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*Chapter 2*

# Milk, Lactose, Lactase: The Medical Adventure

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## Abstract

Lactose is a disaccharide which is the main carbohydrate present in milk of most mammals, including humans. At birth mammals possess lactase for digestion but lose this enzyme after weaning. In humans however, about 1/4 to 1/3 of the world's population retain lactase throughout adulthood, allowing continued consumption of milk.

The consequences of this human divide are the incurring of digestive symptoms in those who have limited intestinal lactase and consume large enough quantities at a time.

The medical issues arising from this dichotomy then include the consequences of lactose associated symptoms and the more important consequences of diary food avoidance. In addition dairy foods are linked or hypothesized to increase or decrease risks for many diseases. Perhaps one of the most interesting aspects of the world lactase dichotomy is the relationship of population distribution and geographic patterns of modern

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day, so called “western” lifestyle linked diseases. The asymmetric distribution and increasing incidence/prevalence of these diseases in developing nations sheds light on the possible co evolutionary impact of lactase pheno or genotype on such diseases. The development of the asymmetric lactase patterns and consequent medical aspects will be discussed.

# Introduction

Lactose is a disaccharide which is the main carbohydrate present in milk of most mammals, including humans. It consists of two hexoses, glucose and galactose (Figure 1).Virtually all newborn mammals are born with the intestinal brush border enzyme lactase phlorizin hydrolase (LCT, or LPH), which hydrolyzes lactose into its components. These are then absorbed through the intestine. However, newborn lose this enzyme after weaning (lactase non persistence, LNP). In some mammals lactase may be induced by reintroduction of lactose into the diet, but humans are different. In about 1/4 to 1/3 of the world’s population enzyme levels are retained throughout adulthood (lactase persistence, LP). As such this is a dominant Mendelian trait and allows continued consumption of dairy foods (including high lactose containing fluid milk) throughout adulthood. In the rest of the population with LNP, enzyme LPH is reduced in intestinal tissue and is not inducible by reintroducing lactose into the diet [1].

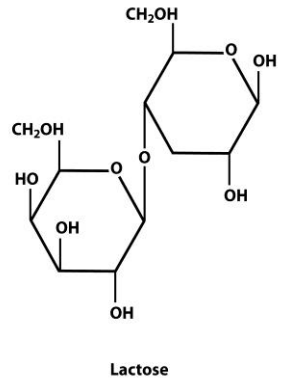


Figure 1. Chemical Structure of the disaccharide lactose consisting of 2 aldohexoses: glucose and D – galactose.

This chapter will outline the evolution and spread of the dominant genetic trait responsible for continued adult digestion of lactose. The genetics involved will be described. Secondary causes of lactose maldigestion will be outlined, but the bulk of the chapter will discuss the medical relevance which divides humanity into those who can and those who cannot digest the sugar. The diagnostic methods used to distinguish LP form LNP will be outlined. A discussion of the effect of this divide on other diseases and modern geographic distributions will be addressed.

## The Evolution and Spread of Lactose Digestion

In order to understand the current distributions of LP/LNP, migrations of early humans and the introduction of animal husbandry and milking practices in culture need to be elaborated. The evolution of lactase persistence and adult ability to digest lactose is a relatively recent event in human history and is believed to still be under selective pressure [2]. The dominant trait is thought to have evolved around 7.5 $x10^3 – 10^4$ years ago [3]. Earlier than this, humans like other mammals were thought to have no adult ability to digest this disaccharide. A popular website outlines over $10^5$ years of human migration (http://www.bradshawfoundation.com/journey/). People of central Asian origin are thought to have travelled over an existing land mass connecting Asia and North America some 18 $X10^4$ years ago during the last Ice Age. From here, territories of modern Canada, United States and Central and South America are thought to have been populated. Comparisons of Y chromosomes between native Americans and Siberians support this hypothesis [4]. Migrations into the south pacific may have occurred more directly from Africa [5. Similarly in Europe early Neolithic man was unable to digest lactose [6]. Then in the last ten thousand years lactose digestion became possible. The explanation for this evolutionary step is now strongly supported by evidence of the introduction of animal husbandry and subsequent milk drinking occurring almost simultaneously in several parts of the old world [3]. This hypothesis is referred to as the gene-culture co evolution or gcc hypothesis. This hypothesis proposed first by Simoons [7, 8] and then Holden and Mace [9] ultimately prevails over two other utilitarian hypotheses. An earlier hypothesis was put forward by Flatz and Rothhauwe to account for a close relationship between a north to south gradient of increasing population

