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Low-carbohydrate ketogenic diets, glucose homeostasis, and nonalcoholic fatty liver disease

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Abstract

Purpose of review—Obesity-associated nonalcoholic fatty liver disease (NAFLD) is highly prevalent, for which weight loss is the generally recommended clinical management. Low-carbohydrate ketogenic diets have been successful in promoting weight loss, but variations in the range of metabolic responses to these diets indicate that the effects of altering macronutrient content are not completely understood. This review focuses on the most recent findings that reveal the relationship between low-carbohydrate diets and NAFLD in rodent models and humans.

Recent findings—Low-carbohydrate diets have been shown to promote weight loss, decrease intrahepatic triglyceride content, and improve metabolic parameters of patients with obesity. These ketogenic diets also provoke weight loss in rodents. However, long-term maintenance on a ketogenic diet stimulates the development of NAFLD and systemic glucose intolerance in mice. The relationship between ketogenic diets and systemic insulin resistance in both humans and rodents remains to be elucidated.

Summary—Because low-carbohydrate ketogenic diets are increasingly employed for treatment of obesity, NAFLD, and neurological diseases such as epilepsy, understanding the long-term systemic effects of low-carbohydrate diets is crucial to the development of efficacious and safe dietary interventions.

Keywords

insulin resistance; ketogenesis; ketolysis; tricarboxylic acid cycle; methionine-choline deficient diets

Introduction

The incidence of cardiovascular disease attributable to obesity, insulin resistance, and diabetes has markedly increased [1, 2*]. Insulin resistance is highly correlated with ectopic lipid accumulation, particularly in the liver. Consequently, the pathogeneses of systemic insulin resistance and diabetes have been linked to nonalcoholic fatty liver disease (NAFLD). NAFLD is an independent predictor of cardiovascular disease – a stronger predictor than peripheral or visceral fat mass [3–4, 5*, 6*]. A critical, but as yet only preliminarily defined influence over the development of NAFLD is distribution of macronutrient classes within the diet. Recently, attention has focused on the use of low-carbohydrate diets and their efficacy in controlling metabolic diseases including NAFLD [7]. However, while low-carbohydrate diets are effective for weight loss, seizure disorders,

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and potentially a host of other neurological diseases, determination of their relationships with metabolic responses, and fatty liver in particular, remains ongoing. It is because therapeutic application of low-carbohydrate diets is likely going to increase that further understanding of the range of metabolic responses observed, and the precise nutritional determinants of these responses, is so timely. The importance of further understanding the impact of low-carbohydrate diets is underscored by case reports of humans that reveal variations in the range of metabolic responses to these diets [8, 9]. This review examines the metabolic responses of rodents and humans to low-carbohydrate diets, and will elucidate critical unanswered questions that merit follow-up pre-clinical and clinical evaluation.

NAFLD: epidemiologic and metabolic considerations

The earliest stage of NAFLD is hepatic steatosis, which is defined by intrahepatic triglyceride (IHTG) concentrations exceeding 55 mg/g liver (5.5%), or when greater than 5% of hepatocytes harbor histological evidence of triglyceride storage [6*, 10]. NAFLD prevalence is 15% in non-obese patients, but increases in obese (BMI=30.0–39.9 kg/m^2) and extremely obese (BMI≥40.0 kg/m^2) patients to 65% and 85%, respectively. In addition to its association with systemic insulin resistance and adverse cardiovascular outcomes, NAFLD can progress to nonalcoholic steatohepatitis (NASH), which is characterized by steatosis and signs of hepatocyte injury, hepatic inflammation with collagen deposition, and elevation of serum alanine aminotransferase (ALT). Approximately 10–29% of patients with NASH will develop cirrhosis within 10 years, which can evolve to hepatocellular carcinoma. Informative reviews on the causes, stages, and implications of NAFLD have been published recently, but whether NASH can develop independently of a NAFLD stage remains to be fully explored [6*, 10, 11*].

