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Review

Dietary carbohydrate restriction induces a unique metabolic state positively affecting atherogenic dyslipidemia, fatty acid partitioning, and metabolic syndrome

Jeff S. Volek [a,*], Maria Luz Fernandez [b], Richard D. Feinman [c], Stephen D. Phinney [d]

[a] Department of Kinesiology, University of Connecticut, 2095 Hillside Road, Unit 1110, Storrs, CT 06269-1110, United States
[b] Department of Nutritional Science, University of Connecticut, Storrs, CT, United States
[c] Department of Biochemistry, SUNY Downstate Medical Center, Brooklyn, NY, United States
[d] School of Medicine, University of California, Davis, CA, United States

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ABSTRACT

Abnormal fatty acid metabolism and dyslipidemia play an intimate role in the pathogenesis of metabolic syndrome and cardiovascular diseases. The availability of glucose and insulin predominate as upstream regulatory elements that operate through a collection of transcription factors to partition lipids toward anabolic pathways. The unraveling of the details of these cellular events has proceeded rapidly, but their physiologic relevance to lifestyle modification has been largely ignored. Here we highlight the role of dietary input, specifically carbohydrate intake, in the mechanism of metabolic regulation germane to metabolic syndrome. The key principle is that carbohydrate, directly or indirectly through the effect of insulin, controls the disposition of excess dietary nutrients. Dietary carbohydrate modulates lipolysis, lipoprotein assembly and processing and affects the relation between dietary intake of saturated fat intake and circulating levels. Several of these processes are the subject of intense investigation at the cellular level. We see the need to integrate these cellular mechanisms with results from low-carbohydrate diet trials that have shown reduced cardiovascular risk through improvement in hepatic, intravascular, and peripheral processing of lipoproteins, alterations in fatty acid composition, and reductions in other cardiovascular risk factors, notably inflammation. From the current state of the literature, however, low-carbohydrate diets are grounded in basic metabolic principles and the data suggest that some form of carbohydrate restriction is a candidate to be the preferred dietary strategy for cardiovascular health beyond weight regulation.

Contents

Abbreviations: ACC, acetyl-CoA carboxylase; AcCoA, acetyl-CoA; AGE, advanced glycated end products; AMPK, AMP-activated protein kinase; ChREBP, carbohydrate response element binding protein; CPT-1, carnitine palmitoyltrasferase-1; CVD, cardiovascular disease; DNL, de novo lipogenesis; DRI, dietary recommended intakes; FABP, fatty acid binding protein; FAS, fatty acid synthase; FAT, fatty acid translocase; FGF21, fibroblast growth factor 21; GK, glucokinase; HDL-C, high-density lipoprotein cholesterol; GLUT4, insulin-dependent glucose transporter; HMG-CoA, 3-hydroxy-3-methylglutaryl-coenzyme A; HSL, hormone sensitive lipase; I-CAM, intercellular adhesion molecule-1; LDL-C, low-density lipoprotein cholesterol; LFD, low-fat diet; LPL, lipoprotein lipase; LPK, liver pyruvate kinase; LXR, liver X receptor; MCP-1, monocyte chemotactic protein-1; MetS, metabolic syndrome; MUFA, monounsaturated fatty acids; NF-κB, nuclear factor-kappa B; NMR, nuclear magnetic resonance; PAI-1, plasminogen activator inhibitor-1; PFK, phosphofructokinase; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids; SREBP-1c, sterol response element binding protein 1c; TG, triglycerides (triacylglycerols); TNF-α, tumor necrosis factor alpha; VLCKD, very low-carbohydrate ketogenic diet; VLDL-C, very low-density lipoprotein cholesterol; WHI, Women's Health Initiative.

* Corresponding author. Tel.: +1 860 486 6712; fax: +1 860 486 1123.
E-mail address: jeff.volek@uconn.edu (J.S. Volek).

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1. Introduction

Recent studies support a shift in our understanding of dietary carbohydrate restriction and its interaction with lipid metabolism. Long considered a stratagem for weight loss, there has been a persistent concern of increased cardiovascular risk if the carbohydrate that was removed were replaced with fat. In fact, numerous experiments over the past four or five years have consistently shown that reduction in dietary carbohydrate leads to improvements in atherogenic dyslipidemia (reduced TG and increased HDL-C), metabolic syndrome and diabetes, even in the absence of weight loss [1–3] or even in the presence of saturated fat.

In addition to experimental demonstrations of its efficacy, the importance of carbohydrate restriction rests on two fundamental ideas. First, in distinction to strategies based on reduction on dietary fat, the rationale for reduction in dietary carbohydrate derives from basic mechanism. Carbohydrate is the major secretagogue of insulin and, beyond its role in providing a source of energy, serves as a control element, either directly via glucose or fructose or indirectly through the effects of insulin and other hormones. Significant advances have been made in unraveling the details of the downstream effects of such stimulation although the links between dietary effects of carbohydrate and the responses in lipid metabolism remain insufficiently explored.

Second, while there is some debate about the clinical applicability of metabolic syndrome (MetS), its intellectual impact has been that it defines a metabolic state encompassing seemingly unrelated processes so that conclusions from the study of diabetes, for example, can cross over to support approaches to dyslipidemia.

A final reason to re-examine low-carbohydrate diets is the historical perspective that aboriginal hunting and fishing cultures survived for millennia with little if any identifiable dietary carbohydrate intake. Examples include the Inuit of the Arctic and First Nations groups in Canada. When these ethnic groups underwent a transition from their high-fat, low-carbohydrate traditional diets, the prevalence of obesity and type-2 diabetes in these populations increased dramatically [4].

Here we present the results of recent dietary intervention studies and try to provide a continuum from clinical markers of health to the underlying physiology and downstream carbohydrate-related regulation of cell signaling. We argue that progress in the field will depend on our ability to integrate these two lines of research.

In light of recent results from dietary studies and the underlying biochemistry, we suggest that current recommendations of health agencies, which downplay or counsel against carbohydrate restriction, need to be re-evaluated.

2. Dietary carbohydrate is a robust regulatory mechanism signaling lipid metabolism

One of the current views integrating physiology and obesity is the idea that metabolic dysfunction arises from exposure of cells to an excess of nutrients [5] independent of their composition. We would argue that the emerging principle is that individual macronutrients, in particular carbohydrate, have different effects on the control of homeostasis. Carbohydrate, beyond its role as a source of energy, has an important regulatory function. Dietary carbohydrate stimulates insulin secretion, and/or affects the availability of energy substrates such as free fatty acids, ketone bodies and glycogen. It also provides a direct source of glucose or fructose, both of which can serve as cell stimuli. Dietary carbohydrate restriction represents a perturbation in upstream response elements that induces a unique metabolic state orchestrated through several key transcription factors (Fig. 1). Even a short-term reduction in dietary carbohydrate has robust effects on transcriptional control. For example, switching from a carbohydrate intake of 49 to 34% of energy for 3 days differentially regulated 369 out of 18,861 genes in skeletal muscle in a manner consistent with a shift in substrate utilization toward fatty acid oxidation [6]. It is difficult to attribute metabolic responses and clinical outcomes to one class of nutrients, but as a principle, one has to consider the inextricable link between dietary carbohydrate and the appearance of plasma glucose and insulin as an important modulator of cellular function.

