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Review article

Inuit metabolism revisited: what drove the selective sweep of *CPT1a* L479?

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ABSTRACT

This article reassesses historical studies of Inuit metabolism in light of recent developments in evolutionary genetics. It discusses the possible selective advantage of a variant of *CPT1a*, which encodes the rate limiting enzyme in hepatic fatty acid oxidation. The L479 variant of *CPT1a* underwent one of the strongest known selective sweeps in human history and is specific to Inuit and Yu'pik populations. Recent hypotheses predict that this variant may have been selected in response to possible detrimental effects of chronic ketosis in communities with very low carbohydrate consumption. Assessing these hypotheses alongside several alternative explanations of the selective sweep, this article challenges the notion that the selection of L479 is linked to predicted detrimental effects of ketosis. Bringing together for the first time data from biochemical, metabolic, and physiological studies inside and outside the Inuit sphere, it aims to provide a broader interpretative framework and a more comprehensive way to understand the selective sweep. It suggests that L479 may have provided a selective advantage in glucose conservation as part of a metabolic adaptation to very low carbohydrate and high protein consumption, but not necessarily a ketogenic state, in an extremely cold environment. A high intake of *n-3* fatty acids may be linked to selection through the mitigation of a detrimental effect of the mutation that arises in the fasted state. The implications of these conclusions for our broader understanding of very low carbohydrate metabolism, and for dietary recommendations for Inuit and non-Inuit populations, are discussed.

1. Introduction

Much recent research has begun to examine the potential of low carbohydrate and ketogenic diets to improve certain aspects of metabolic health (see, for example [1–5]). A ketogenic metabolic state has been tentatively associated with the prevention of some autoimmune conditions, neurological disorders, cardiovascular events, and even cancers (reviewed in [1]). This research has suggested some positive aspects relating to the use of fatty acids and ketone bodies rather than glucose as a primary metabolic fuel (summarized in [6]). However, these scholarly literatures remain in their infancy, leading researchers and clinicians to warn that our understanding of the long term effects of very low carbohydrate intake is not sufficient to justify broader claims regarding optimal health effects [7–10].

Evolutionary biologists have suggested turning to historical evidence from traditional hunter-gatherer societies, as well as the last remaining contemporary hunter-gatherer populations, in our efforts to understand the effects of nutrition on human health [11–13]. Thus, advocates of very low carbohydrate diets in popular and scholarly forums have returned to case studies from historical and contemporary Inuit communities to support the safety and efficacy of very low carbohydrate diets. The Inuit and their ancestral communities, who have inhabited regions of Siberia, Alaska, Canada, and Greenland [14] for thousands of years, have consumed such a diet for the same period [6,15]. Traditionally, the Inuit have consumed a diet high in marine organisms and land animal foods, with much less plant matter than other global indigenous populations [16], resulting in a higher intake of protein and fat and a lower intake of carbohydrate than almost all other populations [17–19]. Apart from the Maasai [20], the Inuit and related populations such as the Yu'pik, are the only known populations to have consistently consumed an extremely low carbohydrate diet, independent of seasonal variability, until the mid-twentieth century.

From as early as the late-nineteenth century, historians, anthropologists, and scientists have pointed to the Inuit [17,18,21–23] as well as to non-Inuit Arctic explorers [24,25] and non-Inuit subjects attempting to replicate the traditional Inuit diet [24,26,27] to illustrate the capacity for humans to survive with very little carbohydrate without obvious detrimental effects. Assessments carried out between the 1950s and the 1970s found exceptionally low rates of diabetes and cardiovascular disease in Inuit communities consuming a traditional diet that conformed to patterns established over several thousand years [28–32]. Combined with the excellent blood lipid levels [33,34] and high glucose sensitivity observed in Inuit communities [17,34], this supported the claim that the traditional Inuit diet is consistent with metabolic health. Conversely, the gradual addition of western foods to the Inuit diet, particularly from around the 1950s [19,22,35–37] is associated with a decline in metabolic health including the appearance of diabetes and obesity [38,39], atherosclerosis [40] and even cancer [41–45] in Inuit populations.

Yet seminal studies carried out on Inuit subjects in the early twentieth century yielded surprising results from a metabolic perspective. Low ketone bodies in the breath and urine were observed in the fed state [17,18,23]. These metabolic results contrast with that which is often registered among healthy non-Inuit subjects, who tend to show a

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marked rise in ketone levels in the breath and urine when consuming a very low carbohydrate diet [24,46]. These results suggest that the traditional Inuit diet may not actually be ketogenic, as commonly assumed, despite being very low in carbohydrate.

More intriguingly, in three studies undertaken by Heinbecker in the 1920s and 1930s, breath and urinary levels of ketones in the *fasted* state were also found to be lower than in non-Inuit subjects [17,47,48]. The fasted state usually corresponds both to a high rate of fatty acid oxidation, indicated by a low respiratory quotient (RQ), and a high production of ketone bodies (see [6]). Similarly, the consumption of fat in the absence of protein and carbohydrate also encourages a high rate of fatty acid oxidation and is expected to result in a low RQ and high production of ketone bodies. The respiratory quotients in the fasted state in Inuit subjects [17], and after ingestion of pure fat [23] were found to be lower than those of non-Inuit subjects, indicating very efficient fatty acid oxidation, but with levels of ketone bodies that were much lower than expected. The detection of low RQs in combination with low ketone bodies were interpreted by Heinbecker to indicate a difference in the metabolism of the Inuit subjects from subjects of non-Inuit descent, which allowed them to oxidize fatty acids efficiently without producing the expected levels of ketone bodies [17,49].

Research on Inuit metabolism was largely discontinued in the second half of the 20th century, leaving us to ask what might account for these historical observations and data, and whether it is in fact helpful or accurate to extrapolate general principles from the Inuit case study that apply to any individual consuming a low carbohydrate diet. If the metabolism of the Inuit does not conform to common definitions of ketosis during consumption of their traditional diet or during fasting, does the health of the Inuit derive from another aspect of their metabolic profile than the production of ketone bodies? And could the Inuit have even undergone genetic adaptations in response to their diet, allowing them to maintain metabolic health in an environmental context that may otherwise be detrimental?

In order to approach these questions, this article reassesses historical studies of Inuit metabolism in light of the recent discovery of a variant of *CPT1a*, which encodes the rate-limiting enzyme in hepatic fatty acid oxidation. This variant, *CPT1a* L479, underwent one of the strongest known selective sweeps in human history in Inuit populations [50]. Given the central role of *CPT1a* in human metabolism, analyzing the selective advantage provide by the L479 variant in the Arctic context should provide insight into the effects of specific environmental factors on metabolism, including low carbohydrate intake, and clarify how the Inuit case study can inform our understanding of human metabolism more generally.

CPT1a encodes the liver isoform of carnitine palmitoyltransferase type Ia (CPT-Ia), an enzyme that converts fatty acid molecules into acylcarnitines for their transport into mitochondria [51] (see Fig. 1). This reaction is the rate-limiting step for β-oxidation in the liver [52]. β-oxidation is required both for the production of energy from fatty acyl molecules and the production of ketone bodies. Ketone bodies are formed during the partial oxidation of fatty acids to acyl coA, which is a precursor for the ketone bodies acetoacetate, β-hydroxybutyrate, and acetone (summarized in [6]). Production of ketone bodies in the liver is particularly important for cerebral metabolism. When blood glucose levels are low, most cells use fatty acids as an alternative fuel source, but the brain is unable to metabolize fatty acids [53] and therefore relies on ketone bodies during periods of low glucose availability [54]. In humans, deficiency in the function of CPT-Ia results in symptoms such as hypoketotic hypoglycemia and hepatomegaly [55], as well as an increased risk of sudden infant death syndrome (SIDS) [56,57]. CPT-Ia is inhibited by malonyl coA, the product of the first step of fatty acid synthesis, which is high in the “fed” state (involving carbohydrate and/or protein consumption) during a high rate fatty acid synthesis, and low in the fasted state, when fatty acid synthesis is inhibited [51].

The L479 variant was first identified in 2001 in a patient with an inherited CPT-Ia deficiency [55]. This variant results in a leucine (L) for proline (P) substitution. The L479 variant is found in several populations including the Inuit and Yu'pik, who descend from ancestral populations that have inhabited Arctic and circumpolar regions for tens of thousands of years. L479 currently exists at high prevalence in Inuit and Siberian populations: 0.93 in Canadian Inuit, 0.73 in Greenland Inuit [58] and 0.68 in northeast Siberians [50], while being absent in South Asian, European Caucasian, and Chinese populations [58]. Its complete absence in populations outside the Arctic demonstrates that its selection is specific to the Arctic environment. Epidemiological studies have associated L479 with an increased risk of sudden infant death syndrome (SIDS) [59–63], which is a leading cause of infant death in modern Inuit populations [64]. Infant death in those carrying the L479 variant is thought to result from defects in fasting ketogenesis: fasting (usually related to illness) is associated with increased risk of hypoketotic hypoglycemia in infants homozygous or heterozygous for L479 [65]. In modern Inuit adults, the L479 allele is associated with reduced adiposity and elevated HDL cholesterol, suggesting a positive effect on metabolic health [66].

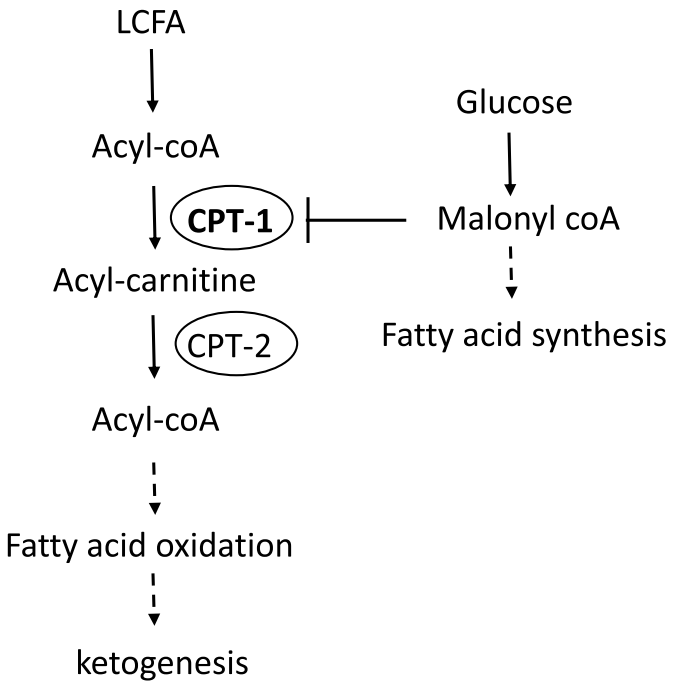

Fig. 1. CPT-1 catalyzes the conversion of acyl-coA into acyl-carnitine, allowing transport across the mitochondrial membrane and fatty acid oxidation. CPT-1 is inhibited by malonyl coA, an intermediate in the synthesis of fatty acids from glucose.

Despite this associated cost of L479 in modern populations, the L479 variant has become the most prevalent allele in ancestral Inuit populations in only the last 6-23 K years [50]. In order to have spread through ancestral Inuit populations so quickly, this variant must have offered a profound evolutionary advantage to those populations. Yet surprisingly, there is currently no consensus on the evolutionary advantage provided by *CPT1a* L479. Several authors have speculated on the possible selective advantage, but have not yet provided convincing mechanistic detail or the necessary supporting data. Examining these hypotheses in greater detail, in light of data from studies inside and outside the Inuit sphere, this article presents a more nuanced view of Inuit metabolism, encompassing the effects of multiple environmental factors and their interaction with genetic factors. While the current state of knowledge does not allow a definitive explanation for the selection of L479, this article aims to clarify what the current state of knowledge allows us to conclude, and what aspects of Inuit metabolism and the selective sweep in *CPT1a* require further study.

A compelling explanation in the literature on *CPT1a* L479 suggests

that it may have been selected as a response to proposed detrimental effects of ketone body over-production arising from a very low carbohydrate consumption, and exacerbated by a high consumption of *n-3* fatty acids due to their effect on the rate of fatty acid oxidation [50,67]. Such an explanation has then been used by other scholars to suggest that a very low carbohydrate diet may not promote metabolic health in other human populations in the long term, due to its association with high ketone production [68].