Increased IHTG is caused by imbalance between hepatocellular triglyceride formation and removal. Therefore, multiple hepatocyte pathways play roles, including rate of fatty acid uptake; esterification of fatty acids into, and lipolysis from, intracellular triacylglycerols (TAGs); rate of fatty acid oxidation (FAO); rate of TAG secretion as very low-density lipoproteins (VLDL); and de novo lipogenesis (DNL). Hepatic TAGs are synthesized from fatty acids that emanate from (i) DNL, (ii) lipolysis of triglycerides stored in adipose tissue, and/or (iii) from diet-derived fats that are packaged as chylomicrons in intestinal enterocytes. A significant contributor to increased hepatic triglyceride content in NAFLD is increased DNL, in which increased hepatocellular carbohydrate is converted to fat. Muscle, and possibly adipocyte, insulin resistance may contribute to increased hepatocyte carbohydrate by diversion of glucose to the liver. In addition, magnetic resonance spectroscopy (MRS) of human livers recently revealed that patients with NAFLD exhibit augmented delivery of fatty acids, through increased peripheral lipolysis; elevated hepatocellular flux through the tricarboxylic acid (TCA) cycle; and increased gluconeogenesis, compared to NAFLD-free subjects [12**].

Classical diet models of NAFLD

A detailed review of rodent dietary models of NAFLD has recently been published [13*]. High-carbohydrate/low-fat, low-carbohydrate/high-fat, and high-carbohydrate/high-fat formulations have all been studied, as have the contributions of *trans*, monounsaturated, polyunsaturated, and chain length of fatty acids [14–18]. The role of nutritional methionine and choline contents are also important, because seminal rodent experiments have revealed molecular underpinnings of NAFLD pathogenesis using methionine and choline deficient (MCD) diets. MCD diets commonly include high sucrose and fat contents, producing exuberant and rapid (within two weeks) NASH, particularly when carbohydrate content is highly enriched as either glucose or the particularly lipogenic monosaccharide fructose, as

opposed to starch [19*, 20*, 21]. Despite robust IHTG and inflammation, MCD models do not exhibit systemic insulin resistance, and MCD diets induce a catabolic state that includes muscle wasting.

Choline is a major constituent of plasma and mitochondrial membranes, as well as the neurotransmitter acetylcholine, and is an essential nutrient that is particularly abundant in animal proteins. The majority of choline is metabolized via either phosphorylation, to supply phospholipids for membrane synthesis, or oxidized and ultimately metabolized to S-adenosyl-L-methionine (SAM), a universal methyl donor [22]. The former pathway has been cited to support the notion that choline deficiency contributes to NAFLD by reducing VLDL packaging and secretion. However, choline deficiency also contributes to mitochondrial dysfunction, and therefore to FAO deficiencies, and has also been linked to increased fatty acid uptake. In a study conducted to understand the contributions of methionine and choline deficiency independently, mice were place on a conventional MCD diet, versus methionine deficient (MD) or choline deficient (CD) diets. Over two weeks, the MD diet reproduced many of the deleterious effects of the MCD diet including weight loss, hepatocellular injury, decreased mitochondrial SAM and glutathione, inflammation and fibrosis, whereas choline deficiency caused only steatosis [23*].

