Title:

Selection pressure at altitude for genes related to alcohol metabolism: a role for endogenous enteric ethanol synthesis?

Authorship:

Connie Sturgess and Hugh Montgomery

Institute for Human Health and Performance; Department of Medicine, University College London, UK.

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Corresponding author: Constance Sturgess

email: constance.sturgess.17@ucl.ac.uk

Tel: 07747325105

Address: 47 Rose Mount, Oxton, Wirral, CH43 5SQ

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New Findings:

What is the topic of this hypothesis?

Highland natives have undergone natural selection for genetic variants advantageous in adaptation to the hypobaric hypoxia experienced at high altitude. Why genes related to alcohol metabolism appear consistently selected for has not been greatly considered. We hypothesise that altitude-related changes in the gut microbiome offer one possible explanation.

What advances does it highlight?

Low intestinal oxygen tension may favour the production of ethanol through anaerobic fermentation by the gut microbiome. Subsequent increases in endogenous ethanol absorption could therefore be a selection pressure for gene variants favouring its increased degradation - or reduced degradation if endogenously synthesised ethanol acts as a metabolic signalling molecule.

Abstract

Reduced tissue availability of oxygen (hypoxia) occurs in diverse disease states, across all age ranges. It also results from ascent to high altitude, where atmospheric pressure, and thus partial pressure of inspired oxygen, fall with ascent (hypobaric hypoxia).

Humans are obligate anaerobes, and adaptation to such hypoxia is necessary if functional capacity is to be retained or survival not to be threatened. These functional changes remain incompletely characterized, although metabolic adaptation (rather than simple increases in convective oxygen delivery) appear to play a fundamental role.

One means to explore such adaptation is through a population genetics approach. Those populations which have remained native to high altitudes have undergone natural selection for genetic variants associated with advantageous phenotypic traits. Many such variants have now been identified, influencing red cell mass and lipid metabolism, amongst others.

Interestingly, a consistent genetic signal has appeared in these studies, implicating a role for alcohol metabolism in the human adaptive response to hypobaric hypoxia. The reasons for this remain unclear, given that human cells appear incapable of alcohol synthesis. Here, we explore one possibility: that increased alcohol synthesis occurs through fermentation by gut bacteria in response to enteric hypoxia.

There is growing evidence that the gut microbiome changes with high altitude exposure, with anaerobes becoming increasingly prevalent and dominant. Many of these have the ability to produce ethanol from fermentation, alongside other products such as short chain fatty acids (SCFA).

We thus hypothesise that (i) ascent to high altitude renders the gut luminal environmental increasingly hypoxic, favouring (ii) an increase in the population of enteric fermenting anaerobes and thus (iii) the synthesis of alcohol which, through systemic absorption, leads to (iv) selection pressure on genes relating to alcohol metabolism. Such proposed selection could operate in one of two directions. In theory, alcohol could be viewed as a toxic product, leading to selection of gene variants which favour its metabolism. On the other hand, alcohol is a metabolic substrate which can also alter mitochondrial function in ways which might be beneficial – leading to selection pressure for alleles associated with reduced alcohol metabolism. Finally, alcohol can influence hypoxic signalling.

Not only could this present a potential mechanism contributing to the overall adaptation in those native to highlands, but it could account for some of the interindividual differences in the effectiveness of lowlander acclimatisation to altitude due to common variation in the effectiveness of exogenous alcohol metabolism, and in the gut microbiome itself. Future research should be aimed at determining any shifts to favour ethanol-producing anaerobes in lowland individuals ascending to altitude.

Introduction

This article represents the exploration of a hypothesis: that alcohol synthesised by bacterial fermentation in the gut might impact upon the process of adaptation to reduced systemic oxygen availability (hypoxia).

Hypoxia occurs in diverse disease states, across all age ranges. It also results with ascent to high altitude: as barometric pressure falls, the resulting reduction in partial pressure of inspired oxygen reduces the diffusion gradient across the alveolar membrane, leading to a reduction in systemic oxygen availability (hypobaric hypoxia).

Humans are obligate anaerobes, and adaptation (acclimatization) to such hypoxia is necessary if functional capacity is to be retained or survival not to be threatened. Near the summit of Mt. Everest (8848m), the lowest recorded partial pressure of arterial oxygen (PaO_2) is 19.1mmHg (compared to values of between 75mmHg – 100mmHg in healthy individuals at sea-level) (Grocott et al., 2009). Instantaneous exposure to such hypoxia would prove rapidly fatal, and yet acclimatised individuals are able to function physically and mentally, even if longer term survival is unlikely if such exposure is sustained for prolonged periods.

Acclimatisation occurs in both the short-term and long-term in those not previously exposed to altitude. In those native to high altitude, natural selection of beneficial phenotypic traits has occurred over generations. The study of such high altitude natives thus presents a valuable opportunity to identify mechanisms of human hypoxic adaptation.

Three major populations have existed for thousands of years at high altitude, located in the Andes, the Tibetan Plateau and the Ethiopian Highlands (Baker, 1987). In the Andes, indigenous high altitude residents demonstrate minimal genetic exchange with those residing in the rest of South America (Gómez-Carballa et al., 2018). The Tibetan plateau has likely been populated by descendants of local native (lowland) Han Chinese for more than 9000 years (Lu et al., 2016). The Sherpa represent a high altitude population derived from native Tibetan founders (Gilbert-Kawai et al., 2014).

Andeans, Tibetans and Ethiopians display distinct differences in phenotypic selection for hypobaric hypoxic exposure (Beall, 2007). For instance, Tibetans maintain a hypoxic

ventilatory response (HVR) and the hypoxia-related rise in oxygen-carrying is blunted. Andean HVR is lower than that in both groups (Beall, 2007), and their rise in haemoglobin concentration greater (Beall, 2002). Other mechanistic differences also exist (Beall, 2007; Beall et al., 2006).

