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Hypothesis

An overlooked danger of ketogenic diets: Making the case that ketone bodies induce vascular damage by the same mechanisms as glucose

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ABSTRACT

Intense debate surrounds the use of low-carbohydrate, ketogenic diets for the promotion of weight loss and avoidance of cardiovascular disease. The rationale behind these diets is that they promote fat oxidation and minimize the addition of glucose to proteins in the formation of adducts that trigger inflammation.
Although nutritional ketosis is widely assumed to be a safe metabolic condition, proper consideration has not been given to the fact that ketones are reactive toward proteins through the same mechanisms as glucose. Here, the case is made that ketone bodies are more potent than glucose in bringing about the protein modifications to which the harmful effects of glucose have been attributed. It is suggested, therefore, that attempts to minimize such protein modifications through nutritional ketosis are futile and may lead to adverse health outcomes.

Introduction

The level of dietary carbohydrate required for optimum health continues to be the subject of intense debate [1–6]. A recent prospective study of 15 428 adults, together with the meta-analysis of seven other multinational prospective studies, concluded that both high- and low-carbohydrate diets are associated with increased mortality [2]. Government guidelines in the United Kingdom and the United States recognize carbohydrates as an important energy source in balanced diets [7,8].

Contrary to these guidelines, the potential benefits of diets very low in carbohydrates have received much attention in recent years [3,9,10]. Such diets induce ketosis, in which the shift to fatty acids as the main respiratory substrate leads to the increased production of ketone bodies (acetone, acetoacetate, and β-hydroxybutyrate), whose serum levels increase into the millimolar range [3,11]. It has been suggested that ketogenic diets provide a safe and effective means of promoting weight loss and improving the health outcomes in a range of conditions, including diabetes and cardiovascular disease (CVD) [3,4].

Ketosis, however, is not without risks: The dangers of ketone bodies at very high concentrations, as seen in diabetic ketoacidosis, are widely documented [11]. There are also compelling reasons to question the safety of the milder elevations in blood ketone bodies achieved by dietary means, especially over longer time periods. For example, cardiac complications have been reported in children with epilepsy on ketogenic diets [12]. Perhaps the most thorough studies demonstrating a link between mild ketosis, inflammation, and vascular injury, however, have been carried out on patients with type 1 diabetes. Jain et al., for example, found patients with mildly elevated plasma ketone bodies (0.49 ± 0.08 mmol/mL) to have significantly higher plasma levels of tumor necrosis factor (TNF)-α compared with those with lower levels of ketone bodies (0.23 ± 0.03 mmol/mL) [13]. Jain et al. also reported a statistically significant correlation between the degree of ketosis and plasma protein carbonyls, which reflect levels of oxidative stress. Although obtained from studies on individuals with diabetes, these findings are of direct relevance to dietary ketosis, not least because the authors demonstrated that the increases in TNF-α (a risk factor for vascular inflammation) and protein carbonyls were related to the degree of ketosis, rather than blood glucose [13]. Other studies have shown that mild hyperketonemia is associated with increased lipid peroxidation, which is also linked to CVD [14].

Whereas there is evidence that ketogenic diets lead to short-term improvements in some cardiovascular risk factors, there is no clear-cut evidence of a long-term benefit [9,10,15]. If anything, the evidence points to a detrimental effect [5,6,9,15]. A study of 43 396 Swedish women, for example, showed that low-carbohydrate diets are associated with an increased risk for CVD [5]. A meta-analysis review, however, found that the association between low-carbohydrate diets and increased cardiovascular mortality was not statistically significant [6].

Notwithstanding the need for further studies into the long-term health implications of ketogenic diets, they have