Ketone metabolism and low-carbohydrate (ketogenic) diets

The liver is a major destination for fatty acids derived from lipolysis of adipose stores and from TAG-derived lipoproteins. Hepatic FAO converts fatty acids to acetyl-CoA, which condenses with oxaloacetate in the TCA cycle, whose electrons are used in the electron transport chain to generate high-energy phosphates. During states of high fatty acid mitochondrial delivery, much FAO-derived acetyl-CoA is diverted from the TCA cycle to ketogenesis, generating acetoacetate (AcAc) and β-hydroxybutyrate (βOHB). Ketogenesis is particularly stimulated during low insulin states [24*]. Liver abundantly expresses the key ketogenic enzyme, mitochondrial HMG-CoA synthase, which is activated by Sirtuin 3-mediated deacetylation, and whose gene *HMGCS2* is negatively regulated through transcriptional mechanisms downstream of insulin signaling [25, 26]. Because hepatic mitochondria lack the enzyme necessary for oxidizing ketone bodies, ketones are secreted and delivered to heart, skeletal muscle and brain, which abundantly express the mitochondrial matrix enzyme succinyl-CoA:3-oxoacid-CoA transferase (SCOT, encoded by *OXCT1*), which is required to convert ketone bodies to acetyl-CoA for terminal oxidation in the TCA cycle [27]. Thus, hepatic ketogenesis helps maintain TCA cycle homeostasis, prevents the accumulation of incompletely oxidized fatty acid intermediates, maintains hepatic redox balance, and supplies extrahepatic organs with energy substrates in glucose-limiting states that include fasting, poorly-controlled diabetes, and during adherence to low-carbohydrate, high-fat ketogenic diets (KD). While individuals with NAFLD have also been reported to exhibit higher circulating ketone concentrations, likely due to increased rates of FAO, βOHB turnover rates are not increased in individuals with NAFLD [12**]. Increased TCA flux in NAFLD livers may partially explain normal βOHB turnover rates, but prospective roles for variation of ketolytic flux through SCOT, and of AcAc turnover, remain to be determined.

Ketogenic diets are actively used for weight loss and anticonvulsant therapy, and are intensively studied as potential adjunctive therapy for brain cancers and neurodegenerative diseases including Parkinson's and Alzheimer's [28–32]. In humans, diets with caloric contents of up to 75–80% fat and ≥15% protein are commonly used for management of seizure disorders (e.g., 4:1 prescriptions, in which calories are derived from four parts fat, and one part protein + carbohydrate), and Atkins diets for weight loss typically consist of 60–70% fat and up to 30% protein. Anticonvulsant benefits of KDs persist for ≥6 years in

patients following discontinuation [33]. While the mechanism(s) of KD's salutary neurological effects have not been fully elucidated, observations from a number of genetic and acquired epilepsy models in rodents have implicated a dysregulation of the mammalian target of rapamycin (mTOR) pathway, neuron-astrocyte cross-talk in neurotransmitter metabolism, and manipulation of hypothalamic hormone signaling [32–36].

Not all patients can tolerate KD regimens, which can be unpalatable, and scrupulous attention to micronutrient content and overall caloric needs is required. Hyperuricemia, hypocalcemia, hypomagnesemia, nephrolithiasis, and of course, ketoacidosis can result. Rare reports implicate the KD in cardiac complications, including cardiomyopathy, prolonged QTc interval and torsades de pointes arrhythmias [8]. However, this response appears to be sporadic, as a systemic analysis of 27 children adhering to prescribed KD for treatment of refractory epilepsy revealed no changes in QTc interval over 12 months [37]. Nonetheless, these data underscore the importance of careful patient monitoring, and perhaps expansion of monitoring guidelines.

Ketogenic diets and NAFLD in humans and rodents

Promotion of weight loss is often recommended to obese patients with hepatic steatosis, and is frequently achieved through a reduction of caloric intake. While intensively studied, the efficacy and safety of shifts in macronutrient content still remain to be determined in a systematic manner – particularly with respect to effects on NAFLD. A small clinical trial of 18 patients used MRS to measure change in IHTG following two weeks of either a calorie-restricted diet, or a carbohydrate-restricted diet that was not calorie-restricted, and found that patients in the carbohydrate-restricted arm exhibited a more profound IHTG reduction, without any difference in overall weight loss, compared to the calorie restricted arm [38**]. A recent two-year multi-center trial that included over 300 patients enrolled in a comprehensive lifestyle modification regimen observed similar weight loss achieved by adherence to low-fat, versus low-carbohydrate diets. The low-carbohydrate diet group exhibited superior HDL cholesterol profiles [39**]. As expected, urinary ketosis was markedly more common in the low-carbohydrate group, but the influence of these regimens on IHTG was not presented. Comparing low-carbohydrate to reduced calorie or low-fat diets in clinical trials to date, systemic glucose homeostasis has not systematically differed.