Adaptation (and acclimatisation) appear dependent on changes in metabolism, rather than in simple convective oxygen availability (O'Brien, Simonson & Murray, 2020; Murray, 2015). Lower muscle mitochondrial volume is found in Tibetans and Andeans at altitude compared to those at sea-level. Mitochondrial mass also falls with high altitude exposure in lowlanders. Conversely, maximum oxygen consumption is relatively preserved at altitude in both Tibetans and Andeans, and is superior to that of acclimatised lowlanders using supplementary oxygen (Beall, 2007).

Genetic analysis of native highlanders has allowed exploration of evolutionary selection pressure at high altitude, and supports a role for changes in cellular metabolism in hypoxic acclimatisation (Murray et al., 2018). Hypoxia-inducible factors (HIFs) are transcriptional regulators, the activity of which rises with hypoxic exposure. During hypoxia, the HIF-1α and HIF-2α subunits escape degradation by propyl hydroxylase, leaving HIF-1α to form a dimer with HIF-1β in the cell nucleus. The HIF dimer then binds to hypoxia-response elements (HRE) in the promotor region of a diverse range of target genes encoding proteins involved in pathways relevant to survival in hypoxia. Target genes include those involved in oxygen delivery, such as erythropoietin (EPO) and vascular endothelial growth factor A (VEGF-A). In keeping with the phenotypic traits demonstrated by high altitude natives, selection for specific variants in genes which encode crucial elements of the HIF pathway - HIF-2α *(EPAS1)* (Beall et al., 2010) and prolyl hydroxylase 2 (*ENGL1*) - has been identified in Tibetans (Simonson et al., 2010).

The HIF pathway is implicated in the regulation of cellular metabolism, including the attenuation of oxidative metabolism through upregulation of pyruvate kinase 1 and subsequent inhibition of pyruvate dehydrogenase (Papandreou et al., 2006). Further confirming the role of metabolic adaptations to high altitude, selection for variants in the gene encoding PPARα (*PPARA*) has been identified, which are associated with a reduction in fatty acid oxidation (FAO) in both Tibetans and Sherpas, and with increased emphasis on glycolysis (Murray et al., 2018). Overall, metabolic adaptation seems to

involve a reduction in oxidative phosphorylation and thus oxygen demand, with more efficient oxygen utilisation (Murray et al., 2018).

However, a mystery remains: there seems a signal for selection of variants in genes regulating alcohol metabolism (Crawford et al., 2017; Huerta-Sánchez et al., 2013). The reasons behind this are not clear, and the associated beneficial phenotypic traits have not yet been identified. This paper aims to review this evidence, and to explore the hypothesis that this selection is driven by changes in synthesis of alcohol by the gut microbiome which might occur as a consequence of hypobaric hypoxia, and which might ultimately have metabolic consequences.

Pathways of alcohol metabolism and associated genes

Humans and many animals have well-developed pathways to metabolise alcohol. Ethanol is absorbed in the gastrointestinal tract by passive diffusion and distributes throughout tissues in proportion to their water content. Ethanol metabolism predominantly occurs in the liver, and though the rate of breakdown is roughly linear, there is some variation with ethanol concentration due to differing enzyme kinetics (Holford, 1987).

Hepatic alcohol metabolism begins with the oxidation of ethanol to acetaldehyde by the alcohol dehydrogenase (ADH) family of enzymes. Acetaldehyde is then oxidised to acetate by aldehyde dehydrogenase (ALDH). Acetate enters the circulation and distributes to peripheral tissues, where it can be converted to Acetyl CoA, an intermediate able to enter various metabolic cycles. Additionally, small amounts of 'first-pass' metabolism is performed by these enzymes located in the gastrointestinal tract (Cederbaum, 2012). The pathways of alcohol metabolism and relevant genes have been extensively reviewed by Zakhari (2006).

ADH enzymes can break down a variety of substrates including those involved in various other pathways including formaldehyde, nitrous oxide and dopamine metabolism. Seven ADH genes have been identified, all located on chromosome 4 (**Table 1**). The enzymes encoded are split into 5 classifications based on their differing properties including kinetics and substrate specificity.

The *ADH1A, ADH1B* AND *ADH1C* genes encode the alpha, beta and gamma subunits respectively, which can be arranged to produce a variety of homo and hetero-dimer *Class 1 ADH isozymes* (Edenberg & Bosron, 2010). They are characterised by a low Km and are the most important for ethanol oxidation in the liver. Class II ADH (*ADH4*) is more active at high ethanol concentrations due to its higher Km. Class III ADH (*ADH5*), also known as formaldehyde dehydrogenase, is also involved in the metabolism of exogenous and endogenous formaldehyde and in nitric oxide metabolism. Class IV ADH (*ADH7*) is present in the stomach and oesophagus and mainly oxidises retinol to retinal. Whilst the gene *ADH6* has been identified and defined as encoding class V ADH, the protein produced has not been characterised.

There is also a large family of ALDH enzymes, which can oxidise a variety of aldehydes (**Table 2**). Three of the 18 ALDH genes (*ALDH1A1*, *ALDH1B1* and *ALDH2*) encode enzymes particularly important for acetaldehyde oxidation in the liver (Hurley & Edenberg, 2012). ALDH activity generally exceeds the rate of alcohol oxidation, preventing the build-up of toxic acetaldehydes. The enzyme ALDH2 is thought to be responsible for most acetaldehyde oxidation produced by ethanol breakdown in the liver (Hurley & Edenberg, 2012).

The substantial numbers of single nucleotide polymorphisms (SNPs) in the gene encoding ADH, and the resultant diverse isoforms of ADH itself, are thought to contribute to inter-individual variation in the ability to metabolise alcohol: those with *ADH1B*2* and *ADH1C*1* alleles have a higher ethanol elimination rate, leading to a build-up of acetaldehyde. A polymorphism in the *ALDH2* gene, *ALDH2*2*, present in some East Asian populations, is associated with loss of enzyme function, again leading to acetaldehyde accumulation (Hurley, Edenberg & T. K. Li, 2002) and associated sweating, nausea and vomiting. These individuals, therefore, are less at risk of alcoholism due to these unpleasant effects.