In Fig. 1, the upstream dietary regulators are a decrease in carbohydrate and increase in SFA input. Normally a high dietary SFA intake would be predicted to increase circulating SFA and promote lipogenesis through SREBP-mediated lipogenic gene expression [7]. If carbohydrate intake were low enough to decrease levels of glucose and insulin, however, a high SFA intake would be processed very differently. We recently showed, for example, a disconnect between dietary SFA and plasma levels of SFA apparently due to the regulatory role of dietary carbohydrate in controlling de novo lipogenesis (DNL) [8].

Most dietary carbohydrate is digested and absorbed as glucose or fructose. In health, plasma glucose levels are normally held within a narrow range despite intermittent feeding and wide variations in intake. Acute regulation of plasma glucose is achieved by a coordinated hormonal system in which insulin has a dominate role. In this way dietary carbohydrate and insulin

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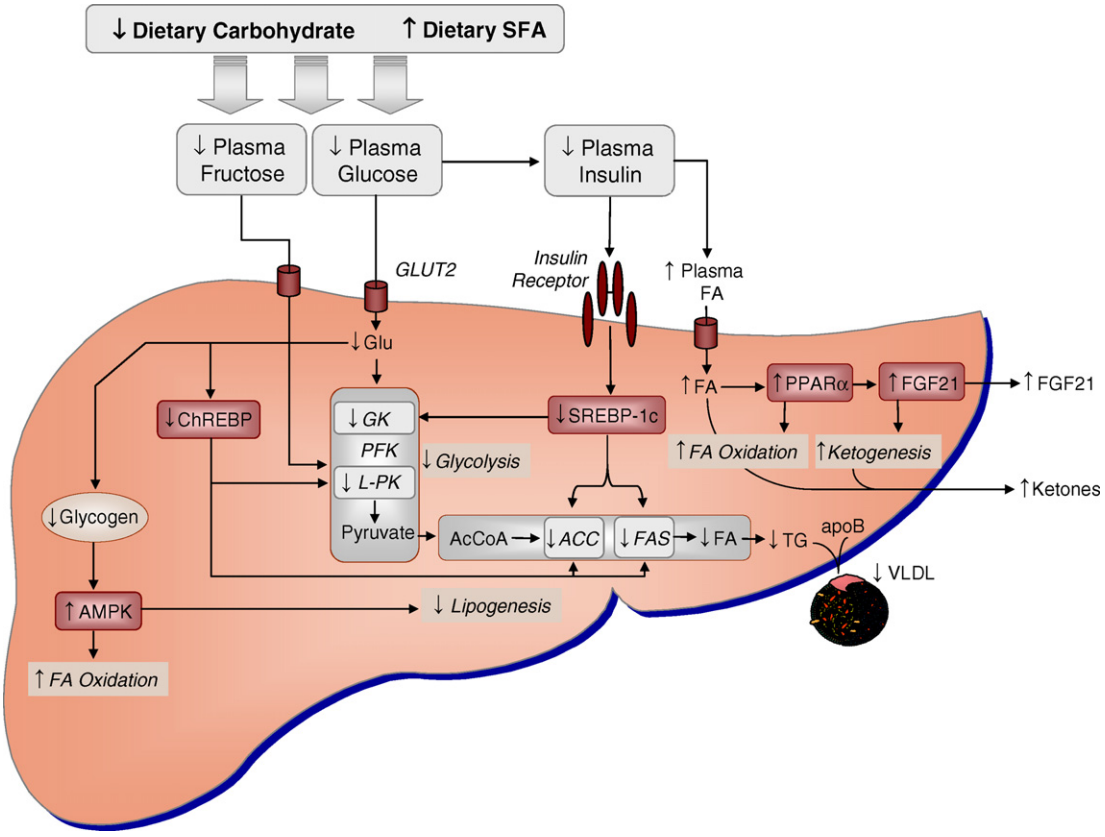

Fig. 1. Schematic of hepatic metabolic regulation induced by a low-carbohydrate diet. Restriction in dietary carbohydrate, even in the presence of high saturated fatty acids, decreases availability of ligands (glucose, fructose, and insulin) that activate lipogenic and inhibit fatty acid oxidative pathways. The relative importance of each transcriptional pathway is unclear, but the end result – increased fat oxidation, decreased lipogenesis, and decreased secretion of very low-density lipoprotein – is a highly reliable outcome of low carbohydrate diets (PPAR, peroxisome proliferator-activated receptor).

stimulation and action are intimately linked. A dysregulation in glycemic control leading to hyperglycemia, as occurs in insulin resistance and type-2 diabetes, contributes to a coordinated breakdown in cellular function that leads to overproduction of free radicals and microvascular damage [9,10]. Brownlee has conducted pioneering work elucidating the mechanisms of hyperglycemia-mediated cellular damage identifying increased flux through the polyol pathway, intracellular production of AGE precursors, PKC activation, and increased hexosamine pathway activation [10]. Dietary carbohydrate restriction, by limiting a source of blood glucose, is a direct method to treat hyperglycemia and hyperinsulinemia and these undesirable consequences. The cellular response elements sensitive to glucose and insulin are now well characterized.

2.1. Glucose as regulatory element

Glucose-responsive promoter regions on genes include the primary lipogenic enzymes acetyl-CoA carboxylase (ACC) and fatty acid synthase (FAS). The recent identification of carbohydrate response element binding protein (ChREBP) has provided evidence for a single transcription factor that is responsible for activation of lipogenic genes [11–13] in response to carbohydrate. High glucose levels result in dephosphorylation and nuclear translocation of ChREBP leading to binding and activation of liver pyruvate kinase, ACC, and stimulation of FAS transcription. Expression of the lipogenic genes occurs without any apparent effect of insulin indicating one way in which glucose directly regulates nutrient partitioning.

2.2. Insulin as regulatory element

Insulin binding on cell membrane receptors activates a series of phosphorylation cascades affecting expression of more than 150 genes. Amplification of insulin signaling has diverse effects – stimulation of peripheral glucose uptake by recruitment of GLUT4 receptors, inhibition of glycogenolysis, gluconeogenesis and lipolysis, stimulation of glycogen storage and protein synthesis point to its generally anabolic effects. At the cellular level, the carbohydrate–insulin axis is coordinated through two interacting pathways involving the ChREBP and a sterol response element binding protein 1c (SREBP-1c), recognized as the primary insulin-responsive mediator of hepatic lipogenic enzyme gene expression. A key regulatory component in nutrient-mediated control of lipid metabolism is the liver X receptor (LXR) that belongs to the superfamily of nuclear hormone receptors. Oral administration of LXR agonists to mice, for example, leads to increased hepatic de novo lipogenesis (DNL) and steatosis, and enhanced secretion of TG-rich VLDL and hypertriglyceridemia. While hepatic DNL can be attributed to LXR-dependent upregulation of SREBP-1c, expression of many lipogenic genes persists in LXR agonist-treated SREBP-1c null mice, due to the presence of ChREBP which is an independent LXR target.

The majority of dietary carbohydrate is digested and absorbed as glucose, but an increasing intake of fructose estimated at 85–100 g/day also exposes the liver to high levels of this monosaccharide. Dietary fructose is readily absorbed and has unique metabolic effects. Fructose by-passes the regulatory control step at phosphofructose kinase-1 and increases dihydroxyacetone phosphate, increasing glycerol-3-phosphate for TG synthesis. Fructose inges-

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tion is thus associated with a general disruption in fuel metabolism and acutely enhances postprandial lipemia [14] and metabolic syndrome [15]. These effects occur despite a minimal impact on glycemia and insulin levels.