Another hypothesis also relates the selection of *CPT1a* L479 to other predicted detrimental effects of the traditional Inuit diet, suggesting that the L479 variant may protect against negative metabolic effects that may occur during the transition from fatty acid to glucose metabolism in the context of a normally ketogenic metabolic state [67]. This metabolic switch is suggested to occur during episodes of particularly high protein consumption in the absence of carbohydrate and fat.

Both hypotheses above assume the Inuit and ancestral Inuit have historically been in the metabolic state of ketosis, and suggest that the selection of L479 are linked to detrimental effects of a chronic ketogenic state. However, the assumption that the Inuit are in the metabolic state of ketosis, which has often appeared in recent literature, is contradicted by the historical data of Inuit metabolism, taken from early-twentieth-century communities who maintained their ancestral nutritional profile, as introduced above.

The association of the L479 sweep to effects of chronic ketosis is also contradicted by the recorded macronutrient intake of historical Inuit communities, irrespective of the data that was collected by Heinbecker. The nutritional and macronutrient profile of the Inuit communities assessed during the early twentieth century is remarkably similar to that which appeared in their ancestral populations, including those that lived during the period of the selective sweep, estimated to have occurred between 6000 and 23,000 years ago on the western side of the Beringia land and maritime area [50]. Anthropologists, ethnographers, and scientists have shown the continuity between the limited carbohydrate consumption that was noted in early-twentieth century Inuit communities and that which archaeological, paleoanthropological, and other forms of data has revealed about earlier Inuit and Yup'ik communities during the last 2000 years, as well as the nutritional profile and macronutrient ratios of ancestral communities in eastern Siberia, Beringia, and the Arctic thousands of years before them. In all cases, spanning millenia, evidence suggests a reliance on marine animals (particularly species of whale, walrus, seal, and salmon) and land mammals [69–71]. Between 10,000 years ago and 23,000 years ago, the progenitors of modern Inuit and Yup'ik communities either side of the Beringia land and maritime area relied to a greater extent on larger land mammals, often described as megafauna, than those later communities who existed after megafauna extinction. Nonetheless, it is clear that the Inuit communities assessed in the early twentieth century relied largely on protein and fat from animals, with few sources of carbohydrate, resulting in similar macronutrient ratio to ancestral communities who lived during the time of the L479 selective sweep in Arctic environments on the western side of the Beringian land and maritime area [18,21].

Yet the continuity of the low carbohydrate macronutrient intake between early twentieth-century Inuit communities and proto-Inuit populations thousands of years earlier need not suggest that the metabolic state of either historical populations *ought* to have resulted in high ketone body production. Theoretical predictions of the anti-ketogenic effect of protein, due to the production of glucose through gluconeogenesis, in fact suggest that Inuit and ancestral Inuit communities may never have registered high levels of ketone bodies when consuming their traditional diet *even before* the appearance of the L479 variant. The state of ketosis requires a specific ratio of ketogenic to anti-ketogenic macronutrients, and thus depends on the relative proportions of protein and fat as well as carbohydrate in the diet. New calculations of the ketogenic ratio based on published estimations of the ancestral Inuit macronutrient ratio in section 2.2 suggest either a non-ketogenic or intermittently ketogenic metabolic state, further challenging those hypotheses that assume the Inuit metabolic state of ketosis and then claim that L479 was selected in response to detrimental effects of chronic ketosis, or the effects of suddenly switching from chronic ketosis to a glucocentric state.

Two alternative hypotheses that have not yet been examined in detail, nor related to other relevant literatures, will subsequently be discussed. They will be shown to be more plausible explanations for the selection of *CPT1a* L479 when the predicted metabolic effects of L479 are considered alongside a comprehensive analysis of the metabolic effects of all relevant environmental factors, including the previously overlooked anti-ketogenic effects of high protein consumption among the Inuit.

The first hypothesis suggests that L479 offers an evolutionary advantage by conserving glucose during a very low carbohydrate (but not necessarily ketogenic) diet, increasing its availability for processes linked to evolutionary fitness [72]. This hypothesis derives indirect support from the existence of several mammalian and human traits recently suggested to have arisen as adaptations to conserve glucose. The glucose-conservation hypothesis has not previously been considered in light of the high protein intake of the Inuit, which may contribute to the selective sweep in two ways. Firstly, high protein intake may potentially decrease a selective pressure for glucose conservation due to the provision of glucose from protein gluconeogenesis. A calculation in Section 2.1 will show that enough glucose can theoretically be produced from protein gluconeogenesis to satisfy minimal needs. But the relative inefficiency of glucose production from protein, and a theoretical upper limit of the process, may mean that the levels of glucose required for *optimal* evolutionary fitness are not met by the ancestral Inuit macronutrient profile. Secondly, the consumption of very high levels of protein in the absence of carbohydrate and fat must also be considered. Such a scenario may have occurred seasonally in Inuit populations due to consumption of large quantities of lean meat as the only available food source. Such a process often results in a condition described as “rabbit malaise”, but may be mitigated by increased availability of glucose arising from the metabolic effects of L479.

A second hypothesis, which may be complimentary to or even synergistic with the glucose conservation hypothesis, suggests that L479 provides an evolutionary advantage in cold adaptation [50]. A new analysis of the literature on cold adaptation inside and outside Inuit populations will suggest the overlooked importance of acylcarnitines for metabolism by brown adipose tissue, as well as morphological changes such as decreased stature, both of which may have contributed to the selection of L479. Given the specific metabolic demands of cold exposure, this analysis will suggest the possibility that any selective advantage in cold adaptation may contribute directly to an advantage in glucose conservation.

These two hypotheses will be related to recent literature on the metabolic effects of high *n-3* fatty acid consumption. Any hypothesis attempting to explain the selection of L479 must be compatible with the high intake of *n-3* fatty acids of traditional Inuit populations, through consumption of marine organisms. *N-3* fatty acids are known to directly increase the rate of hepatic fatty acids oxidation [73] and therefore affect the metabolic context in which L479 was selected. The important role of *n-3* fatty acids on Inuit metabolism has been noted in a few previous discussions of the selection of L479 [50,67]. In these discussions, *n-3* fatty acids are suggested to have potentially contributed to the possible “over-production” of ketone bodies during a ketogenic metabolic state by increasing *CPT1a* activity and thereby the overall rate of hepatic fatty acid metabolism. This article will provide data that challenges the suggestion that *n-3* fatty acids may increase ketone body production to harmful levels in the context of the traditional Inuit diet, and thus contribute to the selection of L479.

However, the discussions that follow will not discount another effect of *n-3* fatty acids that may have contributed to the selection of L479, which relates to a potential detrimental impact of the L479

mutation in the fasted state: a decrease in the rate of hepatic fatty acid oxidation which is linked to an increased occurrence of hypoketotic hypoglycemia and sudden infant death syndrome (SIDS) [74]. Linking the *CPT1a* L479 variant to a new study on the role of *CPT1a* in glucagon secretion, section 2.3 will suggest that the increased risk of hypoketotic hypoglycemia in L479 carriers arises specifically from a reduced rate of fatty acid oxidation in pancreatic α cells, which decreases the ability of L479 carriers to maintain blood glucose levels during episodes of fasting [75]. Such an assessment concurs with the suggestion that the consumption of high levels of *n-3* fatty acids by the ancestral Inuit may have been crucial to the selection of *CPT1a* L479: the occurrence of hypoketotic hypoglycemia might have prevented the selection of *CPT1a* L479 in the absence of the counteracting effect of *n-3* fatty acids. Accordingly, this article also clarifies the suggestion that modern Inuit populations may decrease the risk of this condition by increasing their consumption of traditional foods high in *n-3* fatty acids, which would be expected to reduce fatalities arising from hypoketotic hypoglycemia [74].

1.1. Proposed selective advantages of L479

Table 1 lists all the selective advantages of L479 proposed to date, and the environmental factors expected to drive selection in each case. These selective advantages need not be mutually exclusive. In particular, there is potential for adaptations related to cold exposure and the metabolic effects of high protein intake to contribute to a selection pressure for glucose conservation.

These possible selective advantages of L479 can be assessed by considering the expected metabolic effects of the unique Inuit environmental context in light of historical data on Inuit metabolism, and much later studies of the metabolic effects of L479 in human fibroblast cells *in vitro*. This approach will provide a platform to assess how the metabolic effects of L479 may have contributed to the evolutionary fitness of the Inuit in the Arctic environment.

2. Metabolic effects of environmental factors in the Inuit: A high-protein, high *n-3* fatty acid context for the selection of L479

This section examines the expected metabolic effects of high protein, very low carbohydrate, and high *n-3* fatty acid intake on the metabolism of the Inuit and ancestral Inuit, independent of the metabolic effects of L479. These assessments will define the likely metabolic context that preceded and continued after selection of the L479 variant. The discussions of the metabolic effects of high protein intake in sections 2.1 and 2.2 corroborate the historical findings of low ketone body readings among Inuit subjects in the fed state, which were attributed by Heinbecker and others to the high protein content of the traditional Inuit diet [17,23,88], leading to the conclusion that the Inuit metabolic state was non-ketogenic during consumption of a traditional diet [17,23,49]. The metabolic state of the ancestral Inuit might be similarly characterized as non-ketogenic during consumption of a diet in which the low carbohydrate and high protein status had remained unchanged for thousands of years.

The low ketone body readings recorded historically in the fasted state were suggested to arise instead from differences in the metabolism of Inuit and non-Inuit subjects, separate from the high protein context in the fed state [17,47,48]. Section 2.3 will suggest that these effects may arise, at least in part, from the metabolic effects of L479, which have been demonstrated *in vitro* [55]. Section 2.4 will suggest that the explanation used historically to account for the low ketone body readings in Inuit subjects, keto-adaptation, is less plausible.

Following these discussions of the combined metabolic effects of environmental factors and their association with L479, section 2.5 will consider the metabolic effects of high *n-3* fatty acid intake, including the hypothesis that the selection of L479 may relate to the detrimental effects of ketone body over-production in the context of high *n-3* fatty acid intake. Section 2.6 will assess the metabolic switching hypothesis, which suggests that detrimental effects result from the transition from a ketogenic to a glucocentric metabolic state. Both these hypotheses will be shown to be inconsistent with the predicted effects of environmental factors and the L479 variant on the metabolism of ancestral Inuit populations.

2.1. Protein and glycerol gluconeogenesis

During a low carbohydrate diet, protein gluconeogenesis provides glucose from amino acids, and glycerol gluconeogenesis provides glucose from the glycerol moiety of the fatty acid molecule. Understanding the process of gluconeogenesis, including its limitations, allows us to question and modify several hypotheses that attempt to explain the sweep.

In the Inuit, who have traditionally consumed a high protein and low carbohydrate diet, protein gluconeogenesis will likely have provided a significant proportion of glucose. A calculation of the amount of glucose required to be produced from protein gluconeogenesis to meet theoretical minimal daily glucose requirements is shown below. The daily glucose requirement in the Arctic environment is unknown, given that glucose demand is dependent on multiple variables. Approximating minimal daily glucose requirements in the Inuit as those measured in non-Inuit subjects in the fasted and rested state, an estimate of the minimum amount of glucose required daily to be generated from protein gluconeogenesis is shown in the following calculation:

If glucose consumption by the brain is 110 g glucose per day (see [54] for estimates of daily glucose consumption by the brain) and the brain consumes 55% of glucose [76] total daily glucose requirements are approximated as:

110/55*100 = 200 g.

Assuming carbohydrate intake is around 54 g per day [77] that leaves:

200-54 g = 146 g glucose to be made *via* gluconeogenesis.

Assuming an efficiency of 57% conversion from protein to glucose [78]:

146* 100/57 = 256 g protein is required to produce 146 g glucose *via* gluconeogenesis.

Protein consumption by Inuit consuming their traditional diet in the early twentieth century is thought to have been around 280 g per day, according to data from Krogh and Krogh [77]. As can be seen in the calculation above, adequate protein is consumed for protein

Table 1
Proposed selective advantages of L479.