The CYP2E1 enzyme (*CYP2E1*) provides an alternative catalyst for the oxidation of alcohol. The enzyme is a member of the cytochrome P450 family of enzymes which revolve around the breakdown of toxins and drugs in the body, oxidising a variety of substrates including steroids, fatty acids, and alcohols. CYP2E1 is mostly expressed in the liver and, exhibiting a high Km for ethanol, is more active at high ethanol

concentrations. Polymorphisms in CYP2E1 are associated with variation in drug response (Sutrisna, 2016). Although less significant, CYP1A2 and CYP2A4 also have the ability to oxidise ethanol (Salmela et al., 1998). However, the cytochrome P450 pathway can result in the production of oxygen free radicals.

The wide substrate specificity of cytochrome P450s, ADH and ALDH also translates into roles in omega fatty acid oxidation, a usually minor alternative pathway to beta oxidation (Rizzo, 2014). Following hydroxylation of fatty acids by members of the CYP4 family, the fatty aldehydes and alcohols produced are oxidised by ADH and ALDH.

Another less significant pathway in the liver uses the enzyme catalase (*CAT*), which normally catalyses the removal of hydrogen peroxide, but can also use hydrogen peroxide to oxidise alcohol to acetaldehyde. Catalase is thought to be more relevant to alcohol oxidation in the brain (Zimatkin et al., 2006). The oxidative pathways of ethanol metabolism are summarised in **Figure 1.**

Other, non-oxidative forms of alcohol breakdown exist. Ethanol reacts with glucuronic acid to produce ethyl glucuronide, a soluble, excretable product. The formation of fatty acid ethyl esters from ethanol and fatty acids is catalysed by fatty acid ethyl synthases in most tissues. This latter pathway is more heavily relied upon when the oxidative metabolism of ethanol is blocked, increasing the levels of potentially toxic esters in the circulation, and has been linked to organ damage in alcoholics (Laposata & Lange, 1986).

Genes related to alcohol metabolism identified in studies of high altitude adaptation

Interestingly, these genes involved in alcohol metabolism are amongst the genes ranked most highly for involvement in adaptation to high altitude, in numerous studies (**Table 3**), although the significance of this finding has yet to be clarified. Nor has such selection been related to known phenotypic traits.

In Ethiopian populations, analysis of SNP data highlighted differences in *ADH6*, *ADH1A*, *ADH1B* and *ADH1C* between high and low altitude Ethiopian groups (Huerta-Sánchez et al., 2013). Genomic comparison between high and low altitude communities within the (native high altitude) Amhara and Oromo Ethiopian ethnic groups also suggested

selection for variants in *ADH6* (Alkorta-Aranburu et al., 2012). Whole genome sequencing revealed evidence of selection in *ALDH2* in high altitude Andeans (Crawford et al., 2017). Another study of Andean highlanders identified a strong (reproduced in >1 test) signature of selection for ALDH3 (Eichstaedt et al., 2014).

The prevalence of specific allelic variants of *ADH7* differs between Tibetans and non-Tibetans of East Asian descent (Yang et al., 2017). Likewise, *CYP17A1* and *CYP2E1* appear to be under selection in Tibetans (Simonson et al., 2010). An investigation of 9 SNPs related to alcohol metabolism in Han, Hui, Tibetan, Mongolian and Uygur Chinese ethnic groups showed that all populations differed significantly at locus *ADH1B* (Wei et al., 2018). Furthermore, Tibetans had the lowest frequency for the *CYP2E1*5B* allele, and very low frequencies of *ADH1B*A* and *ALDH2*A* compared to the other groups. In fact, from this latter finding it was inferred that Tibetans were less likely to suffer from adverse reactions of alcohol consumption as these alleles are associated with acetaldehyde accumulation.

Genome wide SNP analysis of high altitude Deedu Mongolians also identified *CYP26A1* and *CYP26C1* as targets for selection (Xing et al., 2013). Three candidate genes for high altitude selection in Tibetans were also highly significant in Mongolians, one of which was *CYP2E1* (Xing et al., 2013). Another study (albeit of only 100 subjects) found 3 new polymorphisms in *CYP2E1* in Tibetan Chinese not previously identified in lowlanders (L. Wang et al., 2017). Differential selection for *ALDH3A1* was identified in genome-wide analysis of Sherpa and Tibetan highland populations (Zhang et al., 2017).

Animals naturally adapted to high altitude environments also demonstrate selection for alcohol metabolism genes - for instance, *CYP1A1* and *CYP7A1* in high altitude Ladakhi cows (Verma et al., 2018). In a study exploring the evolution of energy metabolism genes in a range of hypoxia-tolerant mammals including whales, Tibetan yak and Tibetan antelope, *ALDH3A1* was identified as a common target (Tian et al., 2017). Genes have been selected for in Andean horses living at high altitude since they were introduced in the 1500s, with the region of chromosome 14 containing several *CYP3A* genes showing significant frequency divergences from their low altitude relatives (Hendrickson, 2013).

A study used 327 genomes from horse, sheep, goats, cattle, pigs, and dogs at high and low altitudes to identify any convergent evolution for high altitude adaptation (Wu et al., 2019). *ADH7* was identified in Tibetan cattle, with 3 different SNPs showing a high level of differentiation from other cattle.

However, a genome-wide analysis of Andean highlanders did not report an association with alcohol metabolising genes (Bigham et al., 2009), nor did an allelic differentiation scan between Tibetans and lowland Han Chinese (Beall, 2010).