Ketogenic diets have been extensively studied in rodents. Using a micronutrient supplemented KD (Bio-Serv F3666) very high in fat (93.3%kcal), very low in carbohydrate (1.8%), and also reduced in protein (4.7%), Meratos-Flier and colleagues observed that mice lose weight, develop ketosis, and induce hepatic gene expression signatures that suggest reduced DNL and increased FAO [40*]. To explore the relationships among KD, IHTG, and NAFLD, Garbow et al. maintained C57BL/6J mice for 12 weeks on either (i) Bio-Serv F3666 KD; (ii) a high-fat (40%kcal) `Western' diet (WD) also enriched in sucrose (40%); or (iii) a standard low-fat (13%kcal) polysaccharide-rich chow control diet [41**]. The KD is reduced in protein content due to the fact that induction of ketosis in rodents requires restriction of not only carbohydrates but also protein [42]. Mice fed the KD for 12 weeks were lean, euglycemic, ketotic, and hypoinsulinemic, but were glucose intolerant, and exhibited NAFLD. MRS revealed that KD-fed mice accumulate hepatic lipid within 3 weeks after initiation of the diet, and the hepatic gene expression signature for DNL (encoded mediators of SREBP-1c, FAS, ACC1, SCD1) was suppressed compared to livers of chow-fed controls. In contrast, mice fed the WD ultimately accumulate higher IHTG than KD-fed animals, but do so much more slowly, and as expected due to the high sucrose content, induce mediators of DNL. Intriguingly, unlike steatotic livers of WD-fed mice, livers of KD-fed mice developed hepatic endoplasmic reticulum (ER)-stress, inflammation, macrophage accumulation, and hepatocellular injury, and only KD-fed mice exhibited

elevated serum ALT concentrations. A number of non-mutually exclusive mechanisms may account for the murine hepatic phenotypes observed with this KD. First is the prospective influence of choline and methionine deficiencies. While choline-replete rodent diets are supplemented to contain ~2.5 g/kg, BioServ F3666 KD is not supplemented, and therefore contains only 200–300 mg/kg naturally-derived from the fat sources. Methionine content in this KD, derived from casein, is also reduced, at 2.2 g/kg, whereas methionine-replete diets contain ~4 g/kg. A second prospective contributor to the NAFLD signatures in KD-fed mice is overall reduction of protein: classical studies indicate that diets containing <14% protein retard normal growth, reproduction, and lactation in mice [43]. A third prospective influence is cellular injury though ER stress-inducing membrane remodeling in periportal hepatocytes that receive more fat than can be oxidized or exported via VLDL secretion. Fourth, ceramide production within macrophages or hepatocytes, favored by high intracellular concentrations of saturated fatty acids, may also trigger the inflammasome, whose biomarkers were selectively elevated in livers of KD-fed mice. Finally, splice variants of the insulin-sensitizing nuclear receptor transcription factor PPARγ may exhibit distinct activities in different steatotic contexts. While the `adipocyte' isoform form, PPARγ2, was induced in livers by WD, the `macrophage' isoform PPARγ1 was selectively induced in livers of KD-fed mice.

Hepatic fibroblast growth factor 21 (FGF21) has emerged as a key regulator of hepatic metabolism, glucose, and fatty acid oxidative flux, and insulin sensitivity [44, 45]. KD feeding of *Fgf21*$^{-/-}$ mice leads to weight gain, reduced ketosis, and hepatic steatosis relative to KD-fed wild type controls in two weeks, consistent with impaired ability of the liver to oxidize fatty acids in the absence of FGF21 [46]. Indeed, serum FGF21 concentrations are elevated in patients with diagnosed NAFLD, and obesity is an FGF21-resistant state [47, 48]. *Fgf21* mRNA is markedly induced in livers of mice fed either KD or WD for 12 weeks [41**].