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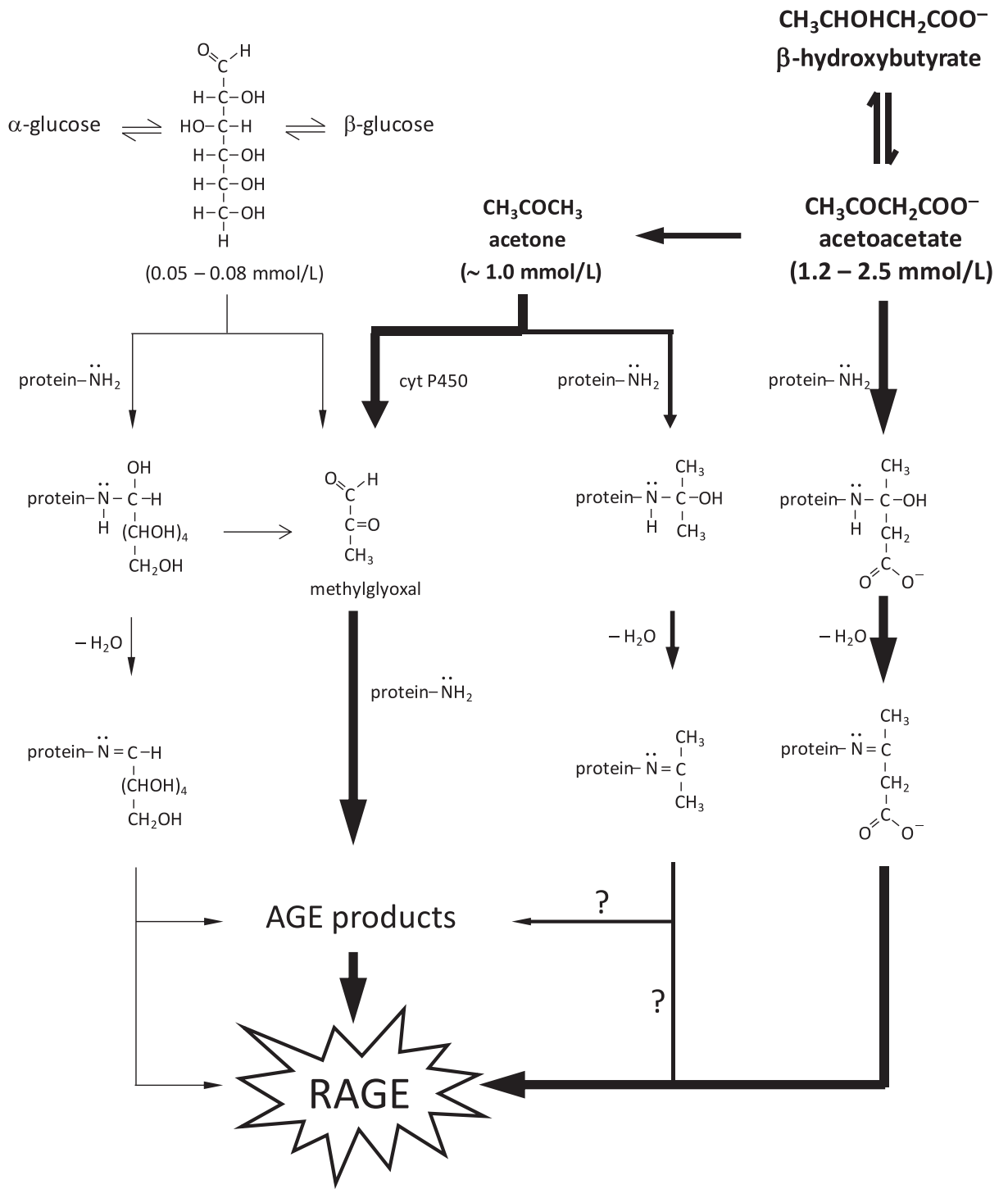

Fig. 1. The major pathways through which glucose and ketone bodies are proposed to add to proteins to form adducts capable of activating receptors of advanced glycation end product. The relative importance of each route is indicated by the thickness of the appropriate arrow. No more than 0.01% of glucose in solution is present in its ring-opened form [31]: the concentration range given in the figure (0.05 – 0.08 mmol/L) assumes a total serum glucose level of between 5.0 and 8.0 mmol/L (encompassing pre-fed levels and those following a meal [30]). The concentration of ketone bodies in nutritional ketosis is usually given as being up to 7 – 8 mmol/L [3]. Based on the values reported for fasting and diabetic subjects with serum levels of β-hydroxybutyrate and acetoacetate similar to those seen in nutritional ketosis [11,35–37], the serum concentration of acetoacetate during nutritional ketosis is estimated to be between 1.2 and 2.5 mmol/L, which is on average approximately 30 times higher than that of glucose in its ring-opened form. The concentration of acetone given is based on reported values for fasted individuals with total ketone body levels similar to those seen in nutritional ketosis [37].

been deemed to be "likely unsafe," and it has been concluded that measures should be taken to prevent the elevation of ketone bodies, except where considered clinically necessary [6,15]. This position is supported by evidence from studies conducted in mice, revealing wider adverse health implications of ketogenic diets, including glucose intolerance, reduction in β- and α-cell masses, and neurodegeneration [16,17].

The harmful effects of excessive glucose are due primarily to its reaction with proteins, resulting in the formation of advanced glycation end (AGE) products, which stimulate inflammation and vascular damage [18–20]. In this article, the pathways through which ketone bodies can induce the same protein modifications as those responsible for the vascular damage caused by glucose are described and evaluated. The case is made that nutritional ketosis is a harmful metabolic state and should not be recommended for the promotion of weight loss or the prevention of disease.