Dietary carbohydrate has a major effect on two other substrates, ketone bodies and glycogen. The hepatic production of ketones that occurs during fasting is reflective of an accelerated rate of lipolysis and is remarkably sensitive to dietary carbohydrate. It is worth noting that many changes in lipid metabolism during fasting are due to the specific removal of carbohydrate as opposed to a general elimination of calories [16]. Ketogenic diets have a profound impact on hepatic gene expression favoring increased activation of genes in lipid oxidation and decreased expression of genes in lipogenesis [17]. Ketogenic diets were recently shown to induce hepatic expression and increase circulating levels of fibroblast growth factor 21 (FGF21) [18,19]. FGF21 induction coordinates lipid homeostasis by inducing hepatic lipid oxidation, ketogenesis, white adipose tissue lipolysis, and TG clearance.

Glycogen, the storage form of carbohydrate in muscle and liver, has historically been viewed in the narrow context of substrate, especially for contracting skeletal muscle. Recent work, however, has linked glycogen metabolism with a range of metabolic processes [20] including transport of glucose into cells. Results from several experimental models have shown that muscle glycogen levels exert an important influence on insulin-mediated and contraction-mediated glucose uptake, as well as basal (unstimulated) glucose entry into cells. Some of the effects of low levels of glycogen may be mediated by stimulation of AMP-activated protein kinase (AMPK) [21]. Glucose uptake is higher in glycogen-depleted muscle and there is an inverse relation between glycogen and glucose uptake across a broad range of glycogen levels [22,23]. Numerous reports have confirmed that exercise induces an increase in skeletal muscle GLUT4 (the insulin-dependent glucose transporter) and a proportional increase in glucose transport capacity. When carbohydrates are fed after exercise there is an increase in glucose entry into cells that is diverted to glycogen formation. As glucose enters into cells and glycogen levels increase, there is inhibition of contraction- and insulin-mediated glucose transport associated with a return of GLUT4 to pre-exercise levels. Prevention of glycogen synthesis, by fasting or feeding a low-carbohydrate/high-fat diet, results in a persistence of contraction- and insulin-mediated glucose transport that lasts as long as carbohydrates are not consumed [24–26]. This line of work shows that low glycogen promotes increased insulin action, whereas high glycogen promotes insulin resistance.

3. Metabolic syndrome

Metabolic syndrome (MetS) represents a group of markers that predispose to obesity, diabetes, cardiovascular disease and hypertension. The underlying defect in MetS is generally considered to be a resistance to the actions of insulin in peripheral tissues that manifests as hyperglycemia, hyperinsulinemia, and atherogenic dyslipidemia (high TG, low HDL-C, and small LDL-C). Definitions are continually being modified and an expanding number of different physiologic effects are correlated with MetS. The intellectual import of the idea is that disparate phenomena, glycemic control, coagulation and hemostasis and body mass regulation can be identified with a single underlying cause, a presumed disruption in insulin control.

3.1. Carbohydrate restriction and MetS

A recent survey of the literature showed that the physiologic markers typically associated with MetS were precisely those that were improved by carbohydrate restriction [3]. Such an association had been noted by other authors in passing and is consistent with the role of insulin in response to carbohydrate and the features of MetS. In the absence of another universally accepted definition, it was suggested that a positive response to carbohydrate restriction might be an operational definition of MetS. Such an idea suggests that an insulin-resistant population is not only the logical clinical target of carbohydrate restriction but might be the best place to determine the effectiveness in an experimental setting.

3.2. Carbohydrate vs. fat restriction in atherogenic dyslipidemia of MetS

We specifically tested the effects of carbohydrate restriction, with isocaloric fat restriction as control, in 40 overweight men and women with the atherogenic dyslipidemia of MetS [27]. Subjects were randomly assigned to a VLCKD (%CHO:fat:protein = 12:59:28) or a low-fat diet (LFD) (%CHO:fat:protein = 56:24:20) for 12 weeks. Both diets led to a reduction in energy intake over the course of the experiment (VLCKD = 1504 kcal, LFD = 1478 kcal) but individuals who undertook carbohydrate restriction had a much higher probability of weight reduction than those on fat reduction. None of the subjects following the LFD lost as much weight as the average for the VLCKD group. Subjects consuming the VLCKD also specifically reduced body fat, improved glycemic control and insulin sensitivity to a greater degree than the LFD subjects.

This experiment was distinguished by measurement of what we believe to be one of the widest spectra of lipid, inflammatory and cardiovascular risk markers. Fig. 2 summarizes data showing that the VLCKD group had consistently more favorable TG, HDL-C and total cholesterol/HDL-C ratio responses compared to the LFD. In addition to these standard markers for MetS, the VLCKD subjects showed more favorable responses to alternative indicators of atherogenic dyslipidemia and cardiovascular risk: postprandial lipemia, apo B, apo A-1, the apo B/apo A-1 ratio, LDL particle distribution, and postabsorptive and postprandial vascular function. Similarly, a generally greater anti-inflammatory effect was associated with the carbohydrate-restricted diet with substantially more pronounced reductions in TNF-α, IL-6, IL-8, MCP-1, E-selectin, I-CAM, and PAI-1. A notable result was that, despite a 3-fold higher intake of dietary saturated fat during the VLCKD, saturated fatty acids in TG and cholesteryl ester were significantly decreased compared to subjects consuming the LFD. That this was due to a decrease in DNL was shown by a corresponding reduction in palmitoleic acid (16:1n 7), an endogenous indicator of this process. Overall, the findings provide support for unifying the disparate markers of MetS and for the proposed intimate connection of the syndrome with dietary carbohydrate.

These results are consistent with many reports in the literature although the differences are somewhat more dramatic than usual, presumably due to the fact that all subjects conformed to the definition of MetS. That patients with MetS might be particularly sensitive to carbohydrate restriction was suggested by Cornier et al. [28] who compared the response of obese insulin-sensitive and obese insulin-resistant subjects randomized to either a high-carbohydrate (60%) or lower carbohydrate (40%) diet. Weight loss was similar for the insulin-sensitive group irrespective of carbohydrate level. The most striking result was that only the insulin-resistant group showed a major change in any lipid parameter with a 42% average decrease in TG on lower carbohydrate, and a 27% increase on higher carbohydrate. That individuals with MetS or insulin-resistance syndrome respond better to restricting carbohydrates than fat is consistent with intolerance to carbohydrate as the fundamental metabolic problem.