Authors and date	Proposed selective advantage of L479	Relevant environmental factors	Discussed in sections
Clemente et al. 2012 [50], Greenberg et al., 2009 [67]	Prevention of ketone over-production	High *n-3* polyenoic fatty acid intake, low carbohydrate intake	2.5
Greenberg et al., 2009 [67]	Decreasing metabolic effects of very high protein intake: increased threshold for "switching off" ketosis	Low carbohydrate, high protein intake	2.6, 4.3, 4.4
Clemente et al., 2012 [50]	Cold adaptation	Cold	3.3, 4.2
Wang et al., 2014 [72]	Glucose-sparing effect	Low-carbohydrate intake, high protein intake (?), cold (?)	4.1, 4.2, 4.3, 4.4

gluconeogenesis to meet this estimate of daily glucose requirements. However, actual glucose requirements may be higher in the Arctic environment, particularly as a result of cold temperatures and physical activity. Metabolic adjustments may occur during low carbohydrate consumption, such that glucose requirements are lowered: in a more recent study just 70-105 g of protein was found to be sufficient for the maintenance of muscle mass on a low carbohydrate diet consisting of 8% carbohydrate [79]. These adjustments are likely to account for the production of adequate glucose from protein in historical Inuit communities, despite their environmental context likely increasing glucose needs above the minimal daily requirement in the fasted and rested state.

These metabolic adjustments might involve the up-regulation of glycerol gluconeogenesis in addition to protein gluconeogenesis. During fasting, a small proportion of glucose is produced from glycerol gluconeogenesis [80,81] (summarized in [6]). The rate of glycerol gluconeogenesis during consumption of a low-carbohydrate diet has not been studied, but may increase alongside increasing protein gluconeogenesis. In the fed state, fatty acids are esterified to form triacylglycerol (TAG) which is stored in hepatocytes or secreted as very low density lipoprotein (VLDL) particles into the circulation (see [82]). In the fasted state, following the depletion of glycogen, hepatocytes synthesize glucose in the process of gluconeogenesis. TAG is catabolized to produce free fatty acids and glycerol [83,84]. Glycerol is phosphorylated in hepatocytes to glycerate-3 phosphate, a precursor for gluconeogenesis (see [82]). A study in which obese subjects were fasted for almost 6 weeks suggests that up to 33 g glucose may be produced from glycerol daily in the fasted state [54]. More recent radiolabeling experiments have demonstrated that glycerol accounts for a significant proportion of glucose formed in both lean and obese individuals during fasting, a proportion that progressively increases as the fast progresses [80]. The average percentage of glycerol converted into glucose was found to be 38–76% in lean individuals fasting up to 8 days and 56–96% in obese individuals fasting up to 23 days. More recent similar experiments involving injection of radiolabeled glycerol into healthy volunteers showed that 68% of glycerol was converted into glucose, accounting for 21.6% of glucose production after 62–86 h of fasting [85]. Future research may be able to clarify whether or not endogenous triglycerides are converted to glucose at a similar rate.

The amount of glucose produced from protein and glycerol gluconeogenesis among the Inuit consuming their traditional diet has not been quantified experimentally. However, it should be noted that during very low carbohydrate intake, the glucose produced from gluconeogenesis, particularly from protein, provides the majority of glucose. The process of gluconeogenesis has important implications for the metabolic state of the Inuit which will be discussed in Section 2.2. Protein and glycerol gluconeogenesis will also be discussed further in section 4.3, which relates high protein intake to the updated glucose-conservation model of the selective sweep presented in this article.

2.2. Metabolic effects of high protein and low carbohydrate intake

Depending on the ratio of macronutrients in any human population, metabolism will be predominantly glucose oxidizing (“glucocentric”) or fat oxidizing (“adipocentric”) at any given time. An adipocentric state is usually associated with hepatic ketone body production. The Randle Cycle describes the mutual inhibition of fatty acid and glucose metabolism (see [86]), which prevents both processes from occurring at the same time in any given cell (this would create a futile metabolic cycle in which fatty acids synthesized from excess glucose would immediately be oxidized). Accumulating evidence suggests that the Randle Cycle operates in the heart, liver, skeletal muscle, adipose tissue, and between tissues on the scale of the whole organism. The Randle Cycle is illustrated in Fig.ure 2.

CPT-Ia is the key regulator required for the inhibition of fatty acid oxidation by glucose: during the metabolism of glucose, malonyl coA is produced as an intermediate in fatty acid synthesis, and inhibits CPT-Ia so that fatty acid oxidation is also inhibited. When glucose levels are low, malonyl coA levels are low, and CPT-Ia is uninhibited [51]. When glucose levels are high, malonyl coA is high and CPT-Ia is inhibited. During a low carbohydrate diet, the intake of protein is crucial in determining the levels of malonyl coA: a high protein diet results in a higher rate of glucose production *via* gluconeogenesis, which will increase the level of malonyl coA and inhibit fatty acid oxidation. Thus the ratio of macronutrients consumed determines whether metabolism is biased toward fatty acid or glucose oxidation.

During the early twentieth century, it was known that protein exerted an anti-ketogenic effect through the conversion of some amino acids into glucose [87]. In order to determine whether a given macronutrient ratio is likely to lead to a ketogenic (“adipocentric”) or glucocentric metabolic state, a calculation of the ketogenic ratio (KR) was used. In the 1920s, Shaffer used the macronutrient ratios published by Krogh and Krogh [77] (see Table 2) to calculate the KR of the traditional Inuit diet, and predicted that the protein content of this diet was too high to allow ketone body production [88]. Shaffer therefore suggested that the high protein intake accounted for the low ketone body readings in Inuit subjects consuming a traditional diet [88]. This theoretical prediction was not proven experimentally, but was supported by studies in human subjects which mostly corroborated theoretical predictions of the ratio of ketogenic to antiketogenic substances required to result in ketosis.

Calculation of the ketogenic ratio (KR) was modified slightly in 1980 in order to formulate Withrow's equation, which is still used today [87,89]. The KR is expressed as the sum of the relative ketogenic and antiketogenic effect of each macronutrient.

$$KR = (0.9\ F + 0.46\ P) : (C + 0.58\ P + 0.1\ F).$$

Where F = fat (grams), P = protein (grams), C = Carbohydrate (grams).

A KR below 1 and above 2 reliably correspond to glucose burning and ketone body production respectively. Between these values, ketosis and glucose burning vary according to genetic factors, metabolic health, and other dietary factors. According to a recent meta-analysis of diets of various macronutrient ratios, a KR of above 1.7 usually results in a ketogenic metabolic state, and a KR below 1.7 results in a glucocentric state [87]. With this calculation in mind, historical evidence of macronutrient intake in the Inuit can now be assessed, in order to determine whether the selection of L479 most likely occurred in a ketogenic or a glucocentric metabolic state.

Estimates of the macronutrient ratios that have been presented in many historical studies are a useful resource in helping to determine the ancestral Inuit metabolic state. They correspond to a dietary profile that remained relatively unchanged among ancestral Inuit communities in Arctic regions during the millennia before extended European colonization of Alaska. However, it should be noted that these estimates may not be entirely accurate. Krogh and Krogh (1914) commented on the difficulty of accurately determining these ratios, suggesting that their figures may be erroneous by 20% or more [77]. The macronutrient ratios given by Bang et al. (1980) were based on a population of “Eskimos” that were consuming western foods, in which the diet preceding the incorporation of those foods was subsequently estimated [19]. In particular, the ratio of protein intake may have been overestimated: given that populations in general appear not to tolerate a diet higher than 35–40% protein it is questionable whether the Inuit could have consumed as much as 47% protein, as in the estimation of Krogh and Krogh [16]. Furthermore, the macronutrient ratios are likely to have varied according to the season due to changing patterns of food consumption. In one population the Arctic explorer Stefansson noted in the 1940s that for around half the year, the only meat available was caribou, and at other times of year the main source of food was fish [90]. This variation in food consumption would have likely had a corresponding impact on the macronutrient ratio.

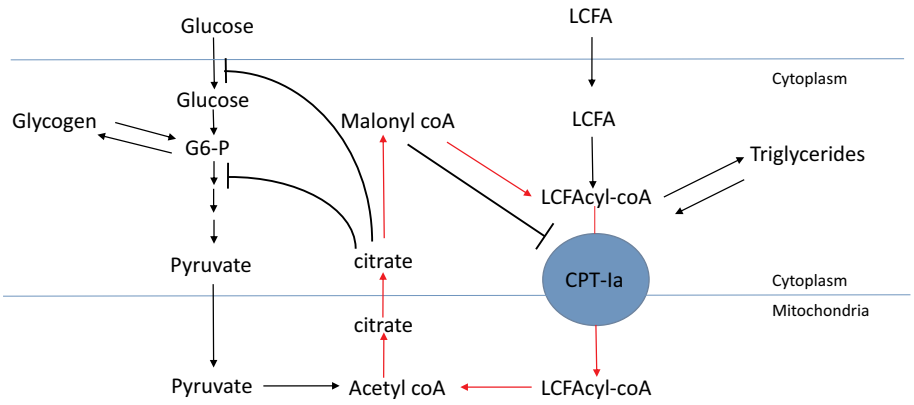

Fig. 2. The Randle cycle describes the mutual inhibition of fatty acid and glucose metabolism. Malonyl coA (an intermediate in fatty acid synthesis) inhibits CPT-Ia, preventing the futile metabolic cycle of newly formed triglycerides undergoing oxidation (red arrows). Therefore, in the presence of malonyl coA, the conversion of long chain fatty acyl coA (LCFAcyl coA) into triglycerides will be favored over transport of LCFAcyl coA into the mitochondria. Citrate and acetyl coA formed during fatty acid metabolism inhibit the uptake and metabolism of glucose.

Table 2
Estimates of macronutrient ratios expressed as a percentage of total calories. Unless stated otherwise, the estimates correspond to a diet almost entirely composed of native foods.

Study	Protein (% calories)	Fat (% calories)	CHO (% calories)
Krogh and Krogh, 1914 [77]*	47.4	44.2	8.4
Rabinowitch et al., 1936 [23] *	43.4	52.1	5.5
Ho et al., 1972 [91]%	30–35	50	15-20
Bang et al., 1980 [19] *&	47	48	7
Average worldwide estimate of hunter-gatherers [16]	19–35	28–58	22–40

* Estimates converted from grams macronutrients based on average calorie intake of 3000Kcal per day and average calories per gram in each macronutrient: fat = 9, carbohydrate = 4, protein = 4.

% In this study, subjects were estimated to consume a small quantity of sugar daily (less than 3 g); other than this, the diet was a traditional Inuit diet, with grain products and simple carbohydrates "virtually absent".

& Based on an estimate of the traditional Inuit diet without incorporation of Western foods. An alternative estimate was given for modern Inuit consuming Western foods: 23% protein, 39% fat and 38% carbohydrate.

Estimates for the macronutrient ratio in the ancestral Inuit diet vary from between 30% and 47% of total calories deriving from protein, but all concur that protein intake is higher, and carbohydrate intake is lower, than in other modern hunter-gatherer societies worldwide [16]. Consistent with a high protein intake, the Inuit were also observed to have a have a high level of non-protein nitrogen in the blood and a high level of urinary nitrogen [17,23].

In order to determine whether this ratio of macronutrients is likely to have led to a ketogenic (adipocentric) or a glucocentric metabolic state in ancestral communities, including those that experienced the L479 selective sweep, the KR can be calculated and compared to the threshold for ketosis defined currently as above 1.7 [87]. Table 3 shows the KR calculated using Withrow's formula above (a slightly modified version of Shaffer's original calculation [88]), for each of the macronutrient ratio predictions in Table 2.

Table 3
Calculation of the ketogenic ratio of each macronutrient ratio recorded historically using Withrow's Eq. [89]. A threshold of 1.7 is used to determine whether the metabolic state is ketogenic or non-ketogenic.

Study	Ketogenic ratio	Ketogenic or non-ketogenic
Krogh and Krogh, 1914 [77]	1.5	Non-ketogenic
Rabinowitch et al., 1936 [23]	1.9	Ketogenic
Ho et al., 1972 [91]*	1.5	Non-ketogenic
Bang et al., 1980 [19]	1.7	Ketogenic

* Conversion of percentage calories to grams of macronutrient based on average intake of 3000Kcal per day, and average calories per gram in each macronutrient: fat = 9, carbohydrate = 4, protein = 4.