Key role of protein glycation in the vascular damage caused by glucose

The initial step in the formation of an AGE product is the addition of glucose to an amine, typically at a lysine residue of a protein [18,19]. This non-enzymatic reaction is responsible for the formation of glycated hemoglobin, the levels of which are used to assess the long-term management of blood glucose in individuals with diabetes [21]. The reactions that immediately follow the attachment of glucose to a protein are well characterized, but then numerous possible chemical changes allow for the generation of a wide variety of AGE products (Fig. 1). Despite the structural diversity of the AGE products, they appear to bind to a common set of cell surface immunoglobulins called receptors of AGE (RAGE) [19,20,22]. Ligand binding to RAGE on the surface of vascular endothelial and smooth muscle cells, and macrophages, results in the activation of proinflammatory pathways that lead to vascular dysfunction and atherosclerosis [23,24]. It is becoming increasingly evident that activation of the RAGE pathway underlies the enhanced risk for cardiovascular morbidity and mortality in individuals with diabetes [23–25].

For glucose to attach to an amine group on a protein, it must be in its ring-opened form [18,19]. It is only in this form that glucose has an aldehyde group, which renders it susceptible to nucleophilic attack by the nitrogen atom of the amine, resulting in the formation of a covalent bond linking the sugar to the protein (Fig. 1). It is well known that ketones undergo essentially identical reactions with nucleophiles, yet this route to protein modification appears to have been overlooked by those extolling the virtues of ketogenic diets.

Protein modification by ketone bodies

Although ketones are slightly less reactive than aldehydes toward nucleophiles, the rates of adduct formation between molecules with these groups and amines at physiologic pH values is limited not by the initial encounter with the amine, but by the ease with which the resulting adduct releases water to form an imine [26,27]. Reported rate constants for the addition of amines to various ketones, including acetone (a ketone body), are of the order 0.1 /M /s [27,28]. The corresponding values for the reactions of glucose with amino acids and proteins are considerably lower [29], perhaps owing to steric factors. Needless to say, studies on protein glycation by glucose involve incubation periods typically measured in days [18].

The relatively high serum concentrations of acetone and, especially, acetoacetate during nutritional ketosis suggest that protein modification by these species is likely to be far more significant than that caused by glucose, even after ingestion of a carbohydrate-laden meal, when serum glucose levels might be in the 6 to 8 mmol/L range [30]. This is because no more than 0.01% of glucose is present in the ring-opened form (the majority is present as the cyclic forms, α-D- and β-D-glucose) [31], thereby diminishing its rate of addition to proteins.

Given the broad specificity of RAGE [20,22], it is expected that proteins modified by the addition of acetoacetate are likely to act as ligands and provoke similar responses to those modified by glucose. This is supported by data suggesting that ligand binding is driven by electrostatic interactions between the positively-charged surface of the receptor and negatively-charged ligands, bearing carboxylate groups [22]. Indeed, one important glucose-derived AGE product that acts as an effective ligand is the carboxymethyl-lysine residue ($lys{-}NHCH_2COO^-$) [22], which is similar in structure and charge profile to the residue formed after the addition of acetoacetate to lysine ($lys{-}NC(CH_3)CH_2COO^-$) (Fig. 1).

Methylglyoxal: A harmful metabolite common to glucose and ketone bodies

Methylglyoxal (CH_3COCHO) plays a key role in the toxicity of glucose [18,32]. It is formed from glycolytic intermediates [32], by the fragmentation of glucose, and following the addition of glucose to proteins (Fig. 1) [18]. Being both an aldehyde and a ketone, methylglyoxal is highly reactive in glycation-type reactions and gives rise to a variety of AGE products, including those involving the amine groups in DNA [32]. It is highly relevant, therefore, that methylglyoxal is formed from ketone bodies, which will constitute a significant source of the species during ketosis [32,33].

The fact that ketone bodies are metabolized to methylglyoxal, a major player in glucotoxicity, further discredits the reasoning behind arguments that the harmful effects of glucose can be avoided through dietary ketosis. In a similar vein, the demonstration that acetol, a metabolite of acetone, reacts with proteins to form carboxymethyl-lysine (mentioned previously as an important AGE product [22]) [34], also argues to the futility of nutritional ketosis in avoiding the harmful effects of glucose.

Conclusions and the wider picture

Although very low-carbohydrate diets may be beneficial to individuals with specific medical conditions, including epilepsy and diabetes [1,3,10], there is sufficient evidence from population, animal, and cell studies to question the long-term safely of such diets [2,5,6,9,10,12–17]. In particular, it cannot be assumed that the beneficial effects of low-carbohydrate diets for those with diabetes—which is, after all, a disorder of carbohydrate handling—are applicable to individuals without the condition.

The pathways described in this article, based on the well-documented chemical properties of carbonyl compounds, including ketone bodies and glucose, provide a credible mechanistic basis with which to underpin the widespread concerns surrounding the long-term safety of ketogenic diets. Specifically, the case is made that ketones can modify proteins through mechanisms that are essentially identical to those responsible for the “toxicity” of glucose. Given that in nutritional ketosis, the levels of ketones in the blood far exceed those of the ring-opened form of glucose (Fig. 1), the case is made that, over the longer term, the dangers presented by ketogenic diets are real and therefore such diets should not be recommended to the general population.

CRediT authorship contribution statement

Mark J. Burkitt: Conceptualization, Writing - original draft, Writing - review & editing.

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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