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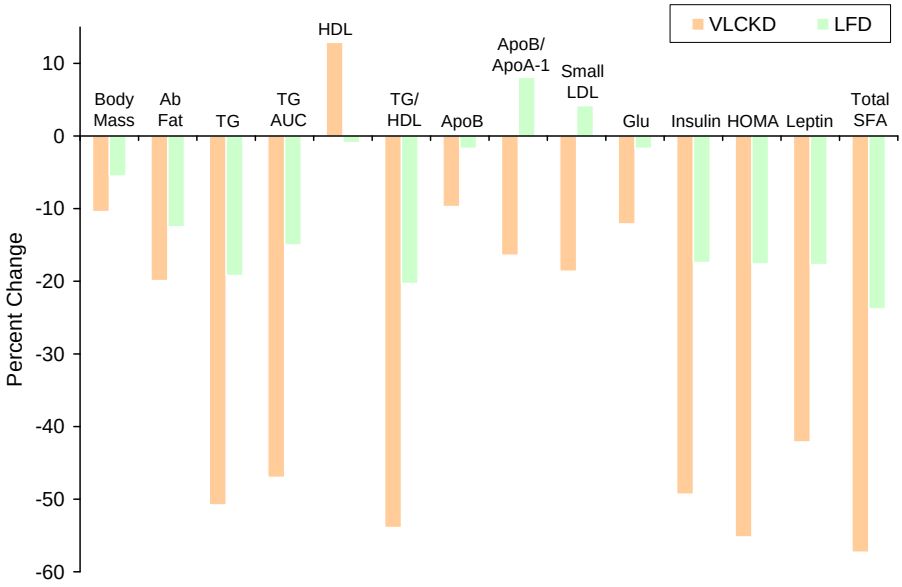

Fig. 2. Summary of changes in subjects who consumed a very low-carbohydrate ketogenic diet (VLCKD) or a low-fat diet (LFD) for 12 weeks. Mean changes were all significantly different between the VLCKD and LFD [27] (Ab Fat, abdominal fat; HOMA, Homeostasis Model Assessment; TG AUC, area under (time) curve).

Shulman and colleagues [29] recently published data that provide insights into the metabolic underpinnings of the abnormal response to dietary carbohydrate in individuals with insulin resistance. The fate of a dietary carbohydrate load in lean insulin-resistant and insulin-sensitive men was determined using a combination of ^{1}H and ^{13}C NMR spectroscopy to assess liver and muscle triglyceride and glycogen synthesis, respectively, and deuterium enrichment to assess de novo lipogenesis. The insulin-resistant men showed impaired skeletal muscle and hepatic glycogen formation following intake of dietary carbohydrate. Consistent with the paradigm presented in Fig. 1, dietary carbohydrate in the insulin-resistant group was instead diverted toward hepatic DNL and TG synthesis that contributed to a significant increase (60%) in plasma TG levels. The findings highlight the importance of dietary carbohydrate as a controlling factor in the dyslipidemia of MetS.

3.3. Carbohydrate restriction stimulates a unique metabolic state

Significant carbohydrate restriction leads to global hormonal and metabolic adaptations. A common theme in the literature is the similarity in these changes and those brought about by fasting or starvation or adaptations to physical activity [30,31]. A classic experiment of Klein and Wolfe showed similar physiologic responses to three days of fasting or fasting with infusion of lipid [16]. The robust metabolic and enzymatic adaptations that are associated with carbohydrate restriction are summarized in Table 1. These adaptations are, to some extent, fiber-type specific and depend on the increase in dietary fat. The time course of metabolic adaptations is also variable; some lipolytic adaptations occur within a week (e.g., gene expression of FAT/CD36 and β-HAD) while others take longer (e.g., FABP and CPT I). Several weeks may be necessary for complete switch to optimal fat utilization.

The increase in the oxidation of lipid substrates is associated with an increase in sympathoadrenal activity, enhanced lipolysis, elevated levels of fatty acids and synthesis and utilization of ketone bodies [32–34]. The hormonal changes that accompany carbohydrate restriction, fasting or continued physical activity lead to inhibition of glycogen synthesis and inactivation of acetyl-CoA carboxylase and a fall in malonyl-CoA levels which, in turn, relieves inhibition of carnitine transport and thereby stimulates fatty acid oxidation.

Table 1
Metabolic and enzymatic adaptations to low-carbohydrate diets

Fat metabolism
" Fat oxidation
" Muscle TG storage
" Muscle TG utilization during exercise
" VLDL catabolism during exercise
" Fatty acid binding protein and fatty acid translocase (FAT/CD36)
" Ketone body production and utilization
" Muscle lipoprotein lipase
" β-Hydroxyacyl-CoA dehydrogenase
" Carnitine acyltransferase I
" 3-Oxoacid CoA thiolase
Carbohydrate metabolism
; Carbohydrate oxidation
; Muscle glycogen storage
; Muscle glycogen rate of utilization during exercise
" Gluconeogenesis
" Phoshoenolpyruvate carboxykinase
; Hexokinase
; Pyruvate dehydrogenase

3.3.1. Ketone bodies

A key metabolic adaptation to carbohydrate restriction resulting from accelerated rates of lipolysis is production and utilization of ketone bodies. Ketosis is frequently viewed as deleterious because of the association with the acidosis from the hyperketonemia seen in untreated type-1 diabetes. Such extreme ketone levels are due to the absence of insulin and unregulated lipolysis and are not seen in fasting or during carbohydrate restriction. A wide range of blood concentrations of ketones are possible (10^{-2}–10^{-5} mol/L) [35], similar to the broad range of glucose levels observed clinically. Thus, both ketones and glucose extend across similar ranges in health and disease with high levels of both manifesting in deleterious outcomes. The concentration of blood ketones (β-hydroxybutyrate plus acetoacetate) in healthy individuals in the carbohydrate fed state is about 0.1 mmol/L and increases to about 0.3 mmol/L after an overnight fast [35]. Prolonged fasting up to

20 days can increase ketone bodies to >10 mmol/L. In healthy men, an isocaloric VLCKD (<20 g carbohydrate/day) resulted in β-hydroxybutyrate greater than 2 mmol/L after two weeks and 3 mmol/L by four weeks [36]. This is an order of magnitude below the levels that occur in untreated type-1 diabetes, which often exceeds 20 mmol/L.

Reviews on ketone body metabolism [37–42] have emphasized their role as efficient fuels, and their therapeutic potential in a variety of clinical states including: (1) diseases of substrate insufficiency or insulin resistance, (2) diseases resulting from free radical damage and (3) diseases resulting from hypoxia [43,44]. Ketone bodies have evolved as important fuel sources and are about 25% more efficient at producing ATP than glucose or fatty acid [45].

There are several levels of regulation of ketone body production. Substrate availability appears to play a major role: accumulation of substantial amounts of acetyl-CoA comes from increased TG hydrolysis in adipocytes, stimulation of fatty acid transport across the inner mitochondrial membrane and partitioning of acetyl-CoA between the ketogenic pathway and oxidation via the citric acid cycle inside the mitochondrial matrix. At rest, the rate of lipolysis is exquisitely sensitive to physiological levels of insulin [46]. Insulin leads to inhibition of hormone sensitive lipase (HSL), promotes glucose uptake into adipocytes and activates several lipogenic enzymes (e.g., fatty acid synthase). The feedback inhibition loop of ketogenesis has been demonstrated by the fact that beta-hydroxybutyrate stimulates beta-cell insulin release [47] and ketone bodies themselves inhibit lipolysis.

Regulation of mitochondrial 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) synthase as a control point in the ketogenic pathway has been the subject of recent research [48]. Short-term regulation of HMG-CoA synthase is brought about by succinylation (inhibitory) and desuccinylation of the enzyme itself. Long-term regulation via changes in HMG-CoA synthase mRNA and protein levels are increased in rats under ketogenic conditions of starvation, diabetes, fat feeding, and injection of cAMP and decreased by insulin and refeeding [48]. The same effectors that increase HMG-CoA synthase mRNA levels also desuccinylate and activate the enzyme.