Two of the macronutrient ratios result in a KR below the threshold of 1.7 and the other two result in a KR above this threshold. Thus a chronic state of ketosis is not well supported by these calculations. The proximity of the two macronutrient ratios that do predict a ketogenic ratio to the threshold for ketosis means that inevitable small fluctuations in the macronutrient ratio from meal to meal would sometimes push the macronutrient ratio below the threshold for ketosis. Therefore, according to these predicted KRs, it is suggested that the Inuit are likely to have been in a glucocentric metabolic state at least some of the time during the period of the L479 selective sweep.

Regardless of the exact portion of time the ancestral Inuit were in the metabolic state of ketosis, is clear that high protein intake needs to be taken into close consideration for its potential to prevent the metabolic state of ketosis, and thus as a compelling explanation for the low ketone body readings observed in Inuit studies historically. The possibility that the Inuit metabolic state is not ketogenic, at least all of the time, has profound implications for hypotheses attempting to explain the selection of L479.

2.3. The in vitro metabolic effects of CPT1a L479 during the fasted and fed states

Since the studies of Heinbecker and others during the early twentieth century, which are corroborated by more recent insights regarding protein metabolism, a further interpretative approach has arisen, which can help to illuminate the differences between the metabolism of non-Inuit and Inuit subjects in the fasted and fed states. The metabolic effects of L479 on the function of CPT-Ia have been studied in human fibroblasts *in vitro*, allowing a new interpretation of early-twentieth century metabolic studies in light of recently discovered genetic factors. Given the very high prevalence of the L479 variant in Inuit populations, the majority of Inuit are homozygous or heterozygous for the L479 allele [50,58,74]. Therefore, it can be assumed that if this allele has a metabolic effect on ketone body production, it would have influenced the results of metabolic experiments on any small group of Inuit subjects.

Experiments measuring the metabolic effects of L479 on the function of CPT-Ia in human fibroblasts *in vitro* demonstrate that L479 affects the activity of CPT-Ia differently in the fed and fasted states. Brown et al. [55] have demonstrated that L479 reduced CPT-Ia activity to 25% of P479 when expressed in fibroblasts, but in addition caused a significantly reduced sensitivity to inhibition by malonyl coA. Thus the metabolic effects of the L479 allele *in vivo* are predicted to be *increased* rates of hepatic β-oxidation in the fed state and *decreased* rates of hepatic β-oxidation in the fasted state [66,67,72]. On a strict ketogenic diet, involving a very high ratio of fat to protein and carbohydrate consumption, malonyl coA levels will remain low as in the fasted state, and thus hepatic β-oxidation may also be decreased by L479 in this

context. In addition to the *in vitro* studies in human fibroblasts, these predicted metabolic effects of L479 are supported by studies in rat hepatocytes in which a malonyl coA-insensitive mutant displayed increased efficiency of fatty acid oxidation compared to wild-type *CPT1a* in the presence of glucose and insulin [108]. These studies suggest that hepatic fatty acid oxidation may be increased in Inuit relative to non-Inuit subjects in the fed (high malonyl coA) state and decreased in the fasted and fed ketogenic states.

Given the results from the *in vitro* studies in human fibroblasts, the observed low breath and urinary ketone body levels in the fasted state [17,47,48] may be attributed to decreased CPT-Ia activity in the fasted state in subjects homozygous or heterozygous for L479. The decrease in hepatic β-oxidation in the fasted state as a result of the L479 allele would be expected to decrease hepatic ketone production, resulting in low breath and urinary ketone bodies, while the rate of β-oxidation in muscle and other tissues will still be high due to a normal level of activity of the alternative *CPT1* isoform, *CPT1b* (see [67]). A high rate of β-oxidation in other tissues would account for the low RQs found in Heinbecker's studies on fasting Inuit subjects [17,47,48].

The proposed lower rate of hepatic fatty acid oxidation is consistent with an increased risk of hypoketotic hypoglycemia in individuals with the L479 allele [68]. A recent publication suggests that glucagon secretion is dependent on CPT-Ia-dependent fatty acid oxidation in pancreatic α cells [75]. According to this study, the lower activity of the L479 variant of CPT-Ia reduces glucagon secretion, decreasing the up-regulation of hepatic glycogenolysis, gluconeogenesis, and fatty acid oxidation that usually occurs during fasting (see [103]). Thus, the lower rate of fatty acid oxidation in pancreatic α cells with the L479 variant may be the causative factor of the high susceptibility of Inuit infants to hypoketotic hypoglycemia. In adults, the rate of fatty acid oxidation in the pancreas may still be high enough to allow blood glucose levels to compensate for lower ketone body levels, preventing the condition of hypoketotic hypoglyemia. This suggestion is consistent with Heinbecker's historical observation that the average blood glucose levels in Inuit subjects after 12 h of fasting was slightly higher than that of non-Inuit subjects [17]. This observation suggests that the metabolism of the Inuit in the fasted state may involve a higher reliance on glucose rather than ketone bodies to fuel the brain compared to non-Inuit subjects, even while the majority of other tissues depend on fatty acids, as is usually the case in fasting human subjects.

The low ketone body readings in the fed state may also be consistent with the predicted metabolic effects of L479, in light of the high protein content of the Inuit diet, as discussed in Section 2.1 above. The anti-ketogenic effect of protein was originally suggested by Shaffer to explain the absence of ketone levels in the Inuit consuming a traditional diet [88]. We now know that CPT-Ia is inhibited directly by malonyl coA produced during the metabolism of glucose, which in the Inuit most often derives from protein gluconeogenesis. Although the L479 variant of CPT-Ia is less sensitive to inhibition by malonyl coA, a high protein intake might still inhibit ketone body production in the modern Inuit, who are predominantly heterozygous or homozygous for L479. Ketone body production depends on a deficiency of oxaloacetate (see [109]), and given that protein metabolism increases the production of oxaloacetate (see [110]), a high protein intake may still inhibit the production of ketone bodies even if fatty acid oxidation is inhibited to a lower degree (see [110]). *In vitro* experiments investigating the effect of oxaloacetate levels on hepatic ketone body production during a high rate of fatty acid oxidation are required to test this hypothesis, and thus to conclusively determine whether high protein intake may explain the lack of ketone body production observed historically in the Inuit.

2.4. Keto-adaptation: An alternative explanation for the low ketone body readings

Aside from these genetic explanations, which are corroborated by *in vitro* assessments, environmental explanations have been suggested to account for historically low ketone body readings in the fasted state. Such explanations have tended to employ the notion of keto-adaptation to sustain the view that the Inuit diet is strictly ketogenic, and may thus also support the notion that the L479 variant is connected to ketone production in some way [111]. Keto-adaptation describes metabolic changes in response to a ketogenic diet or fasting that result in lower levels of breath and urinary ketone bodies (see [22,112]). This phenomenon has also been used to explain the results generated from fasting obese subjects [112] and army personnel subjected to hypocaloric diets, exercise, and cold temperatures [113]. Levels of ketone bodies excreted in the breath and urine decreased during the course of each study. The keto-adapted state is not yet completely understood but may result in part from the faster metabolism of ketone bodies being produced. This may, for example, result from increased expression of the rate-limiting enzyme for ketone metabolism, succinyl-CoA: 3-ketoacid CoA transferase (SCOT), which occurs after a period of starvation [114]).

Although the phenomenon of keto-adaptation has not been disproved as an explanation for the low ketone body levels in the fasted state, it is contradicted by the high protein content of the ancestral Inuit diet: keto-adaptation is only expected to occur if the state of ketosis is maintained chronically. The calculations of the KR from the macronutrient intakes recorded historically all give a KR either below the threshold of ketosis, or very close to it, suggesting that if ancestral Inuit populations have maintained the metabolic state of ketosis, this state is unlikely to have been maintained chronically. Therefore, the metabolic effects of L479 in the fasted state, describing a decreased rate of hepatic fatty acid oxidation and ketone production, offer a more plausible explanation of the low ketone body readings in the fasted state among communities descended from ancestral Inuit populations who experienced the L479 sweep.

2.5. Metabolic effects of n-3 fatty acids and the ketone body overproduction hypothesis

In addition to examining the low carbohydrate and high protein intake of the Inuit, it is necessary to consider a further dietary factor that affects the metabolic state of the Inuit, and which may be linked to the selection of L479: high *n-3* fatty acid consumption. The traditional diet of Inuit and ancestral Inuit people is exceptionally high in *n-3* polyenoic fatty acids: the fat of many of the arctic marine animals that were consumed during the era of the proposed selective sweep, and in the centuries since, is particularly low in saturated fat and high in *n-3* polyenoic fatty acids [92,93]. The exceptionally low rates of heart disease in Inuit populations have been attributed the anti-inflammatory effects of *n-3* polyenoic fatty acids [94–97] and the excellent glucose sensitivity observed in the Inuit has been suggested to result from high *n-3* fatty acid intake due to a strong correlation between these variables [98,99]. Indeed, the appearance of diabetes in modern Inuit populations [38,39] may derive in part from a decline in *n-3* fatty acid consumption in these populations due to a corresponding decline in insulin sensitivity alongside increasing carbohydrate consumption.

The effects of *n-3* fatty acids on metabolism, which are often overlooked in discussions of ketosis, are important to consider in any assessment of the metabolic context in which L479 was selected. Indeed, it is plausible that L479 may not have been selected in the absence of high *n-3* fatty acid intake. CPT-Ia, which is the rate-limiting enzyme for hepatic fatty acid oxidation, is activated by *n-3* polyenoic fatty acids [73]. Clemente and Greenberg both predict that the rate of hepatic fatty acid oxidation is therefore increased in Inuit populations consuming a traditional diet as a result of high *n-3* fatty acid intake [50,67]. In line with this prediction, the consumption of *n-3* fatty acids has been shown to up-regulate hepatic fatty acid oxidation compared to the consumption of saturated fatty acids in rat liver homogenates [100,101]. As well as acting as a substrate for CPT-Ia, this process has been shown to involve the increased expression of many mitochondrial enzymes in the β-

oxidation pathway, including CPT-Ia [100–102].

Discussions of the effects of *n-3* fatty acid consumption and low carbohydrate intake have tended to suggest its association with the selection of L479 against the "over-production" of ketone bodies. Clemente and Greenberg, for example, suggest that high *n-3* fatty acid consumption in the context of a ketogenic metabolic state may result in the over-production of ketone bodies [50,67]. In these discussions, the authors assume that the Inuit are in the state of ketosis. In this metabolic state, the predicted effect of L479 is a decrease in the rate of hepatic fatty acid oxidation and ketogenesis relative to the P479 allele. Thus, the selective advantage of L479 is related in these discussions to a decrease in the production of ketone bodies during consumption of the traditional Inuit diet.

The assumed detrimental effects of ketone body over-production in discussions of the L479 selection usually pertain to the pathological condition of ketoacidosis - the only known condition associated with ketone body over-production. Ketoacidosis is a serious and life-threatening condition that occurs in diabetic and alcoholic [115] patients, which involves high blood glucose and insulin levels, alongside uncontrolled production of glucagon and other hormones [116]. 'Physiological ketosis' is distinct from the state of ketoacidosis. This term was coined historically by Hans Krebs to describe a metabolic state that occurs in healthy subjects when their carbohydrate intake is low [117]. In physiological ketosis, normal blood pH is maintained, preventing the occurrence of life-threatening symptoms of ketoacidosis [118]. Ketoacidosis has been documented in extremely rare cases in non-diabetic patients: one as a result of fasting during lactation [119], another during consumption of a low carbohydrate diet [120], and another in a pregnant patient experiencing starvation [121], demonstrating the potential for the condition to occur in healthy patients.

In all cases of ketoacidosis described, symptoms arise when ketone body over-production occurs in an insulin-resistant state (increased insulin resistance accompanies the states of pregnancy and starvation). In order to assess the theoretical likelihood of ketoacidosis occurring in Inuit and ancestral populations, it is therefore necessary to explore the likelihood that environmental factors such as high *n-3* fatty acid consumption, high protein consumption, and low carbohydrate consumption would encourage the simultaneous occurrence of ketosis and insulin resistance.