Insulin resistance and ketogenic diets in rodentsM

KD-fed mice develop systemic glucose intolerance, and their livers exhibit ER stress, steatosis, cellular injury, and macrophage accumulation. However, indices of insulin resistance were not observed [41**]. Compared to chow-fed and WD-fed mice, KD-fed mice exhibited reduced homeostatic model assessment of insulin resistance (HOMA-IR) values, normal insulin-induced hepatic and skeletal muscle Akt phosphorylation, and increased whole-body glucose disposal by insulin tolerance test (ITT). Badman et al. also observed insulin-sensitizing effects of 7 weeks of this KD on obese leptin-deficient *ob/ob* mice: while neither obesity nor IHTG of *ob/ob* mice was ameliorated by feeding the KD, the diet did improve glucose intolerance, hyperinsulinema, and glucose disposal by ITT [49]. In apparent contradistinction, Shulman and colleagues observed a 350% increase in hepatic diacylglycerol content, PKCε activation, and decreased insulin signaling at the level of insulin receptor substrate IRS-2 tyrosine phosphorylation in wild-type mice fed this KD for 5 weeks [50*]. Furthermore, hyperinsulinemic-euglycemic clamp studies revealed impairment of insulin-mediated suppression of glucose production by livers of KD-fed wild-type mice. The apparent discordance of these findings by this latter group of investigators may be explained by two factors. First, liver and muscle may exhibit distinct phenotypes in KD-fed mice. Although KD-induced hepatic insulin resistance was observed in the clamp studies, impairment of insulin-stimulated peripheral glucose disposal in KD-fed mice was much more subtle. Therefore, enhanced systemic response to insulin (by ITT) in KD-fed mice likely reflects augmentation of insulin-mediated peripheral glucose disposal, relative to the chow group, that overrides any prospective impairment of insulin-mediated suppression of hepatic glucose production that may exist in KD-fed mice. Second, hyperinsulinemic-euglycemic clamp studies in mice have proven controversial, due to variations of (i)

methods that normalize hepatic glucose output, (ii) insulin dosing regimens among investigators, and (iii) responses of humans versus anesthetized mice – especially those fed a KD, which creates an unusual hypoinsulinemic-euglycemic metabolic state, accompanied by markedly increased IHTG [51*, 52].

Important conclusions emerge from the heretofore performed studies of KD-fed mice. First, the relevance of the NAFLD signatures in KD-fed mice to human NAFLD pathophysiology remains to be determined. While it is intriguing that increased IHTG, inflammation, and ER stress may be dissociable from systemic insulin resistance, this constellation of findings is not unique in that it has been observed with MCD diets in rodents, which have also been criticized as less relevant to human NAFLD. Second, the combination of hypoinsulinemia, euglycemia, glucose intolerance, and enhanced insulin responsiveness by ITT all suggest that glucose-stimulated insulin secretion by pancreatic β-cells could be abnormal in KD-fed mice. Finally, while the use of this low-protein, very high-fat, low-carbohydrate diet is not directly relevant to macronutrient distributions ingested by humans, it does provide an important foundation to elucidate the range of metabolic responses that occur in states relevant to human physiology.

Conclusions

KDs are prescribed with increasing frequency for NAFLD, obesity, and neurological disease, and while they have beneficial attributes, their metabolic effects are not yet completely understood, and patient responses to these diets can be variable. Recent studies have provided insight into the contribution of macronutrient content on liver health and demonstrate the influences of shifting macronutrient class distributions. Therefore, future studies of low-carbohydrate diets in rodents and humans must take into consideration additional factors including the effects of low overall protein, choline and methionine content, plus the saturation and length of dietary fatty acids. Achieving macro- and micronutrient balance will be essential to developing efficacious diets that promote weight loss while maintaining systemic health.

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Key Points

- Low-carbohydrate ketogenic diets are effective for weight loss, seizure disorders, and potentially a host of other neurological diseases
- Attention has focused on the use of low-carbohydrate diets and their efficacy in controlling metabolic diseases including obesity and NAFLD, but determination of their relationships with metabolic responses remains ongoing
- Ketogenic diet-fed mice ultimately develop NAFLD signatures and systemic glucose intolerance, but whole-body insulin responsiveness is not impaired
- Clinical and rodent research studies demonstrate a range of metabolic responses to low-carbohydrate diets, underscoring the importance of studying the metabolic effects of macro- and micronutrient dietary balance