A novel mechanism has been proposed whereby ketone bodies control levels of lipolysis through a G protein-coupled seven-transmembrane receptor expressed in mice, the so-called PUMA-G receptor (Protein Upregulated in MAcrophages by IFN-Gamma). PUMA-G, and the human homolog HM74a, appear to inhibit lipolysis in response to β-hydroxybutyrate as its endogenous ligand [49]. It is significant that the receptor was discovered as the target of high levels of nicotinic acid, one of the few drugs widely used for lowering TG and raising HDL-C and was established as the link to prostaglandin-mediated flushing, the major side-effect of therapeutic doses of nicotinic acid [50]. This observation raises the intriguing possibility that the ability of carbohydrate-restricted diets to improve atherogenic dyslipidemia discussed below may be due, in part, to a direct effect of ketone bodies, per se.

3.3.2. Exercise

The effects of VLCKD on metabolism have been studied in the context of physical exercise. High-fat/low-carbohydrate diets consumed for more than 7 days reduce muscle glycogen content and carbohydrate oxidation. This is compensated for by markedly increased rates of fat oxidation [33,34,51–54] even in well-trained endurance athletes who already demonstrate increased fat oxidation [52]. The substrates for enhanced fat oxidation appear to be circulating fatty acids, ketone bodies, and TG derived from VLDL [32–34], the latter probably resulting from enhanced skeletal muscle lipoprotein lipase (LPL) activity [55]. Enhanced fat oxidation and muscle glycogen sparing after a high-fat diet persist even when carbohydrate is provided before or during exercise [56]. Carbohydrate-loading after chronic adaptation to a high-fat diet is the subject of current investigation: some studies show enhanced performance during ultra-endurance exercise compared to a conventional diet [53,57] whereas others have failed to show a clear benefit despite increased fat oxidation [56,58,59]. However, given two or more weeks of adaptation to either a hypocaloric or eucaloric ketogenic diet, endurance exercise performance is not impaired despite a marked shift to fat as the primary oxidative fuel [36,60].

4. Dietary carbohydrate as a controlling factor in atherogenic dyslipidemia

4.1. Carbohydrate restriction and triglyceride levels

One of the most dramatic results reported by Volek et al. [27] is the differential effect of VLCKD vs. LFD on TG levels. The low-carbohydrate regimen reduced TG from an average at baseline of 211 ± 58 mg/dL to 104 ± 44. The corresponding change in the LFD group was 187 ± 58 to 151 ± 38. This kind of decrease in plasma TG is a hallmark of the response to a reduction in dietary carbohydrate and has been reported numerous times [61]. Well-controlled feeding studies indicate that low-fat/high-carbohydrate diets increase TG concentrations unless there is significant weight loss or increased physical activity [62,63]. The greater the amount of carbohydrate that is substituted for fat, the greater the increase in TG [64]. The response is sufficiently consistent that measurement of an *increase* in TG has been used as a biomarker of compliance to a low-fat, high carbohydrate diet [65]. High carbohydrate diets also exacerbate postprandial lipemia [66–68], whereas low-carbohydrate diets improve postprandial lipemia with [69] or without [70,71] weight loss.

The general phenomenon of carbohydrate-induced triglyceridemia has a long history. Overfeeding of dietary carbohydrate increases hepatic lipogenesis [72]. Isoenergetic replacement of dietary fat by carbohydrate also stimulates de novo fatty acid synthesis [73] which, in turn, correlates with increased TG [74]. At the 1985 Marabou Symposium, somewhat ominously entitled "The Nutritional Re-emergence of Starchy Foods", Ahrens recalled [75]:

> "In 1957 we noted that substituting CHO for fat calories in a eucaloric feeding regimen produced a prompt rise in plasma triglycerides. This ought not to have surprised us because in 1955 Hatch, Abell and Kendall showed that an experimental rice diet produced hypertriglyceridemia... The deeper implications of these findings were recognized in the relationship between atherogenesis and hypertriglyceridemia first described in 1959 by Albrink and Man and by Antonis and Bersohn in 1960 (references in [75]). Whether hypertriglyceridemia is an independent risk factor for CHD is still being debated today."

The debate described by Ahrens is currently leaning heavily in favor of characterizing triglyceridemia as a risk factor. Most recently, Tirosh et al. [76] measured fasting TG in 13,953 healthy, young men at intervals of 5 years and reported that a decrease in TG from an elevated baseline was associated with a decrease in CHD risk compared to maintenance of high TG levels, although the reduced risk was still higher than in subjects with persistently low TG levels. Yuan et al. have provided an excellent summary of the evidence supporting the role for high TG in atherogenic risk [77].

Given the historical association of dietary carbohydrate and hypertriglyceridemia and the recent results from dietary interventions, it has to be considered remarkable that the renewed interest in TG as a risk factor has not been accompanied by recommendations for reductions in dietary carbohydrate. Neither Yuan et al.'s review [77], Tirosh's recent study [76] nor an accompanying edito-

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rial [78] mentions the value of carbohydrate reduction in ameliorating high TG.

4.2. Mechanisms of carbohydrate-induced hypertriglyceridemia

Humans have a limited capacity for storage of carbohydrate. Carbohydrate, in excess of storage and oxidative capacity, particularly under conditions of insulin resistance [29], readily stimulates hepatic DNL in proportion to intake [79]. Even under isocaloric conditions, high carbohydrate diets stimulate DNL with proportional increases in plasma TG [74,80]. The source of carbohydrate affects DNL (e.g., fructose is more lipogenic than glucose). In addition to directly providing newly formed fatty acids for VLDL synthesis/secretion, hepatic DNL indirectly contributes to TG production. Malonyl-CoA is a substrate in the synthesis of fatty acids and is a potent inhibitor of carnitine palmitoyl transferase, the rate limiting enzyme in transporting long chain fatty acids into the mitochondria for oxidation. These direct and indirect effects of high DNL contribute to the production of VLDL TG and subsequent hypertriglyceridemia.

In addition to overproduction of VLDL, carbohydrate-induced hypertriglyceridemia may also be driven by impaired TG clearance. Subnormal post-heparin lipoprotein lipase (LPL) activity has been implicated in the pathogenesis of atherogenic dyslipidemia and enhanced postprandial lipemia in insulin-resistant states [81–83]. In individuals with angiographically-defined coronary artery disease, TG concentrations were elevated and post-heparin LPL activity was 22% lower compared to healthy normolipidemic controls [84]. Significant inverse correlations between postprandial adipose tissue LPL mRNA and postprandial lipemia in individuals with elevated plasma TG concentrations [85] or insulin resistance [86] have also been reported. These studies suggest that an additional effect of the insulin-resistant state is the down regulation of adipose tissue LPL expression and activity contributing to the elevated plasma TG levels that is a characteristic marker.

4.3. Carbohydrate restriction and HDL-C

Because of its established role in reverse cholesterol transport as well as other antiatherogenic properties (e.g., antioxidant, anti-inflammatory, and nitric oxide production) [87] high HDL-C is of increasing clinical importance as a therapeutic target [88]. Lifestyle changes (e.g., aerobic exercise, smoking cessation, reduction in alcohol consumption, weight loss and n 3 PUFA supplementation) are universally recommended as the first approach to increase HDL-C [88–90] but because the effects on HDL-C elevation are small (0–10%) there is an emphasis on pharmacotherapy.