Although insulin sensitivity has been shown to decrease during a strict ketogenic diet [122,123], insulin sensitivity in the Inuit ought to be maintained in the context of high protein intake, which is predicted to prevent a chronic state of ketosis (see section 2.2). Consistent with this assumption, Heinbecker's first study demonstrated excellent insulin sensitivity in the Inuit [17]. The results of the early metabolic studies showing low ketone body levels during the fed and fasted states [17,18,23,47,48] combined with the calculations of the KR from the predicted macronutrient ratios of the traditional Inuit diet (see Table 3) suggest that the Inuit are unlikely to be in the state of ketosis chronically: a high protein intake likely prevented this metabolic state, at least some of the time, in ancestral Inuit populations. Therefore, in light of evidence of high protein consumption in ancestral Inuit populations, it is unlikely that the ancestral Inuit would have experienced insulin resistance. Confirmation of this assumption requires measuring insulin sensitivity in Inuit subjects with the ancestral (P) allele of *CPT1a* while consuming their traditional diet.

The possibility of an intermittent rather than chronic state of ketosis remains plausible in light of the historical studies of ketone production and recorded macronutrient ratios. Here, the effects of *n-3* fatty acids on intermittent ketone body over-production cannot be ruled out, owing to an increased rate of hepatic fatty acid oxidation and ketogenesis [73,100–102]. A future study examining the levels of ketone bodies produced in response to a low carbohydrate diet with a high intake *n-3* fatty acids, and associated negative effects, would provide further insight into the potential negative effects associated with high *n-3* fatty acid consumption, and the possibility of these negative effects driving selection of L479. However, at this time, the existence of such effects in the absence of insulin resistance remains highly speculative.

Rather than looking to the prevention of ketoacidosis as an explanatory model for L479 in the context of high *n-3* fatty acid consumption, it is worth considering the possibility of a different effect of this dietary factor. On a few occasions high consumption of *n-3* fatty acids have been referenced to explain the "paradox" of the selection of a variant that has such a devastating effect in the encouragement of infant mortality in modern Inuit populations: a high intake of *n-3* polyenoic fatty acids, which some modern communities have lost, may remove or decrease a cost of *CPT1a* L479. Such an effect may have been crucial for its selection [67,74]. Since *n-3* fatty acids activate CPT-Ia, the high *n-3* fatty acid content of the traditional Inuit diet may have decreased the occurrence of hypoketotic hypoglycemia in L479 carriers, which has been linked to the higher rate of sudden infant death in the modern Inuit compared to other populations [74]. Hypoketotic hypoglycemia is thought to result from reduced secretion of glucagon from pancreatic islet α cells during fasting (see [103]). Glucagon is secreted by pancreatic islet α cells when blood glucose levels are low, and stimulates hepatic glycogenolysis and gluconeogenesis, the release of nonesterified fatty acids from adipocytes, and oxidation of nonesterified fatty acids. A recent publication suggests that glucagon secretion is dependent on CPT-Ia-dependent fatty acid oxidation in pancreatic α cells [75]. According to this study, the lower activity of the L479 variant of CPT-Ia may reduce glucagon secretion from the pancreas, decreasing the up-regulation of hepatic glycogenolysis, gluconeogenesis, and fatty acid oxidation that usually occurs during fasting, thus explaining the predisposition of L479 carriers to hypoketotic hypoglyecemia.

Theoretically, without the intake of high levels of *n-3* fatty acids, the occurrence of hypoketotic hypoglycemia in individuals homozygous and heterozygous for L479 may have prevented the selection of the variant. According to the biochemical models of CPT-Ia activity, the high *n-3* fatty acid intake of the Inuit consuming a traditional diet would have likely increased the activity of CPT-Ia in the pancreatic α cells in the basal (low malonyl-coA) state, thereby reducing the severity of hypoketotic hypoglycemia during fasting, and the associated likelihood of sudden death in infants. The removal of this cost of L479 by high *n-3* fatty acid intake may therefore have been crucial for its selection. As Collins et al. note, "the high population frequencies of the variant suggest a historically low penetrance for adverse outcomes". The high rate of SIDS in the modern Inuit may therefore derive specifically from a shortage of *n-3* fatty acids in the modern Inuit diet as a result of recent dietary changes, including a decrease in the consumption of traditional foods such as *n-3* rich fish [59–63]. Given that breastmilk contains *n-3* fatty acids in proportion to those consumed in the maternal diet [104,105], the changes to the maternal diet in breastfeeding infants may increase the likelihood of episodes of hypoketotic hypoglycemia and SIDS.

The hypothesis that high intake of *n-3* polyenioc fatty acids may be a relevant factor in the selection of *CPT1a* L479 is supported indirectly by the existence of two other genetic variants whose selection has been linked to the high intake of *n-3* polyenioc fatty acids. A study by Fumagalli et al. revealed signatures of positive selection in *FADS1* and *FADS2,* which encode enzymes involved in the desaturation of long-chain *n-3* and *n-6* polyenoic fatty acids, in the genomes of the Greenland Inuit [106]. Those signature have subsequently been found to exist in several Native American populations thought to share common ancestry with the Greenland Inuit [107]. Several variants of *FADS1* and *FADS2* present in the Greenland Inuit were found to be associated with lower blood levels of *n-3* fatty acids, leading the authors to suggest that that high *n-3* fatty acid intake may have driven the selection of these variants in the Greenland Inuit. Given that these variants were also associated with lower stature, it was suggested that these they may have altered growth hormone signaling, an effect which may explain their selection [106].

2.6. The metabolic switching hypothesis

Alongside their discussion of *n-3* fatty acids and ketoacidosis, Greenberg et al. have also suggested that the selective advantage of L479 might relate to the metabolic effect of the variant during the relatively sudden switch from a normally ketogenic state to exceptionally high protein consumption [67]. A condition described as "rabbit malaise" or "rabbit starvation" has been documented in several northern populations, which occurs during episodes of reliance on lean meat sources [16,124]. The high protein intake in the absence of carbohydrate and fat sources is thought to create specific metabolic demands [16]. It has been suggested that the symptoms of rabbit malaise may occur specifically as a result of the abrupt "switch[ing] off" of ketosis when large quantities of lean meat are consumed during a normally ketogenic metabolic state [67]. This switch is said to occur as a result of high levels of malonyl coA being produced during the production of glucose from protein (see section 2.1 and Fig. 2). It is proposed that the lower sensitivity of the L479 variant of *CPT1a* to malonyl coA would increase the threshold of glucose required to switch from ketogenic to glucocentric metabolism in the liver, thus allowing a greater use of fatty acids and ketone bodies during extremely high protein intake. If the Inuit are in the state of ketosis chronically, as per the interpretation of Greenberg et al., an exceptionally high protein intake may cause a rapid switching off of ketosis, and the subsequent effects of rabbit malaise.

However, the notion of "switching" from chronic ketosis to high protein consumption is confounded by the discussion in Section 2.2 above, which suggests that the state of ketosis may not be maintained chronically in the Inuit: a ketogenic ratio of above 2 is likely to be required for the state of ketosis to be maintained consistently. Intermittent ketosis would involve constant fluctuations between metabolic states such that an episode of very high protein intake would be unlikely to be metabolically disruptive.

Even if ketosis is maintained chronically in the Inuit, there is currently no documentation of negative effects of this metabolic transition in the literature. In fact, studies in sports performance have suggested that a high rate of fatty acid oxidation is maintained following carbohydrate consumption in a normally ketogenic state [125,126], suggesting that ketosis may not be switched off in the Inuit following high protein intake even if the metabolic state of ketosis were chronic.

Although the notion of "switching" from ketogenesis is currently unsupported, the broader suggestion made by Greenberg and others that a benefit may be gained by increasing the rate of hepatic fatty acid oxidation during periods of exceptionally high protein intake need not be discounted. Such a suggestion remains useful concept to consider in relation to the selection of L479. This possibility will be considered in Section 4.4, in a discussion of a selective advantage in glucose conservation during periods of switching from high protein and high fat consumption (but not necessarily a ketogenic state) to sole consumption of lean protein.

In the absence of experimental and theoretical support for the ketone over-production and the metabolic switching hypotheses, alternative explanations of the selection of L479 should also be considered and assessed in light of available data. Section 3 will thus consider the context of cold metabolism, which has not been explored previously in any detail in relation to the selection of L479. Section 4 will consider a possible glucose-conservation effect of L479. The role of cold-adaptation and glucose-conservation in the selection of L479 will both be examined in light of additional biochemical data as well as the effects of other genetic variants associated with those environmental factors.

3. A role for cold exposure in the selection of L479

Clemente et al. suggest that L479 may enhance cold adaptation [50]. The authors do not specify how the variant may enhance cold adaptation but suggest that it depends on the increased rate of hepatic fatty acid oxidation in the fed state. An examination of the literature on cold adaptation suggests that the increased rate of hepatic fatty oxidation in the L479 variant may improve cold adaptation in two ways. Firstly, it may increase the production of acylcarnitines which are exported from the liver to brown fat tissue, providing an energy-rich fuel source that bypasses malonyl coA inhibition [127]. Secondly, it may have altered the metabolism of *n-3* fatty acids, disrupting growth hormone signaling in a way that decreased stature, as has also been suggested in the examples of *FADS1* and *FADS2* in the Greenland Inuit, described in Section 2.5. Given that cold exposure increases the demand for glucose, these adaptations may be synergistic with a selection pressure for glucose conservation.

3.1. The controversy of cold adaptation in the Inuit

Many studies have attempted to address the metabolic effects of cold exposure specifically in the Inuit. However, due to the conflicting nature of the results, determining the extent to which cold exposure may affect the metabolism of Inuit populations specifically, and may have therefore driven the selective sweep of *CPT1a* L479, is complicated by several factors. These should be noted as part of a discussion of the possible role of cold exposure in the selection of this variant.

First, the level of cold to which the Inuit are exposed may be greatly diminished by their clothing [128]. There is little evidence for greater physiological responses to maintain core temperature in Inuit subjects [129]. This contrasts with historical data for Australian Aboriginals, who have historically slept unclothed at night when air temperature drops to around 19 °C. In response to cold exposure, they have been shown to exhibit several physiological responses that conserve body temperature: a decreased surface temperature, lower body heat conductance, and vasoconstriction of peripheral vessels [130–132]. Although the Inuit are also known to sleep lightly clothed at night in temperatures as low as 10 °C in order to avoid the problem of profuse sweating [90], comparable physiological responses have not yet been found in the Inuit.

Rather, the greatest physiological challenges of the Inuit are thought to be temperature homeostasis of the extremities (particularly the hands and face) and the prevention of over-heating and profuse sweating (which could freeze and cause a loss of insulation) during physical activity when wearing heavy clothing [128]. Several historical studies support this consensus. In a study involving immersion of Inuit, Caucasian, and African American subjects in water baths of different temperatures, Rennie et al. found evidence for increased cutaneous blood flow in Inuit subjects, necessary for the maintenance of temperature of extremities, and increased tissue heat conductance, necessary for heat loss, during cold exposure [133]. One study involving immersion of the arm in a cold water bath revealed a bigger increase in blood pressure in Inuit subjects compared to acclimated Caucasian controls, suggesting a greater ability of Inuit subjects to maintain temperature of extremities [129]. Several additional studies have demonstrated increased ability to maintain warmth in extremities in Inuit compared to non-Inuit subjects [134–138]. The Inuit have also been shown to exhibit greater sweating efficiency and to have a greater number of sweat glands on the face, but a reduced number on their trunk and lower limbs (which are heavily clothed compared to the face) compared to Caucasians, a possible adaptation to aid heat loss [139]. However, the apparent lack of *physiological* adaptations to conserve core temperature in the Inuit does not exclude the possible existence of *metabolic* adaptations. Furthermore, metabolic changes in response to cold exposure may also occur as a result of another effect which cannot be negated by external insulation: the warming and humidification of inspired cold air. In a theoretical calculation, it has been suggested that 4073 cal would be required daily to warm and humidify air at a temperature of -40 °F [140]. This has not been verified experimentally, but suggests that increased metabolism of the Inuit in response to cold may not entirely be negated by efficient clothing.

