Induction of Hepatic Uncoupling Protein 2 May Mediate the "Metabolic Advantage" of Ketogenic Diets

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Abstract

Several clinical studies demonstrate that weight and body fat drop more rapidly on ketogenic diets than on diets of identical calorie content that are higher in carbohydrates; Dr. Robert Atkins referred to this as the "metabolic advantage" of ketogenic diets. It is reasonable to suspect that this phenomenon reflects hepatic thermogenesis driven by rapid oxidation of fatty acids – analogous to the increase in metabolic rate provoked by the ingestion of medium-chain triglycerides. In mice, ketogenic diets have been shown to markedly boost the expression of uncoupling protein 2 (UCP2). During ketogenesis, when fatty acyl-CoAs are oxidized in hepatic mitochondria at a high rate and electron transport chains become glutted with electrons, mitochondrially-generated oxidative stress may lead to UCP2 induction as a compensatory antioxidant response. Foxo transcription factors, the activity of which is often boosted by oxidative stress, and which can promote transcription of the UCP2 gene in certain tissues, are likely mediators of this induction. If ketogenic diets do indeed indeed increase hepatocyte UCP2 expression in humans, this evidently would amplify hepatic thermogenesis. Intermittent ketogenic dieting may be useful for weight control and neuroprotection; this may be optimally safe and health protective if plant-based foods are employed and potassium citrate or bicarbonate is administered concurrently.

Thermogenesis May be Evoked by Ketogenic Diets

Dr. Robert Atkins, who popularized very-low-carbohydrate, high-fat ketogenic diets as a weight loss strategy, long maintained that such diets had a "metabolic advantage", in the sense that, at any given daily calorie intake, they achieved a more rapid loss of weight and body fat than diets with a higher carbohydrate content.[1] In support of this view, he cited several clinical studies in which the impact of hypocaloric ketogenic diets on the weight and body composition of overweight volunteers was compared to that of higher-carbohydrate diets of identical calorie content. In four such studies, all calories were provided as formula diets, or all food was carefully measured and consumed in a clinical setting; in this way, the calorie and macronutrient intakes of the volunteers were rigorously controlled, and the impact of the diets on appetite control was rendered irrelevant as a determinant of weight outcomes.[2-5] Three of these studies did indeed conclude that weight was lost considerably faster on the ketogenic diets; the study of Young et al., which measured body composition by immersion densitometry, further concluded that fat loss was greater on such diets.[3] For example, Rabast and colleagues determined that average daily weight loss over 30 days of dieting was 298 g on the high-carbohydrate diet, versus 362 g on the ketogenic diet ($p<0.05$).[4] In the Young study, fat loss over 9 weeks (on 1800 kcals daily) averaged 8.4 kg in the high-carbohydrate group, and 14.8 in the ketogenic group (30 g of carbohydrates daily).[3]

Although it is commonly held that loss of body water and glycogen accounts for the initial rapid weight loss on ketogenic diets, the authors of these studies concluded uniformly that this phenomenon could not account for their findings. One would expect the loss of body water and glycogen evoked by a ketogenic diet to be maximized within several days – yet the differential in daily weight loss persisted throughout

the studies. For example, the weight differential after 5 days in the Rabast study was 0.64 kg; this differential was 1.96 kg after 30 days.[4] There is no indication that the subjects receiving the high-fat diets experienced notable fat malabsorption that might have explained their greater weight losses; in the earliest of these studies, actual measurement of fecal fat confirmed the absence of malabsorption.[2] And, although ketone bodies are lost in urine during a ketogenic diet, this likely influenced calorie balance only marginally. For example, urinary ketone levels are in the ballpark of 1 g per liter during ketogenic diets;[6] if one assumes a daily fluid intake of 3 liters and a calorie content of 5 kcal/g for ketone bodies, urinary ketone loss would amount to only 15 kcals daily, or 450 kcals per month – equivalent to about 50 g of stored fat. The subjects in the Young study were encouraged to maintain their usual activity levels, and a post-study survey confirmed that differences in physical activity were unlikely to explain the weight loss differential. In aggregate, these considerations strongly suggest that greater thermogenesis associated with the ketogenic diets was primarily responsible for their greater impact on weight and body fat.