Whilst the genes related to alcohol metabolism identified are not consistent in each study, populations have followed differing mechanisms in other areas of altitude adaptation, and could have varied mechanistic alterations in their alcohol metabolism. Additionally, candidate gene analyses may have put heavier emphasis on those involved in the HIF pathway than those relating to alcohol metabolism. It is also not always clear if the alleles selected for are trait-increasing or trait-decreasing. The question remains as to what process may have led to such selection, given that human cells cannot synthesise alcohol, and with what advantageous phenotypic traits the genetic selection is associated.

Examining their traditionally emphasised role in the breakdown of ethanol, a possibility is presented. Assuming exogenous alcohol intake is not vastly different amongst low and high altitude populations, variation in these genes could relate to alterations in the metabolism of endogenous alcohol produced by the gut microbiome.

Evidence for endogenous ethanol production by gut microbiota

Despite the extensive pathways for alcohol metabolism, human cells cannot (as far as we know) produce ethanol. Exogenous alcohol sources (fermented products and drinks) require degradation. However, significant amounts of ethanol are found in the portal vein of rats and humans not exposed to exogenous alcohol (Blomstrand, 1971; Sarkola & Eriksson, 2001). This appears derived from endogenous synthesis by gut microbiota through fermentation.

More than half of faecal weight comes from microorganisms. Although the majority of the gut microbiome is comprised of bacteria (with over 1000 species present), viruses, fungi and archaea are also present (Lozupone et al., 2012). Both bacteria and fungi are capable of performing fermentation. There are multiple forms of anaerobic fermentation which can produce a range of substrates. Different microorganisms have varying dominant end products.

Anaerobic bacteria are divided into three main categories; obligate anaerobes that cannot survive in oxygen, facultative anaerobes that can grow without oxygen but use oxygen if it is present, and aerotolerant bacteria, which cannot use oxygen for their growth but equally are not harmed by it. Anaerobic bacteria can use a variety of substrates to produce ATP without oxygen. There are multiple anaerobic pathways utilised by bacteria, including anaerobic respiration (which involves an alternative final electron acceptor other than oxygen, such as sulphate) and multiple forms of fermentation. Fermentation usually begins with glycolysis followed by extra steps to revert NADH back to NAD+ so that it can be reused. Ethanol fermentation, mixed acid fermentation and heterofermentation can all produce ethanol as a product.

A broad range of gut commensal bacteria are capable of ethanol synthesis (**Table 4**). For example, obligate anaerobes in the *Bacteroides* genus and facultative anaerobes in the *Enterobacteriaceae* family can both produce ethanol from mixed-acid fermentation. Others use heterofermentation to produce both lactic acid and ethanol. Significant amounts of ethanol are produced by the gut commensals *Lactobacillus fermentum* and *Weissella confusa* from glucose and fructose, and lesser amounts by *Bifidobacterium Longum, Escherichia coli* and *Bacteroides thetaiotaomicron* (Elshaghabee et al., 2016). The gut microbiome is intricately linked with health. Dysbiosis of the commensal flora is associated with disease states such as inflammatory bowel disease (Ohland & Jobin, 2015). The effects of an overgrowth of ethanol-producing microbes can also contribute to disease, allowing identification of those highly reliant on ethanol fermentation as an energy source and giving further evidence of endogenous ethanol production. *Escherichia* was found to be abundant in non-alcoholic steatohepatitis (NASH) patients alongside high serum alcohol concentrations (Zhu et al., 2013), and endogenous ethanol produced

by *Klebsiella pneumoniae* is linked to 'non-alcoholic' fatty liver disease (Chen et al., 2020).

'Gut fermentation syndrome', or 'auto-brewery syndrome' is also recognised. Those affected become intoxicated from a high rate of microbiota ethanol production in the context of high carbohydrate intake. Case studies suggest a role for high alcohol producing yeasts, including *Saccharomyces cerevisiae* (Cordell & McCarthy, 2013), *Candida albicans* (Kaji et al., 1976) and *Candida keyfr* (Jansson-Nettelbladt et al., 2006). The same syndrome, with a background of Crohn's disease, has identified *Candida glabrata* as the culprit (Welch et al., 2016). Whilst there are many triggers for pathological dysbiosis, oxygen availability can also affect the dominant species in the gastrointestinal tract.

Changes in oxygen tension alters the composition of the gut microbiome

The GI tract is subject to a longitudinal and radial oxygen gradient (Espey, 2013). In normoxia, the oxygen tension diminishes quickly from the luminal surface to the centre of the lumen, where there is almost complete anoxia. This gradient favours facultative anaerobes such as *Escherichia coli* and *Enterococcus faecalis* nearer the luminal surface, and obligate anaerobes further into the lumen, reflecting the impact of oxygen tension on microbial communities. In fact, a switch to the predomination of aerotolerant species such as lactobacilli and enterobacteria occurred in ileostomies exposed to air, which then returned to normal balance after closure - demonstrating the rapid response of the microbiota to changes in oxygen availability (Hartman et al., 2009). Likewise, a 4 day exposure to hyperbaric oxygen therapy, resulting in an increase in the luminal partial pressure of O_2, causes an increase in oxygen-tolerant organisms adherent to the mucosa (Albenberg et al., 2014).

Anatomical variation in the oxygen tension of the GI tract impacts the dominant bacterial phyla. In the more oxygenated stomach, firmicutes and actinobacteria dominate, while around the (lesser oxygenated) duodenum, firmicutes and proteobacteria dominate

(Espey, 2013). In the more hypoxic colon, firmicutes and bacteroidetes dominate (Espey, 2013).

It thus appears possible that the low partial pressures of oxygen experienced at high altitude could favour the growth and survival of anaerobic bacteria and yeast, and importantly result in increases in endogenous ethanol production (**Figure 2**). This could perhaps explain the highly ranked genes related to alcohol metabolism in those native to high altitude.

The gut microbiome at high altitude

Not until more recently has the gut microbiome begun to be the subject of studies of altitude adaptation.

