adapt to this lifestyle [194,206]. In fact, low-carbohydrate diets such as the ketogenic diet may be easier to adapt to than expected since one of the diet's hallmarks is the absence of calorie restriction [102]. In a study done by Amari and colleagues, it was found that 29 epileptic children (ages 2 - 17) expressed higher preferences for fatty foods such as butter, cream cheese and bacon, than carbohydrates [207]. However, investigators did acknowledge the possibility that these youth may display different food choices outside of the experimental setup [207,208].

Ultimately, one of the first steps that can be taken towards adopting a low-carbohydrate diet is acquainting oneself with a list of preferred foods, and understanding that gradual steps, such as first reducing carbohydrates to just 30 percent of total caloric intake, are essentially what will render the greatest health benefits in the long-term. Since the most beneficial effects of low-carbohydrate diets on CVD

risk factors in youth with type 2 diabetes are likely to be observed in those diets with the smallest carbohydrate content, we will discuss some details regarding the framework of the ketogenic diet. Following another type of low-carbohydrate diet would involve similar considerations, albeit with less restriction on the amount of carbohydrate consumed. Since the ketogenic diet is predominantly focused on fat intake (about 80% of the diet is composed fat [58]) our discussion will begin with the types of fat that should be included in the diet.

Sources of medium chain fatty acids or medium chain triglycerides, such as unrefined coconut oil and palm kernel oil, are the best sources of fat because they are directly absorbed into the blood and therefore are the easiest for the body to metabolize. Other good sources of fats include saturated fatty acids (SFA) from natural, unprocessed meats and dairy products; MUFA from nuts, vegetables, and fruits such as avocado; and naturally-occurring PUFA from animal protein, fish, nuts, and seeds such as walnuts, flaxseeds, soybeans, safflowers, and olives [209-211]. In fact, when investigating the effects of the ketogenic diet on obese adults (some of whom had type 2 diabetes), Dashti and colleagues predominantly resorted to using PUFA and MUFA such as olive oil (5 tablespoons) [84,212]. Independent of its role in the ketogenic diet, olive oil may reduce CVD risk through its effects on cholesterol levels [212,213]. The worst types of fats, and ones that should be completely avoided, are processed PUFA, such as margarine, and all forms of trans and hydrogenated fats. These fats are highly processed to improve shelf life, and often found in baked goods and sweets. As an alternative, healthy sources of chemically stable SFA and MUFA include butter, macadamia nuts, avocado, egg yolks, and coconut oil [209].

When consuming a high-fat diet, it is important for adolescents to maintain a well-balanced ratio between omega-3 and omega-6 fatty acids. Omega-3 and omega-6 fatty acids are known as "essential fatty acids" (EFA) because they cannot be made by the body, and must be supplemented through diet. Omega 3s have been associated with decreases in inflammation as shown in a study conducted by Paoli and colleagues who found that overweight men who followed a Mediterranean ketogenic diet supplemented with Omega-3s for 4 weeks experienced decreases in levels of the inflammatory cytokines IL-1β, IL-6 and TNF-α. On the contrary, men on the same diet but without the omega-3 supplementation only experienced a decrease in TNF-α levels [214]. In addition to their anti-inflammatory actions, consumption of omega-3 fatty acids is also associated with lowering of blood pressure, and mitigation of heart disease. Omega-6s are recognized for lowering cholesterol levels and maintaining healthy skin. Furthermore, all EFA promote a wide variety of other functions, such as transpiration of oxygen into the blood stream, promotion of healthy brain function, and maintaining fluidity in cell membranes. On a ketogenic diet, the ratio of omega-6 to omega-3 EFA should optimally be balanced at 2:1 (compare this to a typical Western diet which oscillates at levels of 20:1 and 40:1). Higher amounts of omega-6s have been associated with increased inflammation and oxidation [215]. Also, oils with a higher PUFA content oxidize much faster during cooking because of their low smoke point, making them impractical choices for supplementation of EFA. Such oils include unrefined coconut and flaxseed oils. Therefore, it is better to opt for oils with a high smoke point, such as safflower and rice bran oil, which can resist high temperatures and oxidation [209].

When incorporating omega-3s into the diet, it is important to note the inefficient conversion of α-linoleic acid (ALA), which is naturally found in foods such as walnuts, chia seeds and flaxseeds, into eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA). ALA's conversion is limited to 8-21% EPA and 4-9% DHA. Thus, it is important for omega-3s to be supplemented through sources such as fatty fish and eggs that contain high amounts of DHA and EPA rather than ALA [216]. While the exact mechanism by which omega-3 fatty acids exert anti-inflammatory effects is not well understood, it may involve binding of EPA and DHA with G-protein coupled receptor 120(GPR 120) [217].

Protein consumption on the ketogenic diet should be moderate, approximately 15% of total energy consumption [58]. Eating too much protein on a ketogenic diet can inhibit ketone production and increase production of glucose (gluconeogenesis). However, weight loss and positive health effects have still been observed with ketogenic diets that contain more protein. For example, in a study done by Johnstone and colleagues it was found that 17 obese men who were fed a high-protein ketogenic diet (4% C, 66% F, 30% P) for 4 weeks were able to reduce their energy intakes, induce ketosis, and undergo significantly greater weight loss (6.34 kg) compared to a moderate carbohydrate diet (35% C, 35% F, 30% P) (4.35 kg) [218]. The best sources of protein include all unprocessed meats, poultry, fish, and

dairy. Processed meats such as bacon, hot dogs, and deli meats, which often contain large amounts of fillers, preservatives and nitrates, are strictly limited [209].