Second, the high protein diet consumed by the Inuit is known to

increase the basal metabolic rate (BMR) [8], which might otherwise be described as an adaptive response to cold. A negative correlation between the BMR and average environmental temperature has been observed among populations worldwide [141,142]. The BMR of Inuit [17,23] populations is particularly high: the BMR of the Inuit was found by Heinbecker to be on average 33% higher [17] and by Rabinowitch et al. to be 26% higher [23] than subjects living in temperate zones. This increased BMR causes an increase in surface skin temperature, and is therefore thought to be an adaptive response to maintain temperature homeostasis [143]. Protein is described as having a high "specific dynamic action," which refers to the elevation in metabolism, or heat production, following its consumption [128]. Ko et al. conclude that a high protein diet is the primary cause of the elevated BMR in the Inuit, with cold exposure having little or no effect [128]. However, other studies provide evidence against such a conclusion. Heinbecker noted that metabolic rate remained high during fasting and suggested that other factors, such as the high vascularity of the subjects, also contribute to the high BMR [17]. Leonard et al. found evidence of raised thyroid activity in indigenous circumpolar groups consuming a non-traditional diet consisting of only 17% protein, and concluded that genetic factors influencing thyroid activity, including in response to external factors such as diet, contribute to the raised BMR [144]. While the high protein Inuit diet may contribute significantly to the elevation of BMR, then, cold exposure may also cause some elevation in BMR. This may be the case due to energy required for warming inspired air, and may involve physiological adaptations such as increased vascularity. Furthermore, cold may have contributed to the selection of genetic variants that increase the BMR, providing a survival advantage in the Arctic climate.

A final complicating factor in determining the extent to which cold exposure may affect the metabolism of Inuit populations relates to the possible presence of brown fat in the Inuit. This aspect of cold adaptation has been studied in other populations exposed to cold but not in native Arctic populations [128]. Several recent studies have demonstrated the presence of thermogenically active brown fat in adult humans [145–148] and the volume of brown fat tissue has been shown to increase during cold exposure in humans [147–149]. Brown adipose tissue (BAT) is highly metabolically active: a study in mice found the uptake of glucose and fatty acids by brown fat to be 2.7 and 2.8-fold higher respectively, and fatty acid synthesis to increase 2-fold, during cold exposure [150]. Therefore, if the Inuit have large volumes of metabolically active brown fat, such a state would be expected to affect whole-body metabolism. Thyroid hormone (TH) is known to induce BAT differentiation [151–153] and thermogenesis *via* increased UCP-1 expression [154] and so the elevations of TH observed in some Inuit populations [155,156] may correlate with increased activity of BAT.

The degree to which the metabolism of the Inuit is up-regulated by cold ambient temperatures, and glucose, fatty acids, and acylcarnitines are metabolized by BAT, is therefore currently unclear. The BMR of the Inuit has been shown to be significantly higher than that of populations in more temperature regions, but this has been widely attributed to the high protein diet; the relationship of cold exposure to this increase remains unclear. Despite efficient clothing, metabolic adaptations such as increased BMR and increased metabolic activity of brown fat may aid survival of Inuit and Siberian populations in the extreme cold. Furthermore, metabolism may be perturbed by the inspiration of cold air, which may result in an increase in the BMR (possibly associated with an increase in thyroid hormone and increased brown fat). Studies comparing the BMR of Inuit and non-Inuit subjects in native clothing in the Arctic, and in response to different levels of protein intake, would help to clarify the effect of cold exposure on the metabolism of the Inuit.

3.2. Associations between cold exposure, genetic variants, and morphological features

Having outlined various caveats and unresolved aspects of cold metabolism in the Inuit, it is now possible to consider how cold may have contributed to the selection of L479. The cold adaptation hypothesis is consistent with the association of several metabolic variants associated with communities who have historically lived in cold climates, which provides indirect support for the hypothesis that cold exposure played a role in the selection of L479. Multiple metabolic variants and morphological features are associated with cold climates, indicating a likely role of cold exposure in the selection of other genetic variants.

Two recent genome-wide association studies have identified a number of genetic variants that are associated with exposure to cold environmental temperatures [157,158]. Several of these are variants are thought to affect metabolism, suggesting that cold exposure creates specific metabolic demands. Signals of positive selection have been found in a number of genes including THADA (involved in thyroid function); LRP5 (involved in cholesterol metabolism and systolic blood pressure) [158]; PON1 (involved in vasodilation and protects lipids from peroxidation); LEPR (associated with BMR); and FABP2 (involved in metabolism of long chain fatty acids) [157]. As Hancock et al. note, these variants may have been selected as a result of other environmental conditions that correlate with temperature, rather than cold itself. Despite this caveat, the association of many genetic variants with cold temperatures provides support for the hypothesis that cold temperatures may have directly driven the selection of specific variants in human populations worldwide, many of which affect metabolism. Therefore, the effects of cold exposure should certainly be considered in attempts to explain the selection of *CPT1a* L479.

Relationships have been found to exist between measurements such as limb length relative to trunk length, bi-ileac breadth, and body weight with latitude and mean annual temperatures, and a correlation has been found between environmental temperature and stature (reviewed in [128,132]). Many of these relationships conform to Bergmann's and Allen's Rules, which predict body form-temperature associations: Bergmann's rule proposes a smaller body size in warmer climates [160]; Allen's rule predicts longer limbs in warmer climates [161]. Features that decrease the surface area: volume ratio of the body are likely to have been selected in colder climates as adaptations to reduce heat loss. The correlation between stature and latitude is much weaker than that of bi-ileac breadth and body weight with temperature. This observation conforms to predictions of heat loss in thermoregulatory models, which suggest that the effect of body breadth on heat loss will be more pronounced than the effect of stature [159]. According to this paradigm, Inuit populations may be expected to experience a selective pressure for features such as increased bi-ileac breadth and shorter limbs, while a selection pressure for shorter stature may be much weaker.

3.3. A possible role of cold exposure in the selection of CPT1a L479

Given the role of cold exposure in the selection of other genetic variants, particularly those related to metabolism, it is certainly plausible that cold exposure played a role in the selection of *CPT1a* L479. In light of recent studies, it is possible to offer two new suggestions of specific mechanisms by which cold exposure might have contributed to the selection of L479.

The first suggests that L479 may have increased the hepatic production of acylcarnitines, which, according to recent studies, appear to have a central role in brown adipose tissue (BAT) metabolism (see Fig. 1 for an illustration of the role of *CPT1a* in the production of acylcarnitines). The activity and amount of brown adipose tissue increases generally in humans in response to cold exposure [145–149]. Heat production in BAT is dependent on fatty acid availability: increased delivery of fatty acids to mitochondria enhances thermogenesis in rat brown adipocytes [162]. In support of a direct role of *CPT1a* in brown fat thermogenesis, a recent study found that both *CPT1a* and *CPT1b* expression in the liver increase in response to cold exposure in

rats, corresponding to increased uptake of CPT-Ia-derived acylcarnitines by BAT [127]. Increased serum free fatty acids (FFAs) released from white adipose tissue during cold exposure were shown to act as a signal to up-regulate *CPT1a* and *CPT1b* expression *via* HNF-4α. The authors suggest that the use of acylcarnitines as a fuel source in BAT may bypass the inhibition of malonyl coA and allow increased thermogenesis during long-term cold exposure. Thus, *CPT-1a* L479 may have a central role thermogenesis in BAT in the Inuit: this variant increases the production of acylcarnitines and may therefore increase the availability of fuels for thermogenesis in BAT. The possible future discovery of increased volume and activity BAT in the Inuit would provide increased support for this interesting hypothesis.

The second way that cold exposure may have contributed to the selection of L479 is by driving an associated morphological change. This may also relate to the metabolic demands of cold exposure – if the morphological adaptation reduces heat loss, this effect might reduce the amount of glucose required for temperature homeostasis. In support of the hypothesis that L479 may have driven a morphological change linked to cold adaptation, an association has been found to exist between L479 and stature. Skotte et al. recently found an association of L479 with several serum lipid markers, including reduced serum concentration of total *n-3* fatty acids and reduced DHA [163]. A similar association of L479 with erythrocyte levels of fatty acids was identified by Anderson et al. [164]. These studies both found L479 to be associated with a reduction in stature, potentially invoking a selection pressure for short stature in a cold environment with high consumption of *n-3* fatty acids. However, this hypothesis should be considered in light of the prediction that the selection pressure for short stature in populations exposed to the cold may not be high enough to drive the selection of genetic variants (see section 2.2). Therefore, while there is evidence suggesting a strong association of L479 with height, evidence that the effect of L479 on stature drove the selection of this variant is lacking. A selection pressure for shorter stature in Inuit populations might be supported by the future discovery of signatures of positive selection in other height-associated variants. *FADS1* and *FADS2* may be relevant examples, though their selection can be attributed to a number of other possible effects. Despite several genome-wide scans being conducted in Inuit populations, signatures of selection have so far not been found at any other loci known to be associated with stature.

Either of the possible mechanisms discussed above offers a plausible explanation for the selection of L479, but additional data is required to support each hypothesis. Given that any adaptation to conserve heat will likely result in a glucose-sparing effect, the hypothesis that L479 provided a selective advantage in cold exposure might be synergistic with the glucose conservation hypothesis.

4. A possible role of L479 in sparing glucose

Having discussed the metabolic effects of environmental factors including cold ambient temperature and dietary context, and related them to the possible selective pressures for L479, this section will suggest a new hypothesis to explain the selective sweep, which is potentially synergistic with the other causal factors examined so far. In the context of a very low carbohydrate diet, L479 may offer a selective advantage in glucose conservation by increasing the rate of hepatic fatty acid metabolism in the fed state [72]. This explanation is supported by data reviewed below, which can be considered in light of the previously discussed factors of cold exposure and high protein intake.

4.1. A glucose conservation selective pressure during human and mammalian evolution

Before exploring the possibility of a glucose conservation effect of L479, it is helpful to briefly outline the concept of a selective pressure for glucose conservation, and the relationship of other adaptations to this selective pressure. In support of the potential for a selective pressure for glucose conservation to drive evolution, a number of adaptations that have arisen throughout mammalian and human evolution have been linked to glucose conservation. A selective pressure for glucose conservation is thought to derive from the obligatory glucose requirement of the brain, which consumes the largest proportion of the body's available glucose. If adequate glucose is not available for the brain, or the supply of glucose is limited for other processes linked to evolutionary fitness, a variant that “spares” glucose for these processes may have provided a profound survival advantage to the Inuit.

Adaptations that have been linked to a selective pressure for glucose conservation include the suppression of the ketone-degrading enzyme, SCOT in the mammalian liver [72]: suppression of hepatic SCOT enabled ketone bodies to be exported from the liver to the brain, thereby sparing glucose for vital cerebral function [165]. In humans, the selection pressure for glucose conservation is thought to be intensified as a result of an exceptionally large brain. The human brain is highly metabolically demanding, consuming between 44% and 87% of the resting metabolic rate between infancy and adolescence and around 20% of the resting metabolic rate of adults [166,167]. Glucose is the primary fuel for the brain: the adult brain consumes around 110-145 g glucose per day [53]. Historically, the Expensive Tissue hypothesis proposed that the metabolic demands of the increasing size of the human brain during evolution was compensated for by a decrease in the size of metabolically demanding gut tissue (which could occur in response to a transition to more nutrient dense foods), allowing the metabolic and nutritional demands of both organs to be met [168]. As noted by Wang et al. [72], the particularly high level of fat mass in human infants compared to other species may reflect the high metabolic demands of the infant brain, and may represent an evolutionary adaptation to enable the metabolic demands of the human infant brain to be met [169].

The function of the immune system has also been linked to cerebral metabolism: Begoña Ruiz-Núñez et al. [170] suggest that the insulin resistance that occurs during immune activation may be an evolutionary adaptation to allow glucose conservation for the brain when glucose supply is threatened by a metabolically-demanding immune response, described as an “energy appeal interaction” [171], in which metabolism is influenced to divert resources to the immune system.