Firstly, animals have demonstrated changes in gut microbiome at altitude. The gut commensals of high altitude antelopes, frogs, pikas, black-necked cranes, mice and Chinese macaques differ from lowland populations (Ma et al., 2019; L. L. Xu et al., 2020; W. Wang et al., 2020; Zhao et al., 2018; Suzuki et al., 2019; H. Li et al., 2018).

Similar results are found in human populations. The gut microbiome of Tibetans at 6 locations across the Tibetan plateau ranging from 2800m – 4500m varied significantly with increasing altitude (Lan et al., 2017). The obligate anaerobe *Bifidobacterium* was more abundant in the populations dwelling above 3000m. The same was true of high altitude Indian natives (3500m), with more Bacteroidetes and less Proteobacteria found (Das et al., 2018).

Human genome interaction leads to differences in the gut microbiome (Goodrich et al., 2014), a fact which might account for distinct differences in the gut microbiome of Tibetans and Han Chinese living at the same altitude (3600m), with anaerobic Prevotella, facultative anaerobe *Enterococcus* and obligate anaerobes *Megasphaera* and *Clostridiales* more prevalent in the Tibetan population (K. Li et al., 2016). Possible confounding factors (such as differences in diet and individual immunology) make drawing conclusions from microbiome analysis complex. Variation has also been linked with body mass index (BMI) and age (Lan et al., 2017), and the harsher, plant based diets

of some high altitude herbivores (Ma et al., 2019). Diet has also been highlighted as a main factor in microbiome differences between human populations at varying altitudes (K. Li et al., 2016; Das et al., 2018).

However, changes in gut microbiota at altitude may be independent of the effects of diet, with alterations in dietary protein to fat ratio having minimal effects on gut composition in lowland humans acclimatising to high altitude (Karl et al., 2018). Furthermore, 15 Indian army men, consuming homogenous army specific-diet, had faecal samples taken in lowland environment and during acclimatization at around 3500m (Adak et al., 2013). Total aerobes decreased 50-fold whereas total anaerobes increased 115-fold after 15 days of high altitude acclimatisation. For example, Lactobacillus numbers rose 74-fold (Adak et al., 2013). In addition, high altitude exposure caused shifts in the microbiome to favour obligate anaerobes in mice, including increases in Prevotella and Anaeroplasma (Suzuki et al., 2019). The differences in gut microbial community between high and low altitude mice persisted when the effects of body mass, diet, reproductive status and population of origin were accounted for.

Adaptation to hypoxia in lowlanders who ascend to altitude does not appear to be related to changes in consumption of exogenous alcohol. Likewise, there are no data to suggest that habitual alcohol consumption is greater in native (genetically-adapted) high altitude populations. Exercise patterns and dietary intake might in theory differ between high and low-altitude native populations, and this may influence the gut microbiome in a manner which alters fermentation processes in addition to the impacts of hypoxia itself.

Is the genetic selection for improved degradation of alcohol?

If allelic selection has indeed occurred in response to increased alcohol biosynthesis, in what way do the function of these variants differ? One possibility is that they favour increased degradation of ethanol to cope with the higher volumes produced by the gut microbes.

There are various obvious reasons why increased ethanol would be harmful to the body. Chronic alcoholics suffer from liver disease, so increases in ethanol degradation could be protective at altitude. In fact, compared to both obese and healthy controls, ethanol-producing bacteria are more abundant, and serum ethanol higher, in patients with non-alcoholic steatohepatosis (NASH) (Zhu et al., 2013). Furthermore, transcription of the genes for ADH, catalase and CYP2E1 were elevated in those with NASH, perhaps to cope with the increased amounts of endogenous ethanol (Baker et al., 2010). The increase in endogenous ethanol synthesis may be causative in the pathogenesis of non-alcoholic fatty liver disease, through the induction of mitochondrial dysfunction and subsequent overproduction of reactive oxygen species (Chen et al., 2020). Meanwhile, ethanol in combination with hypoxia causes greater cellular damage than either ethanol or hypoxia alone in rat hepatoma cells (S. M. Wang & Wu, 2009).

Therefore, selection may have occurred at altitude for genetic variants associated with increased alcohol oxidation. However, increased alcohol oxidation also has adverse effects. Alcohol metabolism results in an increased hepatic redox state. The NAD+/NADH redox ratio is lowered as NADH is produced during the conversion of ethanol to acetaldehyde and acetaldehyde to acetate. This can inhibit glycolysis, pyruvate dehydrogenase, fatty acid oxidation, gluconeogenesis and the citric acid cycle (Ontko, 1973; Badawy, 1977). The inhibition of fatty acid oxidation due to the increased redox state during ethanol breakdown is a possible cause of fatty liver development in alcoholism. Interestingly, decreased FAO is already though to contribute to high altitude adaptation. Furthermore, the re-oxidation of NADH to maintain alcohol metabolism and other processes is mainly done by the mitochondrial electron transport chain, and oxidative phosphorylation appears to be downregulated in those adapted to altitude (Murray et al., 2018).

Significantly, hypoxic damage to the liver is noted in chronic alcoholics. This occurs due to increased oxygen demand through increased alcohol oxidation, so less oxygen reaches perivenous hepatocytes. CYP2E1 has been implicated in contributing to ethanol-induced hypoxia, oxidative stress and activation of HIF-1α (X. Wang et al. 2013). Conversely, HIF-1α was also suggested to play a part in CYP2E1-dependent ethanol-induced toxicity (X. Wang et al., 2013). While endogenous ethanol production might not reach levels

sufficient to have such impacts, this pathophysiology could be somewhat more pronounced in hypoxic conditions.

The raised oxygen requirements for extra ethanol metabolism at high altitude could therefore point towards adaptation favouring a more efficient mechanism of alcohol breakdown or more reliance on non-oxidative pathways. It is also possible that even if altitude induces more endogenous ethanol production, it is not enough to be relevant to any of these effects.