The only major sources of carbohydrate consumed on the ketogenic diet come from cruciferous or leafy green vegetables – basically any plant that grows above ground. Starchy root vegetables that grow below ground, as well as nightshades (e.g. tomatoes, potatoes, peppers etc.) should be consumed in moderation due to their high carbohydrate content. Ultimately, the ketogenic diet emphasizes that low-carbohydrate vegetables, such as spinach, cabbage, and mushrooms, should form the basis of every meal, especially because of their high nutrient and fiber content. Unlike vegetables, fruits are given a lower priority on the ketogenic diet because of their high carbohydrate content. Berries, citrus fruits and avocados can be consumed in moderation, but all other fruits, most notably tropical fruits like pineapple, banana and mango, should be eliminated, or at least strictly limited [209]. In addition to their low-carbohydrate content, berries contain high levels of polyphenols, anti-oxidative and anti-inflammatory compounds which have been associated with reduction of CVD risk through improvement of lipid profiles [219].

Other sources of acceptable, but nevertheless limited, sources of carbohydrate on the ketogenic diet include nuts and seeds. As with anything ketogenic, the lower the carbohydrate content the better, and nuts that meet such criteria include brazil, macadamia, pecan, and walnuts, all of which contain high amounts of EFA which help reduce inflammation and improve lipid profiles [209]. Walnuts are particularly beneficial because of their high PUFA content (38% linoleic acid and 9% ALA), along with their dietary fiber, antioxidants, and phytosterol [220]. In a dietary study with 365 participants, Banel and colleagues found that consuming a diet supplemented with walnuts resulted in a large decrease in total cholesterol and LDL levels, improvements in antioxidant capacity and decreased levels of inflammatory biomarkers [221].

Dairy is a particularly popular option on the ketogenic diet due to its high fat and low carbohydrate content. Highly processed dairy should generally be avoided, while raw and organic yogurts, cheese, creams, and milk should be consumed in moderation. Full-fat dairy products are preferred over low-fat ones, since they have fewer carbohydrates and a more satiating effect [209]. Probiotic and lactobacillus-containing dairy products, such as yogurt, are found to be especially beneficial as well [222].

In general, whole foods are always the best option to choose on a ketogenic diet. Although foods such as chips, hamburger patties, hot dogs, French fries and other processed foods are high in fat, they are not good choices because of the fillers and types of fat (e.g. trans fat) they contain. Fresh or frozen vegetables, fruits, meats, poultry, fish, and dairy; pure spices such as cinnamon, oregano, salt, and pepper; and decaffeinated, low-carbohydrate beverages such as herbal tea, almond, and coconut milk, are the best options, and should be consumed in healthy amounts [209].

Conclusion

As reviewed previously [8,18] and mentioned herein, low-carbohydrate diets are effective tools for reducing excess weight. This significant reduction of BMI is likely to reduce risk for CVD through effects on adiponectin, leptin, CRP, and possibly fetuin-A, IL-6 and TNF-α. Adherence to a low-carbohydrate diet in particular is also likely to improve subjects' lipid profiles, increasing levels of HDL and decreasing levels of triglycerides and numbers of small, dense LDL particles. Concomitant with this reduction in CVD risk through attenuation of dyslipidemia, low-carbohydrate diets may improve the health of the endothelium that interacts with these molecules, decreasing risk for atherosclerosis. Also contributing to this amelioration of risk could be lower levels of FGF-21 and IL-1β, as suggested by preliminary ex vivo and animal studies using low-carbohydrate diets. This compilation of data, collected from both youth and older adults (Table 1), can serve as a promising foundation for additional intervention studies in youth with type 2 diabetes that can verify the effects of low-carbohydrate diets on these markers.

CVD Risk Factor	Role in CVD	Changes Induced by a Low-Carbohydrate Diet		
		Adolescents with/ at risk for diabetes	Adults with/at risk for diabetes	Healthy adults
Dyslipidemia	Increases plaque formation	Decreases	Decreases	Decreases
IL-1β	Increases inflammation	Not discussed	Decreases	Not discussed
TNF-α	Increases inflammation	Not discussed	Decreases (related to body weight)	No change
IL-6	Increases inflammation	Not discussed	Increases, decreases (related to body weight)	No change
CRP	Increases inflammation	Not discussed	Decreases (related to body weight)	No change
Markers of endothelial dysfunction	Increase inflammation, decrease vascular function	Not discussed	Decreases	Not discussed
Fetuin-A	Impairs insulin and glucose metabolism	Decreases (related to body weight)	Decreases (related to HDL)	Not discussed
FGF-21	Impairs insulin and glucose metabolism	Decreases (related to body weight)	Not discussed	Not discussed
Adiponectin	Inhibits inflammation and plaque formation, improves insulin and glucose metabolism	No change (diets contained 50% C)	Increases	Increases
Cortisol	Increases inflammation, dyslipidemia and hypertension, impairs insulin and glucose metabolism	Not discussed	Increases (in a diet with 4% C)	No change
Leptin	Increases vascular calcification and stiffness	Decreases (related to body weight)	Not discussed	Not discussed

Table 1: *Summary of the effects of low-carbohydrate diets on CVD risk factors in youth with type 2 diabetes.*

Conflict of Interest

The authors have no conflicts of interest.

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