A study by Golay and colleagues, also cited by Atkins, reached a dissenting conclusion.[5] In this study, obese subjects consumed 1000 kcal whole-food diets, providing either 15% or 45% carbohydrates, for 6 weeks. Loss of weight and body fat did not differ significantly between the two groups at the end of the trial. However, the absolute losses – 8.9 kg of weight, 9 kg of fat for the low-carbohydrate group, vs. 7.5 kg of weight, 7 kg of body fat for the higher-carb group – show the same trend (roughly 20% greater for the low-carbohydrate group) as reported in the Rabast study. Therefore, the Golay study cannot be viewed as a robust refutation of the previously cited studies or of the "metabolic advantage" concept.

Other studies have compared *ad libitum* low-carbohydrate and high-carbohydrate diets, or calorie-controlled diets in which the subjects have prepared their own food. In these studies, the superior appetite control associated with ketogenic diets – presumably reflecting such factors as rapid hepatic fatty acid oxidation, elevated ketone levels, and stable blood glucose – may be largely responsible for the greater weight loss typically seen (at least within the first few months) in the low-carbohydrate dieters. Therefore, such studies cannot provide definitive insight into the possible thermogenic effects of ketogenic diets. However, in some studies in which daily calorie intakes were assessed by food diaries, calorie intakes of subjects on the ketogenic diets were found to be higher (whether by intent of the investigators or by free choice of the subjects) – yet fat loss proved notably greater on the ketogenic diet.[7, 8]

At least one study challenges the notion that ketogenic diets can promote thermogenesis. Brehm and colleagues studied two groups of dieters; one was asked to consume an *ad libitum* low-carbohydrate diet (maximum 20 g of carbohydrate daily), the other was prescribed a 1200 kcal diet that provided about half of calories from carbohydrates. At the end of the 6 month study, body fat loss averaged 6.20 kg and 3.23 kg, respectively ($p<.05$), even though self-reported daily calorie intakes did not differ significantly between the two groups.[9] Nonetheless, resting energy expenditure, measured by open-circuit indirect calorimetry, did not differ between the two groups throughout the study. The authors concluded that systematic under-reporting of daily calorie intake by the higher-carbohydrate group likely rationalized the outcome, and that ketogenic diets do not evoke thermogenesis. However, if the higher-carbohydrate group was indeed consuming more calories daily, it seems likely that this would have boosted their resting energy expenditure; perhaps it did, thereby masking a modest thermogenic impact of the ketogenic diet. So a definitive determination of the resting energy expenditure associated with hypocaloric diets

that are either ketogenic and higher in carbohydrate requires a study in which daily calorie intakes are kept constant.

These considerations suggest that, whereas good appetite control is likely to be largely responsible for the superior short-term impact of *ad libitum* ketogenic diets on loss of body fat, the possibility that evoked thermogenesis may also contribute in this regard is compatible with the results of a number of clinical studies, and remains a very credible possibility. Indeed, some researchers consider "metabolic advantage" a well established fact, despite the fact that medical orthodoxy pays it little heed.[10, 11]

If ketogenic diets do indeed evoke thermogenesis, what could be its source? Some note that energy expenditure for gluconeogenesis should be greater on ketogenic diets.[7, 10] This evidently would be true for ketogenic diets that are higher in protein than control diets, but metabolic advantage has been observed with ketogenic diets that are identical in protein content to control diets (as in the Rabast and Young studies). Even when daily protein intakes are identical, post-absorptive gluconeogenesis is about 14% higher on ketogenic diets than on diets of moderate carbohydrate content;[12] but this modest effect would seem unlikely to account for the large increment of fat loss often seen with ketogenic diets. Moreover, this effect would tend to be offset by the fact that the thermic effect of dietary lipid, the chief component of ketogenic diets, is exceptionally low: 2-3%, as opposed to 6-8% for carbohydrate.[13]

Ketogenesis May Induce Uncoupling Protein 2

A recent mouse study by Kennedy et al. may provide pertinent insight in this regard.[14] These researchers put mice on four different diets – a standard carbohydrate-rich chow diet, a high-fat diet containing 35% carbohydrate, a ketogenic diet devoid of carbohydrate, and a calorie-restricted chow diet. Although the first three groups had *ad libitum* access to food, the mice eating the high-fat diet gained weight rapidly, whereas those on the ketogenic diet initially lost weight – indeed, their weights were quite comparable to those of the calorically-restricted mice throughout the study. The *ad libitum* chow-fed group gained only a modest amount of weight. Analysis of the livers of these animals after 9 weeks on their respective diets yielded some striking findings: mRNA expression of uncoupling protein 2 (UCP2) was 6-fold higher in the ketogenic diet group than in the other groups, and AMP-activated kinase (AMPK) activity was approximately twice as high in the ketogenic group than in the chow-fat or high-fat diet groups.