Interindividual variation in ADH and ALDH genes between lowlanders, and related differences in alcohol metabolism, could thus contribute to differences in performance at altitude - as could genetically-determined differences in the gut microbiome.

Or is less efficient alcohol degradation advantageous?

Alternatively, alcohol could be advantageous at high altitude and selection may have been for alleles associated with reduced alcohol degradation.

Alcohol consumption reduces the risk of coronary disease (Rimm et al., 1991), and long-term moderate consumption protects against myocardial ischaemic-reperfusion injury by mimicking ischaemic preconditioning (Miyamae et al., 1997). Raised endogenous ethanol production could have a similar effect. Furthermore, the previously discussed downregulation of mitochondrial metabolic processes as a result of alcohol oxidation could equally be deleterious at altitude, meaning that reduced alcohol metabolism would be favourable. A deeper understanding of how metabolic pathways are altered at high altitude is needed to determine this.

Numerous studies have demonstrated the influence of alcohol in altering HIF-1α levels in various tissues (Morris & Yeligar, 2018). For example, chronic alcohol exposure can increase HIF-1α expression in the brain cortex (Reddy et al., 2013). Although most of the effects of alcohol on HIF are linked to organ damage, this is generally in the setting of large amounts of exogenous ethanol consumption. The effects of low-level chronic alcohol are less well known, and often the effects of alcohol on hypoxic signalling differ

depending on chronic or acute alcohol consumption. Therefore, selection for variants in alcohol metabolism genes could be related to the effects of increased endogenous ethanol on hypoxic signalling and HIF pathways.

Or is the genetic selection based on other functions of the gene products, unrelated to alcohol metabolism?

The products of those genes under selection may have roles other than the metabolism of alcohol.

Anaerobic fermentation may yield products other than alcohol, such as Short Chain Fatty Acids (SCFA, albeit that the very anaerobes producing SCFAs commonly also synthesise ethanol as another product of fermentation (Miller and Wolin, 1979)). Expanded anaerobic populations have thus been hypothesised to drive an increase in SCFA production, triggering more efficient energy intake with increased utilisation of this energy source at high altitude (Mazel, 2019). In support, Ruminococcaceae and Clostridium populations (both of which can synthesise SFCA) are expanded in high altitude antelope (Ma et al., 2019). Similarly, high altitude pikas had increased total SCFA concentration (H. Li et al., 2018). Lachnospiraceae and Pesudobutyrivibrio were high in a native Tibetan population living at 3600m, and both are able to produce butyrate (a SCFA) (K. Li et al., 2016).

It is possible that increased SCFA absorption at altitude has a role in driving selection of some of variants described, given the roles of cytochrome P450s, ADH and ALDH in omega fatty acid oxidation, and the role of altered fatty acid metabolism in high altitude adaptation (Murray et al., 2018; O'Brien et al., 2019). This suggests an alternative role for variants of these genes at altitude in relation to fatty acid metabolism (Foll et al., 2014). As previously discussed, CYP2E1 can also metabolise steroids and fatty acids.

In addition, ADH5 is also known as S-nitrosoglutathione reductase (GSNOR), and is involved in nitric oxide homeostasis (Liu, 2004), and nitric oxide has been suggested to play a role in high altitude adaptation (Beall, 2007).

Finally, ALDH (ALDH2 particularly) may play a role in protecting against harmful reactive oxygen species (T. Xu et al., 2017), with the ALDH3A1 playing a possible role in protecting against oxidative stress in hypoxia-tolerant mammals (Tian et al., 2017).

Future Directions

Future research into adaptation to high altitude should further explore genes related to the different pathways of alcohol metabolism and use them in candidate gene studies along with hypoxia-related genes in those native to high altitude. To determine the shift to favour anaerobes capable of producing ethanol, a future study could examine individuals' shift in gut microflora to specifically ethanol-producing microorganisms in those ascending from lowland residencies to high altitude (>3000m), taking faecal samples before ascent and throughout acclimatisation. To account for factors other than high altitude affecting the microbiome, diet should be as controlled as possible. It could be particularly valuable to consider individual shifts over the course of acclimatisation due to varying baseline interindividual microbiota. The functional effects on alcohol metabolism of the alleles associated with high altitude, might be explored. Finally, the putative presence of increased SCFAs at altitude, and whether this accounts for the allelic enrichment observed (given that many reported genes have both SCFAs and ethanol as substrates) requires further study.

Conclusion

Whilst the metabolic changes which contribute to high altitude adaptation are increasingly understood, they remain incompletely described. High altitude exposure is associated with increasing enteric hypoxia, and with increases in the population of fermenting anaerobes, capable of synthesising ethanol and SCFAs. Selection for alleles in genes which metabolise alcohol (and often also SFCAs) is consistently observed in both animals and humans, and may thus represent a response to the increased biosynthesis of such substrates. Selection might in theory favour degradation of potentially toxic

ethanol. Alternatively, selection for reduced degradation might occur if substrates assist in hypoxic adaptation. Future research should seek to identify increases in ethanol-producing anaerobes in lowland individuals ascending to altitude, whether endogenous biosynthesis does increase, and the functional impact of those genetic variants so far identified. Results may have consequences beyond the field of high altitude physiology, indeed suggesting the gut to be a sensor for tissue hypoxia, with ethanol and SCFAs as its signalling molecule.

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Additional information

Competing interests

None declared.

Author contributions

CS and HM developed the hypothesis and co-authored the manuscript.

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Tables and Figures

Table 1. Human alcohol dehydrogenase genes approved by HUGO Human Gene Nomenclature Committee.