Volek et al. [61] summarized experiments comparing low-fat and low-carbohydrate diets and showed that every one of 15 studies reported greater increases in HDL-C on carbohydrate-restricted diets compared to low-fat diets (average absolute difference 11%). Counter-intuitively, the increase in HDL-C in response to traditional lifestyle modification is greater in individuals with normal or elevated initial HDL-C levels with the least improvement in those having low concentrations [91]. This is not true of low-carbohydrate diets where, e.g., individuals recruited on the basis of low HDL-C [<40 (men) or >50 (women) mg/dL], showed a 13% increase in HDL-C (35.8 ± 6.9 to 40.4 ± 9.6 mg/dL) with 12 of 20 subjects showing a >10% increase in HDL-C [15]. An unexpected finding in this study was a gender by diet effect, with women exhibiting a more pronounced increase in HDL-C on the low-carbohydrate diet (17% women vs. 8% men). This is consistent with the striking increase in HDL-C (33%) observed in response to a very low-carbohydrate diet in normolipidemic, normal weight women [71]. Although the effect of carbohydrate restriction on HDL-C is not as prominent as the TG-lowering effects, the consistency and magnitude of increase is superior to other standard lifestyle modifications. In terms of CVD indicators, the ratio of TG/HDL-C is considered one of the better indicators of risk. Because both components improve with carbohydrate restriction, this ratio emphasizes benefits of such interventions.

4.4. Carbohydrate restriction and LDL particle size

An odd conundrum confronting both researchers and clinicians is an apparent dichotomy, as risk markers, between LDL-C and the other indicators, TG and HDL-C. An evolving picture of how this problem may be resolved lies in the heterogeneity of LDL particles. Small, dense LDL particles are believed to be associated with greater atherogenic potential. Krauss and coworkers have carried out comprehensive studies of this effect and have identified a genetically influenced state, referred to as pattern B, characterized by a preponderance of small LDL particles. People with this pattern, estimated to be 30% of the American population, respond to low-fat diets by lowering LDL although the pattern B persists [92,93]. In distinction, people with so-called pattern A, whose plasma is characterized by larger, more buoyant particles, respond to fat reduction with a shift to the more atherogenic pattern B. While genetic predisposition and particular mutations affect the response to diet, it appears that in most cases, replacing carbohydrate with fat or protein leads to improvement in LDL size distribution [94] and Krauss made the generalization that "carbohydrate rather than fat is a major dietary determinant of expression or phenotype B in susceptible individuals". It appears, in fact, that almost everybody is susceptible and it is now clear that carbohydrate intake is strongly and linearly related to the prevalence of pattern B [94]. In summary, improvements in LDL-C seen in low-fat dietary interventions must be considered imprecise in the absence of information about particle size.

The incorporation of small, dense LDL particles as an indicator of MetS provides a unifying view of atherogenic dyslipidemia. Amelioration of the phenomenon with reduced carbohydrate provides further support for a central role for response to carbohydrate as the underlying physiologic trigger. Internal correlations among these markers are as expected and pattern B is usually associated with elevated TG and low HDL. In seeking a convenient marker of CVD risk in insulin-resistant people, McLaughlin et al. [95] measured LDL particle diameter and subclass in comparison with traditional markers. They found that the TG/HDL ratio was the best predictor of insulin resistance and LDL particle diameter and came up with a cut-off of >3.5 as a simple means of identifying dyslipidemic patients.

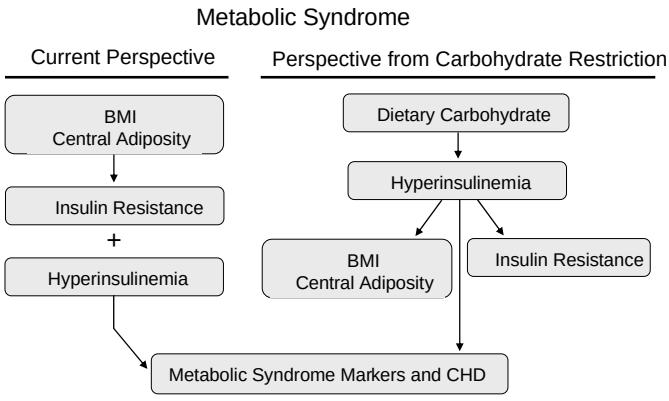

Fig. 3. Perspectives of metabolic syndrome (BMI, body mass index; CHD, coronary heart disease).

4.5. Mechanisms of carbohydrate associated atherogenic dyslipidemia

A low HDL-C concentration and the prevalence of small LDL particles leading to high concentrations of apo B-containing lipoproteins are often metabolically linked to and appear in parallel with hypertriglyceridemia. Overproduction of apo B-100-containing very low-density lipoprotein (VLDL), decreased catabolism of apo B-containing particles and increased catabolism of apo A-I-containing HDL-C represent converging pathways linking this dyslipidemic triad [96]. Each is reinforced by high dietary carbohydrate and ameliorated by carbohydrate restriction. Low-carbohydrate diets reduce very low-density lipoprotein (VLDL) production through several mechanisms. Lower glucose and insulin concentrations concomitantly reduce ChREBP and SREBP1c expression that activate key lipogenic enzymes (discussed in Section 2) thereby decreasing hepatic lipogenesis and $VLDL_1$ production [97]. Conversely, high dietary carbohydrate leads to overproduction of larger TG-enriched VLDL particles [98–100] contributing to the formation of small LDL particles and low HDL-C [94]. Secretion of larger $VLDL_1$ particles that are enriched with TG is associated with the generation of small LDL particles [101] and increased catabolism of HDL-C [102,103].

In addition to the effect on VLDL metabolism, as noted above, reduced lipogenesis associated with carbohydrate restriction leads to decreases in malonyl-CoA concentration and reduced inhibition of carnitine acyltransferase allowing for enhanced mitochondrial transport and oxidation of fatty acids. Decreased glucose also limits glycerol-3-phosphate production for the re-esterification of free fatty acids.

4.6. Weight loss: stimulus or response?

The current paradigm for explaining the pathogenesis of MetS emphasizes excess body fat as the primary impetus for inducing dysregulation in glucose and lipid metabolism and other metabolic risk factors associated with the syndrome [104]. This viewpoint provides a serial mechanism whereby increases in body fat act as a *stimulus* to cause MetS, and it is proposed that improvements in metabolic risk factors are contingent on decreases in body fat. Weight and fat loss, however, do not occur spontaneously. They are a *response* to a change in dietary input. Our theory that the response to carbohydrate restriction might be an operational definition of MetS [3] is embodied in an alternative parallel mechanism (Fig. 3). According to this mechanism, hyperinsulinemia is the key stimulus and increase in body fat, dyslipidemia and insulin resistance are parallel responses.

5. Role of carbohydrate in fatty acid partitioning and inflammation

5.1. Fatty acid composition in metabolic syndrome and diabetes

Consistent with the underlying role of insulin resistance in dysregulated lipid metabolism, development of MetS has been shown to be associated with a unique fatty acid composition characterized by higher circulating saturated fats (14:0, 16:0) and lower levels of 18:2*n* 6, with higher proportions of palmitoleic acid (16:1*n* 7, the MUFA product derived from palmitic acid) and dihomo-γ-linolenic acid (20:3*n* 6, the precursor to arachidonic acid) [105]. We emphasize the role of saturated fats because of their historical link with cardiovascular risk and the long chain *n* 6 PUFA arachidonate because of its connection with inflammation.