Ketogenic diets are ketogenic because fatty acids are taken up into liver mitochondria at such a high rate that the Krebs cycle is incapable to disposing of all of the evolved acetyl-CoA. This evidently should be associated with a notable increase in the redox potential across the mitochondrial inner membrane that drives ATP synthase activity; this increase in redox potential slows the flow of electrons through the respiratory chain and thus limits the rate at which the Krebs cycle can process acetyl-CoA. Under these circumstances - in which mitochondrial UCP2 would tend to be activated owing to increased superoxide production by highly reduced mitochondrial respiratory complexes[15, 16] - a notable increase in the mitochondrial level of UCP2 could be expected to promote an increased leakage of protons back into the mitochondrial matrix, lessening the proton motive force and thereby accelerating the Krebs cycle.[17] As a result, a portion of the free fatty acids taken up by hepatic mitochondria would be converted to CO2 and heat, with no net production of ATP. This mechanism is quite analogous to the thermogenesis mediated by brown fat in rodents, and I propose that it may be largely responsible for the "metabolic advantage" conferred by ketogenic diets. Indeed, if human hepatic metabolism during ketogenic diets is roughly

comparable that observed in mice fed such diets, it is straightforward to predict that ketogenic diets will evoke hepatic thermogenesis in humans.

Indeed, there is already proof that rapid hepatic oxidation of fatty acids can evoke a thermogenic response in humans. Several clinical studies have shown that ingestion of medium-chain triglycerides (MCTs) – in comparison to the ingestion of long-chain triglycerides – induces an acute and chronic thermic effect.[18-21] MCTs are immediately oxidized in hepatic mitochondria because they do not require carnitine palmitoyltransferase-1 (CPT-1) activity for mitochondrial uptake (whereas CPT-1 activity is rate-limiting for mitochondrial oxidation of long-chain fatty acids). The acute thermogenic response to MCTs presumably indicates that the uncoupling capacity of hepatic mitochondria in healthy people not engaged in ketogenic dieting is sufficient to enable a measurable thermogenic response if free fatty acid uptake is sufficiently high; this may reflect a modest baseline expression of UCP2, or perhaps "spontaneous" backflux of protons across the mitochondrial inner membrane which becomes significant when the proton motive force is elevated. UCP2 induction would be expected to potentiate such a thermogenic response.

How could a ketogenic diet induce hepatic UCP2 expression? One likely possibility is that increased oxidative stress originating from electron-overloaded mitochondrial respiratory complexes mediates this induction. In obese animals with hepatic steatosis, in whom superoxide production by damaged hepatic mitochondria is increased, UCP2 expression is elevated.[22, 23] Increased UCP2 expression has also been noted in the liver of patients with steatohepatitis.[24] Moreover, treatment of rat hepatocytes with tumor necrosis factor-alpha - which increases mitochondrial superoxide production – or with various drugs which likewise increase superoxide generation by mitochondria, has been shown to induce UCP2.[23, 25-28] UCP2 induction is also evoked by exposure of hepatocytes to moderate concentrations of hydrogen peroxide in conjunction with glucagon – the activity of which is of course elevated during ketogenic diets.[29] Homeostatically, it would make excellent sense for an increase in mitochondrially-generated oxidant stress to induce increased UCP2 expression, since UCP2 would tend to alleviate the glut of respiratory chain electrons that promotes mitochondrial superoxide production.

Foxo Factors May Mediate This Induction

But what could mediate the link between oxidative stress and UCP2 expression? Although PPARalpha activity promotes UCP2 transcription in mouse hepatocytes, it does not appear to do so in human hepatocytes;[30] moreover, a link between oxidative stress and PPARalpha activation is not clear. A more likely mediator may be the Foxo family of transcription factors, which have been shown to promote transcription of UCP2 in human endothelial cells and in brown adipose tissue of mice, in conjunction with coactivator PGC-1alpha.[31, 32] Foxo factors induce the expression of a number of antioxidant enzymes, and, not surprisingly, oxidative stress can boost their transcriptional activities by various mechanisms, including phosphorylation via JNK and increased association with Sirt1 and beta-catenin.[33-37] The low serum insulin associated with ketogenic diets would evidently be compatible with Foxo activity.[38] Moreover, the increased AMPK activity observed in the livers of ketotic mice could be expected to boost the activity and expression of PGC-1alpha, thereby expedited Foxo transcriptional activity.[39] Indeed, AMPK activation has been reported to increase the mRNA expression of UCP2 in the liver and in a number of other tissues.[40-48]

This latter point raises the intriguing possibility that concurrent treatment with AMPK activators such as metformin or berberine might potentiate a thermogenic response to ketogenic dieting. These agents, via

inhibition of acetyl-CoA carboxylase and resultant disinhibition of carnitine palmitoyltransferase-1, might also help to sustain hepatic fatty acid oxidation and ketone production when people who are attempting ketogenic diets consume more carbohydrate than might be prudent.