Gene symbol	Gene name
ADH1A	Alcohol dehydrogenase 1A (class1), alpha polypeptide
ADH1B	Alcohol dehydrogenase 1B (class1), beta polypeptide
ADH1C	Alcohol dehydrogenase 1C (class1), gamma polypeptide
ADH4	Alcohol dehydrogenase 4 (class II), pi polypeptide
ADH5	Alcohol dehydrogenase 5 (class III), chi polypeptide
ADH6	Alcohol dehydrogenase 6 (class V)
ADH7	Alcohol dehydrogenase 7 (class IV), mu or sigma polypeptide

Table 2. Human aldehyde dehydrogenase genes approved by HUGO Human Gene Nomenclature Committee.

Gene symbol	Gene name
ALDH1A1	Aldehyde dehydrogenase 1 family member A1
ALDH1A2	Aldehyde dehydrogenase 1 family member A2
ALDH1A3	Aldehyde dehydrogenase 1 family member A3
ALDH1B1	Aldehyde dehydrogenase 1 family member B1
ALDH1L1	Aldehyde dehydrogenase 1 family member L1
ALDH1L2	Aldehyde dehydrogenase 1 family member L2
ALDH2	Aldehyde dehydrogenase 2 family member
ALDH3A1	Aldehyde dehydrogenase 3 family member A1
ALDH3A2	Aldehyde dehydrogenase 3 family member A2
ALDH3B1	Aldehyde dehydrogenase 3 family member B1
ALDH3B2	Aldehyde dehydrogenase 3 family member B2
ADLH4A1	Aldehyde dehydrogenase 4 family member A1
ALDH5A1	Aldehyde dehydrogenase 5 family member A1
ALDH6A1	Aldehyde dehydrogenase 6 family member A1
ALDH7A1	Aldehyde dehydrogenase 7 family member A1
ALDH8A1	Aldehyde dehydrogenase 8 family member A1
ALDH9A1	Aldehyde dehydrogenase 9 family member A1
ALDH16A1	Aldehyde dehydrogenase 16 family member A1
ALDH18A1	Aldehyde dehydrogenase 18 family member A1

Table 3. Method details and genes related to alcohol metabolism identified in high-altitude genetic studies of humans and animals

Study	Type of gene analysis	Measure used for selection	Gene(s)	Advantageous allele	Functional attributes of allele
Alkorta-Aranburu et al., 2012	Genome-wide analysis	Population branch statistics and multiple regression	ADH6	rs12510722	None yet reported
Beall et al., 2010	Genome-wide analysis and candidate gene analysis	Genome-wide allelic differentiation scan	N/A	N/A	N/A
Bigham et al., 2009	Candidate gene analysis	Locus-specific branch length, the natural log of the ratio of heterozygosities, Tajima's D and the whole genome long-range haplotype test	N/A	N/A	N/A
Crawford et al., 2017	Genome-wide analysis	Population branch statistics	ALDH2	N/A	N/A
Hendrickson, 2013	Genome-wide analysis	SNP-by-SNP allelic model and sliding window F_{ST}	CY3PA	CYP3A393 (rs7283092) CYP3A89 (rs7359845) CYP3A94-97 (rs7369733)	None yet reported
Huerta-Sánchez et al., 2013	Genome-wide analysis	Population branch statistics	ADH6, ADH1A, ADH1B, ADH1C	No SNP/allele details found	N/A
Eichstaedt et al, 2014	Genome-wide analysis	Integrated haplotype score, Cross-population extended haplotype homozygosity, population branch statistics and pairwise F_{ST}	ALDH3	ALDH3B2	None yet reported
L. Wang et al., 2017	Analysis of CYP2E1 gene polymorphisms	X^2 test	CYP2E1	CYP2E1*1A most frequent allele in the Tibetan population	Normal enzyme activity
Simonson et al., 2010	Genome-wide analysis and candidate gene analysis	Cross-population extended haplotype homozygosity and the integrated haplotype score	CYP2E1, CYP17A1	No SNP/allele details found	N/A
Tian et al., 2017	Candidate gene analysis	Phylogenetic analysis by maximum likelihood branch-site model	ALDH3A1	No SNP/allele details found	N/A

Verma et al., 2018	Transcriptome analysis	Fold change statistic	CYP1A1, CYP7A1	No SNP/allele details found	N/A
Wei et al., 2018	Analysis of 9 selected SNPs	Chi-squared test and Fisher's exact test	ADH1B (rs1229984), ADH1C (rs698), ALDH2 (rs671) AND CYP2E1*5B (rs2031920) studied	Low frequencies of CYP2E1*5B, ALDH1B*A and ALDH2*A in Tibetans*	Low frequencies of CYP2E1*5B, ALDH1B*A AND ALDH2*A lead to reduced acetaldehyde accumulation *(Wall et al., 1997)*
Wu et al., 2019	Genome-wide analysis	Cross-population extended haplotype homozygosity, ΔDAF and F_{ST}	ADH7	No SNP/allele details found	N/A
Xing et al., 2013	Genome-wide analysis	Cross-population extended haplotype homozygosity and the integrated haplotype score	CYP26A1, CYP26C1	No SNP/allele details found	N/A
Zhang et al., 2017	Genome-wide analysis	Integrated haplotype score, extended haplotype homozygosity, Cross-population extended haplotype homozygosity and population branch statistics	ALDH3A1	Novel missense variant: Chromosome 17 ALDH3A1, GRCh37	None yet reported

Table 4. Examples of ethanol-producing bacteria genera in the human microbiome. Adapted from Wiegel (1980).