A selective pressure for glucose conservation is therefore suggested to have operated throughout human and mammalian evolution. The low carbohydrate content of the traditional Inuit and ancestral Inuit diet may have intensified the selection pressure for glucose conservation by severely limiting the amount of glucose consumed in a form immediately available for metabolism.

4.2. The glucose-sparing effect of L479

The notion of glucose conservation as an evolutionary adaptation can be explored more specifically in relation to the potential “glucose-sparing” effect of *CPT1a* L479. The effect has been tentatively raised by Wang et al. to suggest that a variant that increases glucose conservation may increase the availability of glucose for processes linked to evolutionary fitness, such as the immune response and reproductive processes [72]. These general claims require further assessment and elucidation in light of more recent discussions, including this present assessment.

A glucose conservation selective pressure is particularly relevant to states in which glucose needs are particularly high, which might include exposure to extreme cold, very high protein intake in the absence of fat and carbohydrate, pregnancy [169], lactation [176] and illness [177]. As a result of the low carbohydrate content of the traditional Inuit diet, any selection pressure for glucose conservation is likely to have been intensified in ancestral Inuit communities. The increased rate of hepatic fatty acid oxidation in the high malonyl coA state associated with the L479 variant is suggested to conserve glucose by inhibiting hepatic glucose uptake and oxidation, thereby sparing glucose for other

processes linked to evolutionary fitness. A selective advantage in glucose conservation may be compatible with the hypothesis that L479 improves cold adaptation: any adaptation that reduces heat loss will also potentially conserve glucose. Indeed, the association of several metabolic variants with cold climates suggests a possible association between cold adaptation and glucose conservation.

The glucose conservation hypothesis is complicated by consideration of the historical data of the lack of ketone body production in Inuit subjects. Section 2.3 suggested that the metabolic effect of L479 in the context of the traditional Inuit diet may be increased hepatic fatty acid oxidation *in the absence of hepatic ketone body production*, a prediction which is supported by the recorded low ketone body levels in Inuit subjects on their traditional high protein diet. In this state, the brain is expected to rely on glucose rather than ketone bodies.

Given the lack of ketone body production in the high malonly coA state, the glucose sparing effect of L479 might arise only from the up-regulation of fatty acid metabolism in the liver and other organs and tissues expressing *CPT1a*. In this scenario, the glucose conserved would only be that which would have otherwise been consumed by these organs, not that which would be conserved by the export of ketone bodies to the brain. A lack of ketone body production would limit the glucose sparing effect of the L479 variant, given that the brain consumes the largest proportion of glucose. Therefore, in the absence of evidence for ketone body production in the Inuit consuming their traditional diet, this hypothesis may need to be modified in light of the metabolic effects of high protein intake and the predicted metabolic effect of L479 in this context.

Two alternative effects of L479, both linked to cold exposure, could plausibly contribute further to a selective advantage in glucose conservation (see Section 3.3). Firstly, L479 may provide increased acylcarnitines exported from the liver to BAT. This may conserve glucose by reducing the consumption of glucose by BAT, rather than reducing the glucose consumption of the brain. Secondly, L479 may have driven a decrease in stature, which may decrease heat loss and may also reduce the amount of glucose used for temperature homeostasis. Either of these effects may contribute to a glucose conservation advantage. Therefore, even if the Inuit are not in the state of ketosis, L479 might still provide a glucose sparing effect.

4.3. The glucose-conservation hypothesis in the context of high protein intake

The process of protein gluconeogenesis, which was introduced in section 2.1, has not previously been discussed in relation to the selection of *CPT1a* L479, but is necessary to consider in the context of a selective advantage in glucose conservation.

Although the production of glucose from protein may potentially mitigate the shortage of glucose that would otherwise arise from a very low carbohydrate diet, the inefficiency of protein gluconeogenesis, and a possible upper limit of this process (see [19]), may mean that sufficient glucose may not be produced from protein, even though the levels of glucose produced in gluconeogenesis are expected to prevent ketosis. Protein metabolism is energetically wasteful: a proportion of the energy produced in protein metabolism is lost due to the up-regulation of BMR that occurs as a result of protein consumption [172,173]. A high protein diet up-regulates the basal metabolic rate, effectively resulting in a loss of energy: as noted by Speth and Spielmann, 'for every 100 calories ingested from protein, up to 30 calories are needed to compensate for the increase in metabolism' [173]. Although protein provides glucose, the efficiency of glucose production from protein is likely to be much lower than that when glucose is absorbed directly from the gut following the consumption of carbohydrate sources.

The calculation in Section 2.1 showed that minimum daily glucose requirements can theoretically be met by protein gluconeogenesis. The existence of a selective pressure for glucose conservation would therefore suggest that while minimal glucose needs are met, increasing the amount of available glucose may still increase evolutionary fitness, for example by providing glucose for processes linked to fertility and immunity. Furthermore, the selective pressure may also derive from the upper limit on the amount of glucose that can be produced from protein gluconeogenesis [22]. While this theoretical limit is likely to depend on multiple variables, it may be reached during consumption of the traditional Inuit diet. As discussed earlier, historical records of macronutrient intake imply that the protein intake of the Inuit exceeds the apparent limits of protein metabolism in the context of very limited carbohydrate consumption [16].

4.4. Re-reading the metabolic switching hypothesis in light of the glucose-conversation paradigm

The metabolic switching hypothesis described in Section 2.6 assumed the Inuit and ancestral Inuit have historically tended toward a chronic metabolic state of ketosis. This article has questioned the hypothesis in light of the high protein intake among the Inuit, and the historical studies pointing to a non-ketogenic metabolic state. But even if ketosis may not have been abruptly terminated during periods of high protein intake, the notion of metabolic switching does not have to be discarded as a possible contributing explanation for the selective advantage of the L479 variant of *CPT1a*. The advantage may have resulted from an increased rate of hepatic fatty acid oxidation relative to glucose oxidation during a relatively sudden seasonal increase in protein intake, irrespective of the state of ketosis.

Decreased sensitivity of the L479 variant of *CPT1a* to malonyl coA inhibition will allow a greater rate of hepatic fatty acid oxidation following a high protein intake [67]. The observation that the symptoms of rabbit malaise can be alleviated by the consumption of carbohydrate or fat suggests that the condition may involve a deficit of glucose [16,67]. Scholars have not yet linked increased hepatic fatty acid oxidation to the glucose-conservation paradigm for the selection of L479. But in light of this discussion, it should be stressed that a high protein intake in the absence of sufficient fat may exacerbate the shortage of glucose that may already be present in Inuit populations due to low carbohydrate intake and cold exposure, by simultaneously up-regulating the BMR and inhibiting fatty acid oxidation and ketone body production. This scenario may have occurred historically due to seasonal reliance on leaner meat sources such as caribou, which has been well documented in discussions of ancestral Inuit diets, including those that may have corresponded to the period in which the selective sweep took place [16,90,124]. In such cases of increasing hepatic fatty acid metabolism during high protein intake, L479 may provide a selective advantage by reducing the shortage of glucose that would otherwise occur during these periods. This effect is compatible with the glucose-conservation hypothesis, but emphasizes the metabolic effects of high protein intake in creating the selective pressure for glucose conservation.

Surprisingly, no scholarly discussions of the negative effects of rabbit malaise among the Inuit or any other community have linked the condition to a shortage of glucose in the context of a switch to high protein, low fat, and low carbohydrate food sources. To date, rabbit malaise has been linked to the limited capacity for the liver to upregulate the enzymes for urea synthesis, resulting in hyperammonemia and hyperaminoacidemia, rather than to a shortage of glucose specifically [16,179]. Furthermore, it is unknown whether or not *CPT1a* L479 reduces the severity of the condition. Therefore, while this hypothesis is credible, further investigation into the condition of rabbit malaise is required to determine whether symptoms may be alleviated by increasing the availability of glucose.

4.5. An updated model of the glucose-conservation hypothesis

In light of the discussions in this section, Fig. 3 provides an updated model of how L479 may conserve glucose and have enhanced the

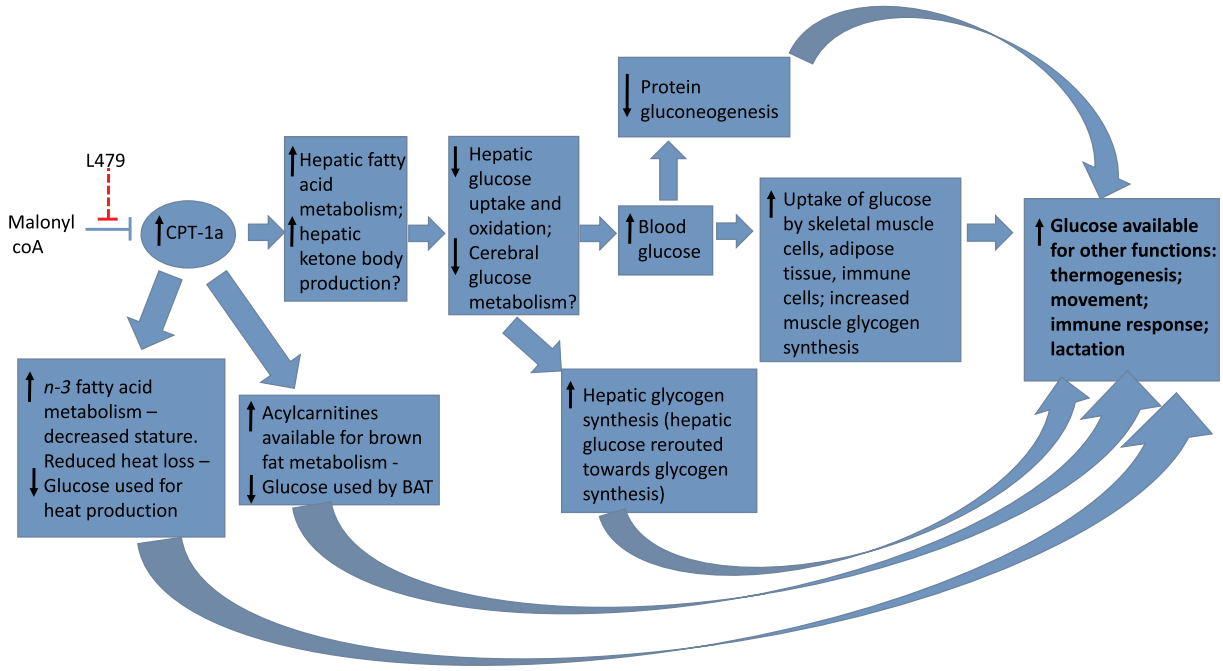

Fig. 3. Model illustrating how L479 may provide a selective advantage in glucose conservation.
Glucose may also be conserved as a result of several mechanisms, either alone or in combination. In the high malonyl coA state, L479 decreases inhibition of *CPT-Ia* by malonyl coA (dashed red line). This results in higher activity of CPT-Ia and a higher rate of hepatic fatty acid oxidation, decreasing hepatic glucose uptake and oxidation and rerouting glucose toward glycogen synthesis in the liver, as described by the Randle cycle (see Fig. 2). Glucose uptake and metabolism in tissues such as muscle and adipose tissue will be increased due to a higher blood glucose level. If glucose uptake exceeds oxidation rate in muscle tissue, glycogen synthesis will occur, conserving glucose for periods of low availability (for a more detailed explanation of the effect of fatty acid metabolism on glucose uptake and glycogen synthesis see [86]). The production of ketone bodies in the liver during consumption of the traditional Inuit diet is currently ambiguous (see section 2.3), but may decrease cerebral glucose consumption. Production of acylcarnitines is increased by L479, potentially decreasing the glucose consumption by BAT. The increase in *n-3* fatty acid metabolism by L479 may result in a decrease in stature, which may result in lower consumption of glucose for temperature homeostasis.
This model suggests that a selective pressure for glucose conservation may have existed in the Arctic environment, and that L479 may have provided a glucose sparing effect in this context. Further support for the model may be provided by animal studies of glucose metabolism by different tissues in response to varying ratios of macronutrients, and by virtual models of natural selection incorporating environmental factors.

evolutionary fitness of the Inuit in their historical environmental context, which has included exposure to extreme cold, very high protein intake in the absence of fat and carbohydrate, and the resulting effects on periods of high glucose demand such as during pregnancy [169], lactation [176] and an immune response 177]. It is also supported by the existence of human and mammalian traits that have been linked to a selective pressure for glucose conservation, described in section 4.1. They provide further support for the hypothesis that L479 may have been selected as a result of the same selective pressure. Those traits include the suppression of SCOT, the high fat mass of infants, and the insulin resistance that occurs during infection.