Safety and Feasibility Issues

The long-term safety of ketogenic dieting has often been questioned in light of the high saturated fat content of such diets that employ fatty animal products. Indeed, a recent analysis of large prospective cohort studies has concluded that people who adopt low-carbohydrate diets centered on animal products tend to experience increased mortality.[49] However, this same study observed *reduced* mortality in people who practice low-carbohydrate diets in which plant products predominate. Indeed, it is perfectly feasible to implement a fully vegan ketogenic diet featuring nuts, edamame and other soy products, green salads and low-carb vegetables, salad oil, olives, and avocados; it would be of interest to study the clinical impact of vegan ketogenic diets. Jenkins and colleagues have reported favorably on the effects of a non-ketogenic reduced carbohydrate plant-based diet, which they dub "eco-Atkins".[50] It is also feasible to base a ketogenic diet on large intakes of fish; a "Spanish ketogenic Mediterranean diet" of this type can have an excellent impact on obesity, metabolic syndrome, and non-alcoholic hepatic steatosis.[51-53]

The mild metabolic acidosis associated with ketogenic diets can mobilize calcium from bone, which in turn increases risk for nephrolithiasis. About 3-6% of epileptic children who sustain ketogenic diets for about 2 years experience a kidney stone.[54-56] Fortunately, there is evidence that this complication can be substantially prevented by daily consumption of potassium citrate, which has an alkalinizing metabolic impact; ingestion of potassium or sodium bicarbonate would likely confer a similar benefit.[57-59] This strategy might also be expected to mitigate the potentially adverse impact of ketogenic diets on bone density.[59-61]

Once substantial weight has been lost, ketogenic diets often become more difficult to tolerate and sustain. This likely explains why, despite superior results in the shorter term, the long-term (e.g. one year or more) outcomes of low-carbohydrate diets tend to be comparable to those of reduced-fat diets.[62] But episodic implementation of such diets may represent a feasible weight control strategy for some, and episodic ketogenesis may also have neuroprotective potential, in light of the neuroprotective impact of ketones. [63]

Conceivably, it might prove possible to achieve episodic ketogenesis within the context of significant carbohydrate consumption through administration of nutraceuticals and drugs that promote vigorous fatty acid oxidation in hepatic mitochondria. In a short-term open clinical study targeting obese subjects, in which subjects were asked to do substantial fasting exercise and choose low-fat foods, administration of carnitine, hydroxycitrate and pyruvate salts, ingested during postabsorptive metabolism, was associated with a surprisingly rapid rate of fat loss suggestive of evoked thermogenesis.[64] Carnitine availability can be rate-limiting for hepatic CPT-1 activity, hydroxycitrate aids this activity by impeding malonyl-CoA synthesis, and supplemental pyruvate – which exerts a thermogenic effect in rats and possibly humans as well[65, 66] - may aid Krebs cycle efficiency via anaplerosis.[67, 68] When insulin levels are low post-absorptively, and especially after prolonged aerobic exercise that depletes hepatic glycogen stores and promotes adipocyte lipolysis, this supplementation regimen could be expected to amplify hepatic fatty acid oxidation – possibly to an extent that could evoke increased UCP2 expression.[69] And such expression would likely be boosted by co-adminstration of metformin or berberine.

Summing Up

Robert Atkins maintained that very-low-carbohydrate ketogenic diets have a "metabolic advantage" that promotes a more rapid loss of body fat independent of their favorable impact on appetite. Several clinical studies do indeed appear consistent with the possibility that ketogenic diets may evoke thermogenesis. The recent revelation that UCP2 expression is markedly elevated in the livers of mice ingesting such diets suggests that evoked hepatic thermogenesis might be responsible for such an effect – and the thermogenic response to ingested medium-chain triglycerides in humans supports the credibility of this hypothesis. The mechanism by which ketogenic diets induce hepatic UCP2 in mice remains unclear, but it is reasonable to suspect that mitochondrially-derived superoxide may trigger this expression, likely via Foxo-mediated signaling. Whether these findings are relevant to humans remains to be assessed in future research. Episodic ketogenic dieting can be conducted in a way that is compatible with long-term health, and may be beneficial from the standpoint of weight control and neuroprotection.

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