Genus	Number of species
Anaerobic and facultative anaerobic bacteria:	
Spirochaeta	3
Treponema	1
Enterobacteriaceae (10 genera)	16
Zymomonas	2
Pasteurella	2
Bacteroides	4
Fusobacterium	2
Lachnospira	1
Desulfovibrio	1
Staphylococcus	1
Streptococcus	2
Peptococcus	2
Peptostreptococcus	2
Ruminococcus	2
Sarcina	2
Clostridium	30
Bacillus	15
Lactobacillus	6
Eubacterium	6
Bifidobacterium	18
Actinomyces	1
Aerobic bacteria:	
Pseudomonas	5
Alcaligenes	1
Paracoccus	1
Bacillus	1

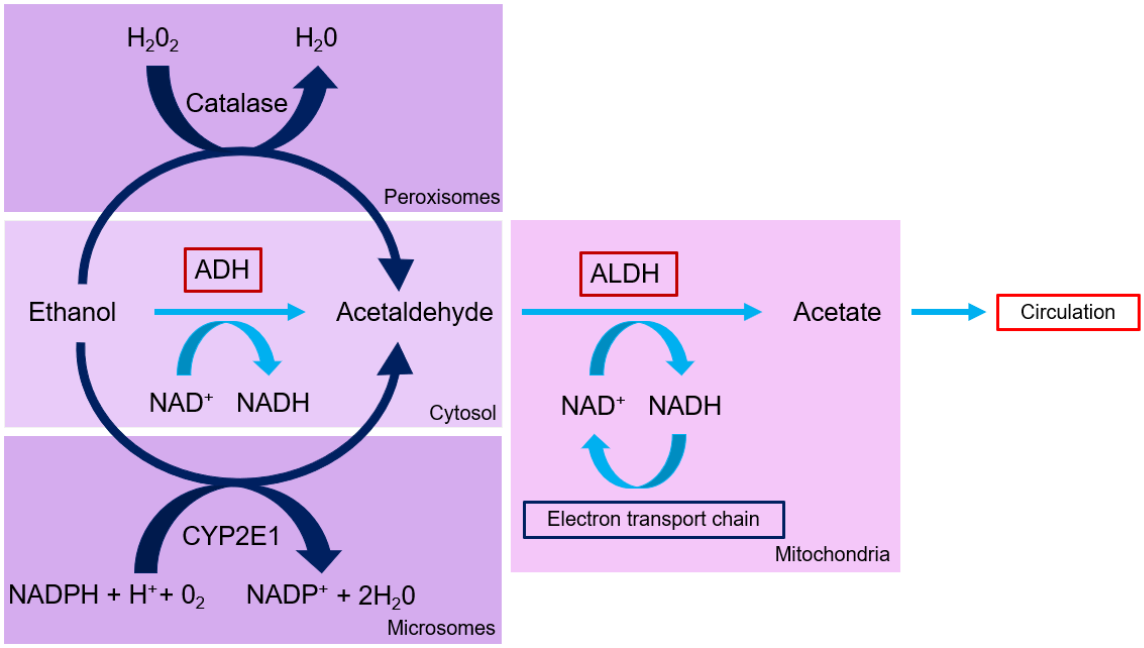

Figure 1. The pathways of alcohol oxidation.

Abbreviations: ADH, aldehyde dehydrogenase; ALDH, aldehyde dehydrogenase; and CYP2E1, cytochrome P450 2E1.

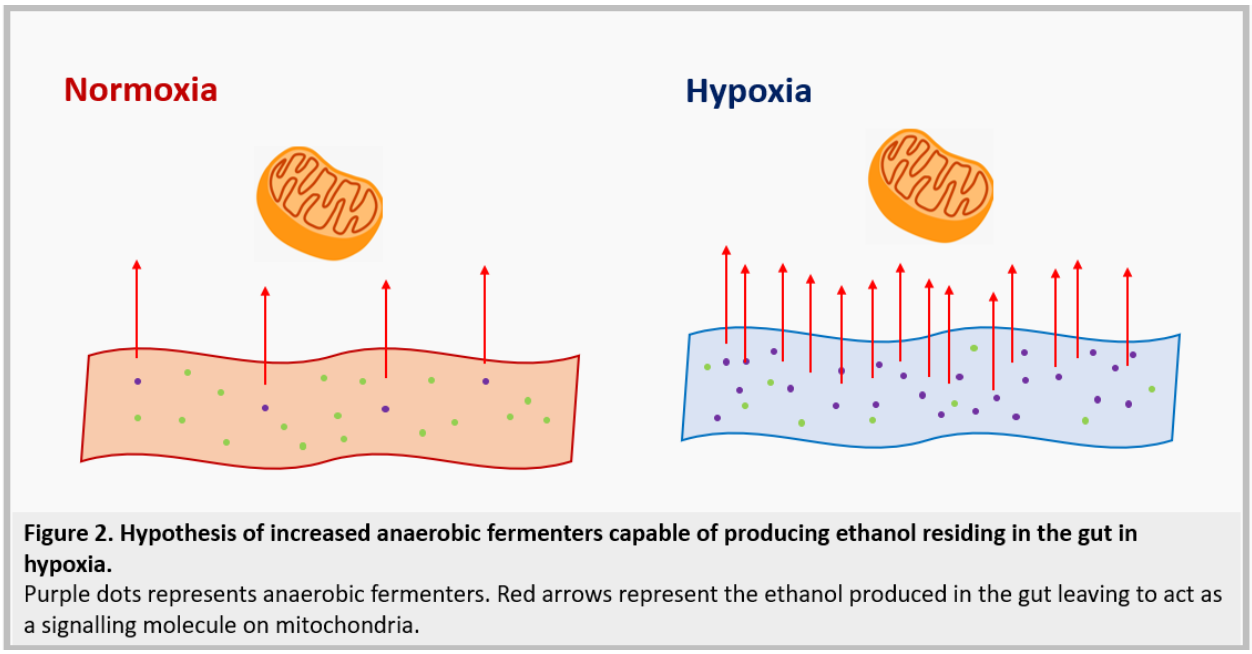

Figure 2. Hypothesis of increased anaerobic fermenters capable of producing ethanol residing in the gut in hypoxia.

Purple dots represents anaerobic fermenters. Red arrows represent the ethanol produced in the gut leaving to act as a signalling molecule on mitochondria.