The model is based on the increased rate of hepatic fatty acid oxidation in the L479 variant of *CPT1a*, which will decrease glucose oxidation in the liver according to the Randle cycle. It takes into account the role of glucose conservation as a result of adaptations to cold exposure. Due to increased hepatic fatty acid metabolism, glucose that is not used by the liver or BAT will be rerouted to glycogen synthesis in the liver, and a higher blood glucose level will promote increased glucose uptake by other cells and tissues such as muscle and adipose tissue. If glucose uptake exceeds oxidation rate in muscle tissue, glycogen synthesis will occur, conserving glucose for periods of low availability (for a more detailed explanation of the effect of fatty acid metabolism on glucose uptake and glycogen synthesis see [86] and [174,175]).

5. Conclusion: Glucose conservation, cold adaptation, and a new hypothesis to explain the selection of L479

By examining the historical findings of low ketone bodies in the breath and urine of fasting Inuit subjects in light of the metabolic effects of *CPT1a* L479, data from those findings can be understood as an effect of the reduced rate of hepatic fatty acid oxidation among subjects with the L479 variant. This suggestion provides an updated explanation of Heinbecker's original interpretation of his results, which concluded that the metabolism of the Inuit differed from that of non-Inuit subjects in the low production of ketone bodies during fasting, despite low RQs indicating a high rate of whole-body fatty acid oxidation.

The possibility of higher levels of ketone bodies being produced but not detected in historical studies of Inuit metabolism cannot be entirely ruled out. Such a possibility may relate to loss of ketone bodies in the period between collection of the samples and analysis, or the faster metabolism of ketone bodies in Inuit subjects. Heinbecker concluded that there was ‘no reason to doubt the reliability of the total acetone body determinations’ in the Inuit samples on the basis of a control sample of urine from a diabetic patient, in which the total concentration of total ketone bodies remained constant during incubation at a high temperature. The possibility of faster metabolism of ketone bodies was not considered as an explanation for lower than expected ketone readings. But in light of the discussions and theoretical predictions above, including the account of keto-adaptation in section 2.4, such a process is unlikely.

There is currently no experimental evidence connecting the selection of L479 with the harmful effects of ketone over-production (specifically, ketoacidosis), or negative metabolic effects of abruptly switching off ketogenesis during relatively sudden periods of exceptionally high consumption of lean protein. Though future scholars may find other evidence to question the health consequences of a very low carbohydrate diet, the Inuit case study does not provide strong

evidence of a selective sweep "against ketosis" in the context of such a diet. Considering all available data, rather, the most plausible current hypothesis suggests the necessity of glucose conservation, possibly in combination with the requirement to mitigate effects of cold exposure and/or very high protein intake.

L479 may provide a glucose-sparing effect in the context of a very low carbohydrate and high protein diet by decreasing the inhibitory effect of malonyl coA in the fed state. This in turn increases the metabolism of fatty acids and reduces glucose consumption by the liver and by other organs and tissues expressing *CPT1a*. Glucose might be conserved further by increased hepatic ketone body production, which will decrease cerebral glucose consumption. But experimental confirmation of ketone production in the Inuit is currently lacking, and is challenged by the historical data of high protein intake in the Inuit, which were suggested in section 2.3 to prevent chronic ketone body production *via* the production of oxaloacetate.

Glucose may be also be conserved by other metabolic effects of the L479 variant, which relate to cold adaptation. L479 may increase the availability of acylcarnitines for BAT metabolism, decreasing the consumption of glucose by BAT. Alternatively, L479 may result in a decrease in stature caused by altered metabolism of *n-3* fatty acids. This effect may also link to glucose conservation: a morphological adaptation to reduce heat loss might provide a glucose-sparing effect by reducing the use of glucose for temperature homeostasis. A selective advantage in glucose conservation may be particularly relevant during episodes of extremely high protein consumption, when glucose demands may be increased due to the up-regulation of BMR that accompanies protein metabolism.

A high intake of *n-3* fatty acids are predicted to decrease a cost of L479 that arises in the fasted state. *N-3* fatty acids increase the basal activity of CPT-Ia, helping to compensate for the decreased rate of activity of the L variant of *CPT1a* in the fasted state. This, in turn, may allow increased glucagon secretion from *CPT1a*-expressing pancreatic α cells during fasting, thereby decreasing the likelihood of hypoketotic hypoglycemia, a condition that can be fatal in infants.

The updated glucose conservation hypothesis presented here is consistent with the effects of L479 *in vitro*, in which L479 causes a lower rate of fatty acid oxidation when expressed in human fibroblasts in the absence of malonyl coA. It suggests that the low ketone bodies observed in Inuit subjects consuming their traditional diet may also result from the anti-ketogenic effect of high protein intake. Although the L479 variant of *CPT1a* is less sensitive to inhibition by malonyl coA, high protein intake may inhibit ketone body production by increasing oxaloacetate levels, therefore inhibiting the hepatic production of ketone bodies even in the presence of a high rate of hepatic fatty acid oxidation.

Given that minimal glucose requirements can theoretically be met by high protein consumption, this updated hypothesis suggests that sufficient glucose cannot be produced *via* protein gluconeogenesis for optimal evolutionary fitness. Such insufficiency may be a result of the low efficiency of protein gluconeogenesis, or a result of a maximal limit of protein gluconeogenesis being reached during consumption of the traditional Inuit diet. In either case, the levels of glucose required for optimal evolutionary fitness would not be produced in the process of gluconeogenesis. Future experimental research and theoretical models may help to clarify the upper limit of protein gluconeogenesis, and the environmental factors most likely to create a glucose demand high enough to drive the selection of a variant that increases glucose conservation.

Further research is needed to assess the hypothesis that a selective pressure for glucose conservation drove the selection of L479. Corroboration of the hypothesis is important for our understanding of human metabolic science more generally. It would also underline the roles of glucose conservation, temperature homeostasis, and high intake of *n-3* fatty acids in driving the selection of metabolic variants, and may provide a framework to identify other genetic variants linked to these environmental factors.

6. Discussion: Implications of the *CPT1a* L479 selective sweep for the metabolic health Inuit and non-Inuit populations

If the low breath and urinary ketone bodies in historical readings of Inuit subjects can be shown conclusively to represent a low rate of production of ketone bodies, the excellent metabolic health of traditional Inuit populations may be attributed to factors other than chronic ketone body metabolism. The low rates of metabolic diseases, high glucose sensitivity, and favorable lipid readings observed in traditional Inuit populations may instead be explained by a high rate of fatty acid oxidation, as indicated by the low RQs during fasting. Low carbohydrate intake and a high intake of *n-3* fatty acids are likely to contribute to a high rate of fatty acid oxidation in the Inuit by decreasing the inhibition of CPT-Ia, and by activating CPT-Ia and other enzymes involved in fatty acid metabolism respectively.

The L479 allele may contribute further to the metabolic health of the Inuit by increasing the rate of fatty acid oxidation in the "fed" (high malonlyl-coA) state relative to the P479 allele, as predicted in the results of biochemical studies on *CPT1a* L479. The higher rate of fatty acid oxidation associated with L479 may explain why the variant is associated with a lower adiposity and higher HDL (see [66]). However, the levels of expression of *CPT1a* in various tissues are not known precisely, and so the effect of L479 on the whole-body rate fatty acid oxidation cannot be accurately determined. Furthermore, the RQ has not been measured in Inuit subjects consuming the traditional diet, and so experimental proof of a higher rate of fatty acid oxidation in subjects homozygous and heterozygous for L479 is lacking. Nevertheless, this hypothesis suggests that it would be worth investigating whether the health-promoting effect of low carbohydrate diets more generally could be attributed to an increased rate of fatty acid oxidation in addition to ketone body metabolism.

A diet with similar macronutrient ratios and *n-3* fatty acid content to the traditional Inuit diet would be expected to promote metabolic health in Inuit and non-Inuit populations alike by increasing the rate of fatty acid oxidation. However, the effects of very high protein consumption, such as those that occur seasonally in the Inuit, may differ according to the copy number of L479: by increasing the rate of fatty acid oxidation in the presence of malonyl coA, L479 may decrease a deficit in glucose that might occur during high protein intake. Glucose sensitivity may also be affected in individuals with different copy numbers of L479: by decreasing sensitivity of CPT-Ia to malonyl coA inhibition, L479 may result in decreased hepatic uptake of glucose when blood glucose level is high due to inhibition of glucose uptake by citrate as described by the Randle cycle (see Diagram 1). This may be an important factor contributing to the increasing incidence of diabetes and obesity in modern Inuit populations in response to increased intake of refined carbohydrates [36,37] which cause exceptionally rapid increases in blood glucose levels. Studies comparing glucose sensitivity and metabolism during very high protein intake between Inuit subjects with different copy numbers of L479 could test these two hypotheses.

The condition of ketoacidosis could potentially occur in Inuit and non-Inuit populations during low carbohydrate and high *n-3* fatty acid intake. However, the possible association of high in *n-3* fatty acids with the condition of ketoacidosis is currently speculative: at this time there are no case studies of ketoacidosis in which *n-3* fatty acids are suggested to be a contributing factor. Future investigation into the effect of high *n-3* fatty acid consumption on ketone body production are required to confirm that ketone bodies are not over-produced in this context, resulting in detrimental effects. In the absence of such evidence, the Inuit case study currently supports the safety and health promoting effects of a low carbohydrate diet in both Inuit and non-Inuit populations, as well as the health promoting effects of *n-3* fatty acids.

The susceptibility of Inuit infants homozygous for *CPT1a* L479 to hypoketotic hypoglycemia can be framed as an evolutionary mismatch between a mutation that has been selected for in the context of a low carbohydrate high *n-3* fatty acid diet and a modern diet including refined carbohydrates and a lower intake of *n-3* fatty acids. Collins

previously suggested that the susceptibility of homozygous carriers of L479 to hypoketotic hypoglycemia may result from the modern dietary changes, and that the historical occurrence of the condition would have likely been much lower [74]. Collins refers specifically to the high carbohydrate content of the modern Inuit diet. A more relevant factor may be the lower *n-3* fatty acid content of the modern Inuit diet. *N-3* fatty acids activate CPT-Ia [73]. A decrease in *n-3* fatty acid intake will therefore decrease the basal activity of CPT-Ia, resulting in insufficient rates of fatty acid oxidation in the fasted state. Reduced rates of breastfeeding may also increase risk of infants for hypoketotic hypoglycemia and SIDS. Importantly, as well as decreasing the incidence and severity of infections (see [178]), breastmilk contains *n-3* fatty acids in proportion to those consumed in the maternal diet [104,105]. *N-3* fatty acids in breastmilk may therefore protect against hypoketotic hypoglycemia by increasing the activity of CPT-Ia in pancreatic α cells, leading to increased glucagon production. Given that efforts to identify infants homozygous for *CPT1a* L479 most susceptible to episodes of hypoketotic hypoglycemia and SIDS have not been successful, dietary interventions to increase *n-3* fatty acid intake (especially in breastfeeding mothers), and to encourage breastfeeding, may prove to be more successful strategies in decreasing SIDS and episodes of hypoketotic hypoglycemia in Inuit infants.

This article has attempted to clarify our current state of knowledge of Inuit metabolism and the selective sweep in *CPT1a*. It challenges previous suggestions linking the selective sweep to the detrimental effects of a low carbohydrate diet, and instead links the selective sweep to a selective pressure to conserve glucose. In our efforts to improve the metabolic health of Inuit and non-Inuit populations, this article highlights the important effects of *n-3* fatty acids, and raises previously overlooked effects of high protein consumption. Future studies into metabolism may benefit from the integrative approach used in this article, which considers simultaneous interactions between all relevant environmental and genetic factors.

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