5.2. Saturated fatty acids

The key question in assessing the effect of carbohydrate restriction is: what are the consequences of replacing dietary carbohydrate with SFA? Fig. 4 provides a schematic view of the questions to be asked. In each case, we will assess the effect of dietary SFA in general and, where this has been studied, whether the effects are perturbed by the reduction in dietary carbohydrate. Again, we think of carbohydrate, via insulin, as a control element in the metabolism of lipid. We anticipate that, for any particular process, if the K_m for insulin is exceeded, the outcome may be very different than when lower levels of the effector are present, that is, effects may not be linear. More generally, in considering the role of dietary SFA, it is *substitution* that must be considered: it is generally agreed that substitution of unsaturated fat, especially MUFA, for SFA is beneficial, while substitution of carbohydrate for SFA is not, and may be harmful.

5.2.1. Correlation of dietary SFA and CVD outcome

Because they address outcome, correlations between CVD incidence and dietary SFA (question 1 in Fig. 4), are usually considered critical. The condemnation of SFA originated with the Seven Countries Study which purported to show that countries with high SFA intake had higher CVD [106]. The study has been widely criticized for not including all available data and, in our view should be considered anachronistic [107,108].

Prospective studies are, in any case, always more substantive. The outcomes on these studies, however, are ambiguous at best [109]. The Women's Health Initiative [110] was one of the largest long-term randomized controlled trial: 48,835 postmenopausal

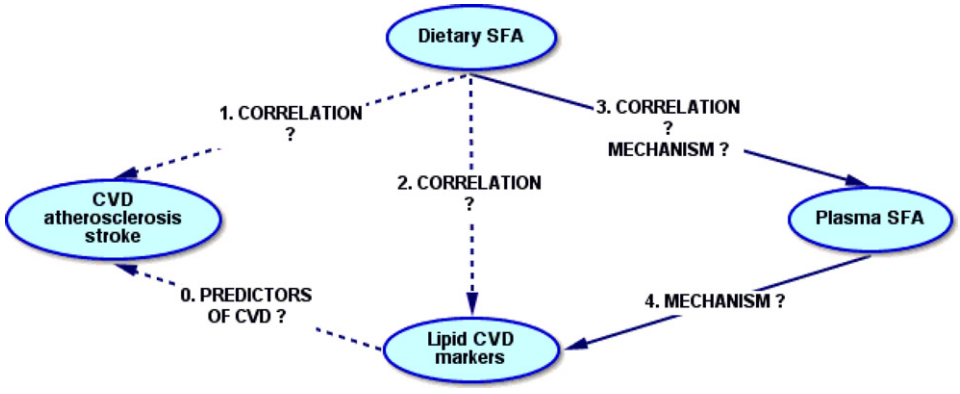

Fig. 4. Questions to be addressed in assessing the role of saturated fatty acids and the effect of carbohydrate restriction. Dotted lines represent correlations that are indirect or for which no mechanism is known. Solid lines represent processes that are directly linked or for which mechanisms are known.

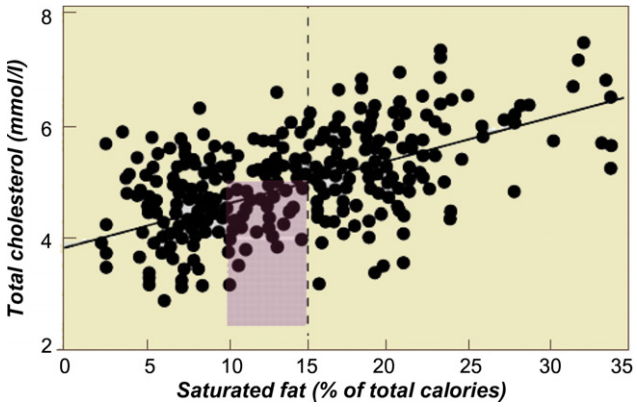

Fig. 5. Correlation of dietary saturated fat with total blood cholesterol. Shaded portion indicated to show expected benefit as saturated fat is lowered.

women were randomly assigned to an intervention designed to reduce total fat intake and to increase intakes of vegetables/fruits, or a comparison group provided with educational material. Results showed insignificant differences between those consuming less than 7% SFA and those consuming more than 14%. The interpretation of this study has to be seen in light of the blanket condemnation of SFA. In other words, given the number of factors in such a study, the failure of the WHI to show an effect of SFA may not exclude a role and may point to a need for further study, but the idea that SFA is inherently and unambiguously detrimental becomes fundamentally untenable and dietary recommendations for across-the-board reduction, fundamentally inappropriate.

5.2.2. Correlation of dietary fat with plasma CVD markers

The DRI guidelines of the Institute of Medicine recommend reductions in dietary SFA [111]. The logic of the argument is that dietary SFA is correlated with increases in plasma cholesterol and that cholesterol is a predictor of CVD; data from a review by Clarke et al. [112] of numerous studies are cited (Fig. 5). There are several limitations to this argument. The slope of the line is 0.067 mmol/L/% total calories (2.6 mg/dL/%), or an average reduction of 13 mg/dL for a reduction from the nominal average of 15% to the generally recommended 10% of calories. There is also a large variation and, although not statistically rigorous, the implication is that the data can be interpreted as a trajectory for changes in dietary saturated fat leading to reduction in plasma cholesterol. The shaded portion of Fig. 5 shows that whereas this is true, the possibility of cholesterol *increasing* with lowered saturated fat is almost as great. In addition, whatever the probability of improvement in cholesterol, it must be multiplied by the probability that the cholesterol will lead to lowered CVD risk. Total cholesterol is not a strong predictor and, overall, it is hard to consider Fig. 5 as evidence of substantial benefit to reducing SFA across the board.

5.2.3. The effect of dietary SFA on plasma SFA

Any effect on CVD outcome due to changes in dietary SFA is assumed to proceed by the corresponding appearance of this species in the plasma and the subsequent physiologic effect of plasma SFA. It is frequently stated that plasma fatty acid distribution reflects dietary intake. This is generally true of PUFA, although the absolute levels may not change much. It is not true of SFA. Dougherty, for example measured dietary intake in Italy (27.2% total fat), United States (43.5%) and Finland (56.7%) [113]. There was little difference in SFA in the plasma (17.4%, 17.4%, 18.9%, respectively). A prospective study by Dayton et al. [114] reduced dietary SFA by 50% at constant total fat. The distribution of stearic and palmitic acid was the same in the experimental and control group. Similarly, Raatz et al. [115] is widely quoted, as indicated by the title, of demonstrating

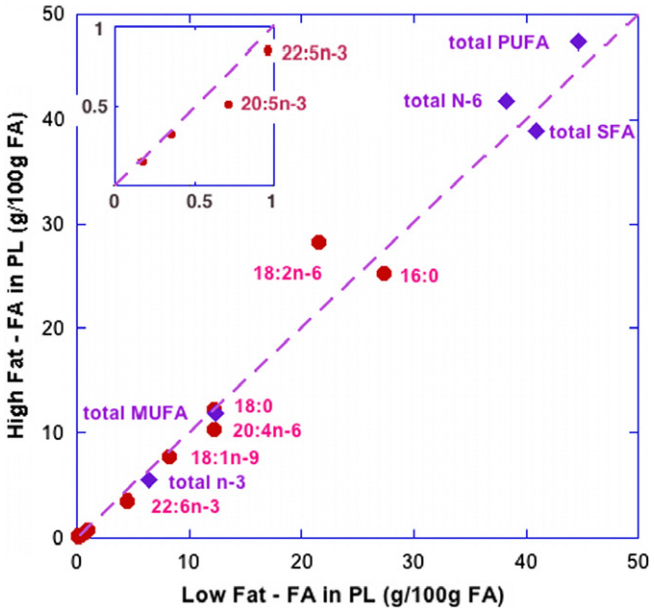

Fig. 6. Effect of total fat on plasma fatty acid profile. Individual FA in red. Total values in blue. Points to the left of the identity line (broken) indicate that higher fat favored the indicated FA: higher fat favored increases in plasma PUFA, particularly *n* 6 while low fat favored *n* 3 although the absolute value was small. Data from Raatz et al. [115] (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.).

"Total fat intake modifies plasma fatty acid composition in humans", but changes were in fact minimal (Fig. 6). Most striking, in the end, is the study from Volek and colleagues [8] which showed a reduction in plasma SFA in the low-carbohydrate arm of a dietary comparison in which this group consumed three times the amount of SFA as the low-fat arm (Fig. 7).

5.3. Arachidonate metabolism and inflammation

Low-carbohydrate diets result in profound alterations in PUFA, particularly arachidonate (20:4*n* 6) [8,116]. Arachidonate in

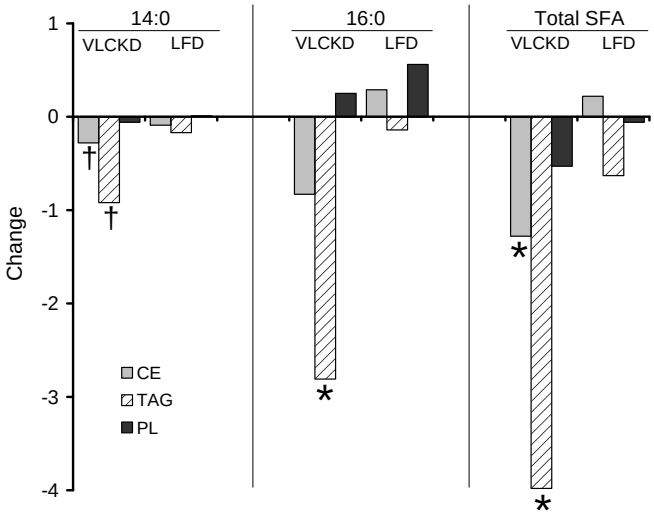

Fig. 7. Changes in circulating saturated fatty acids in response to 12 weeks of a very low-carbohydrate ketogenic diet (VLCKD) or a low-fat diet (LFD) in subjects with metabolic syndrome. $^{*}P < 0.05$ or $P < 0.01$ from corresponding LF value for that lipid fraction in plasma. Data from Forsythe et al. [8] (CE, cholesterol ester; TAG, triacylglycerol; PL, phospholipid).

membranes is commonly assumed to have a deleterious effect on overall inflammatory balance because of its enzymatic conversion to proinflammatory, prothrombotic, and vasoconstrictive eicosanoids (e.g., prostaglandin E_2, thromboxane A_2, leukotrienes B_4). Arachidonate is also capable of non-enzymatic conversion to other proinflammatory bioactive products (F_2-isoprostanes) via interaction with molecular oxygen. In contrast, eicosanoids derived from the 20-carbon *n* 3 PUFA, eicosapentaenoic acid (20:5*n* 3), have less potent inflammatory effects [117]. The negative connotation of increased arachidonate derives from the emphasis on its metabolites, but the intact fatty acid has a range of biologic functions including anti-inflammatory and lipid partitioning effects [118,119], and muscle membrane arachidonate is also positively correlated with insulin sensitivity [120]. Counter-intuitively, then, rather than being a negative factor within lipid membranes, increased arachidonate appears to be a beneficial outcome of weight-reducing diets associated with greater lipolysis [121].

In our recent study comparing low fat to VLCKD [8], we proposed that the increase in plasma arachidonate in response only to the low-carbohydrate diet was best explained by decreased degradation presumably due to less interaction with reactive oxygen species [122]. Increased production from 18:2*n* 6 was unlikely since the metabolic intermediates 18:3*n* 6 and 20:3*n* 6 were reduced in all three circulating fractions measured (TG, CE, and PL) and there was no increase in 20:3*n* 9, which typically occurs in cases where PUFA anabolism is increased. Since arachidonate was elevated in all circulating fractions in the low-carbohydrate diet, a shift from other pools was unlikely. Thus, an increase in the proportion of arachidonate resulting from a diet that restricts carbohydrate may be due to lower catabolism (i.e., better preservation) and, therefore, reduced formation of proinflammatory products.

Scenarios associated with less oxidative stress should result in better preservation of the substrate arachidonate since free radicals take part in several steps in its metabolism. Inflammatory cytokines are known to increase production of hydroxyl radicals which in turn initiate arachidonic acid release and breakdown. The VLCKD in this study resulted in significantly greater reductions in several proinflammatory markers including TNF-α, E-selectin, ICAM-1, and IL-8, that were associated with the increase in arachidonate. The significantly greater reduction in TNF-α in subjects following the VLCKD is of interest in that it is one of the agents known to activate NF-κB a major transcription factor regulating cytokines, chemokines and adhesion molecules (TNF-α, MCP-1, IL-8, E-selectin, and ICAM-1) [123,124]. The reduction in all of these agents by the VLCKD suggests that the anti-inflammatory effects of carbohydrate restriction may be mediated via down regulation of NF-κB expression [125]. In macrophages, SFA activate toll-like receptor signaling leading to activation of nuclear factor-kappa B (NF-κB) and expression of cyclooxygenase-2 [126]. NF-κB is a transcription factor that regulates over 100 genes, many with an established role in inflammatory responses and atherosclerosis, and may therefore represent a crucial link between fatty acids, MetS and atherogenesis [123]. As noted above, TNF-α is an activator of NF-κB expression and we have previously found that guinea pigs fed high-cholesterol atherogenic diets demonstrated significant increases in aortic TNF-α, an effect that was attenuated by reduction in dietary carbohydrate [127].

6. Discussion and recommendations

Discussion or even acknowledgement of carbohydrate restriction as an effective lifestyle modification to treat atherogenic dyslipidemia is notably absent from much of the current literature [88–90,128]. Among researchers examining cellular mechanisms of hypertriglyceridemia there is a general disregard for carbohydrate restriction as a potent modulator of TG levels [29,129]. The generally negative response of the medical community to low-carbohydrate diets has meant that the links between dietary effects of carbohydrate and downstream metabolic changes in lipid metabolism have remained unexplored. It is argued here that progress in the field will depend on our ability to put historical controversies behind us and to integrate nutritional outcomes with underlying biochemical and cell biological processes.

The results of several prospective low-carbohydrate diet studies have been presented that point to a consistent improvement in traditional features of MetS. Metabolic syndrome consists of additional features related to defective insulin action including inflammation and altered fatty acid partitioning. The role of dietary carbohydrate in contributing to these emerging aspects of MetS has been presented. The preponderance of evidence indicates that restricting dietary carbohydrate positively impacts fatty acid composition and inflammation, even when the isocaloric replacement of dietary carbohydrate by fat increases ones intake of SFA.

We encourage continued cellular work that considers dietary carbohydrate as an upstream control element in regulating cellular metabolic responses. Based on metabolic principles and the experimental evidence to date, we believe there is a strong case for conducting a major prospective trial of carbohydrate restriction, compared to a fat restriction control group, on cardiovascular endpoints.

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