frequency of LNP status in Europe [10]. They postulated that lactose digestion dominance preceded introduction of herding. The development of LP status occurred as a compensation for low population exposure to sunshine and skin synthesis of vitamin D. The ability to digest lactose would allow milk drinking with exposure to large amounts of calcium. In turn this reduces risk of rickets an important cause of poor bone formation. This is referred to as the calcium assimilation hypothesis. Another hypothesis by Vullo and Anderson postulated that LNP status protects against malaria [11]. There is little support for this hypothesis and is discussed below.

Most genetic anthropological studies and simulated models favor the gcc hypothesis, but in Europe the latitudinal pattern is still not fully explained [12, 13]. According to the prevalent gcc hypothesis, milk drinking originated in the central Balkans and populations from here migrated north and then east [12, 13]. Indeed several reports favor such migrations into central Russia and subsequently into northern India and possibly northern China [14-16]. At around a similar time frame in the Middle East and various tribes in Africa also domesticated camels and other cattle resulting in convergent evolution of different genetic polymorphisms controlling LPH and ability to digest lactose [3, 17]. Then after the rediscovery of the new world by Columbus, migration of north western and south western Europeans to the new worlds both in North/South America and Australia/New Zealand introduced LP populations in large numbers.

## Genetics of Lactase

The gene for lactase has been located on Chromosome 2 [18]. With the exception of congenital lactase deficiency the normal regulation of LCT controlled via transcription from a promoter region located upstream from the 5’ region of LCT, 14kb pairs in a cis position. Such cis position regulation leads to an imbalance of alleles and is characterized by allelic instability. Cis acting genes generally have promoter, enhancer, and silencer functions or have an insulating effect on target genes. These cis acting genes facilitate adaptation to soft environmental sweeps [19]. Even prior to identification of specific single nucleotide (SNP) polymorphisms it was recognized that several haplotypes (A haplotype in genetics is a combination of alleles (DNA sequences) at adjacent locations (loci) on the chromosome that are transmitted together) are in evolved with adult LP status [20] In 2002 Ennatah et al identified a locus (chromosome 2q21-22) within an intron of a nearby gene;

minichromosome management 6 (MCM6), 13910 bp downstream from LCT [21]. This SNP correlated 100% with LP status. In this paradigm substitution of Thymidine for Cytosine rendered the individual LP, such that T/T is homozygous dominant LP, T/C is heterozygous LP but on intestinal biopsy enzyme of lactase was intermediate and C/C is homozygous LNP with <10units (u) lactase/g of tissue [22]. Enattah et al also described a second SNP in Europeans, the G/A -22018 but does not seem to have a promoter control function in Europeans. This SNP may be responsible for LP status in Northern Chinese [16] in addition to other polymorphisms.

**Table 1. The various polymorphisms described for lactase persistence are shown**

| Polymorphism | C/T 13910 | G/A22018 | C/G13907 | G/C14010 | T/G13915 |
|---|---|---|---|---|---|
| Described by | Ennatah[21] | Ennatah[21]<br>Xu [16] | Tishkoff[3] | Tishkoff[3] | Tishkoff[3]<br>Imtiaz [24] |
| Populations affected | N Europe<br>Ancestors in<br>NA*,SA*<br>Aust*<br>Central Asia<br>N India | Similar to<br>C/T13910<br>N China | African<br>Tribes<br>(eg Beja in<br>Sudan) | African<br>Tribes<br>(eg Kenya<br>And<br>Tanzania) | African<br>Tribes<br>(eg Kenya)<br>Middle East<br>(eg Saudi<br>Arabia) |

* NA- North America, SA – South America, Aust – Australia.

These SNP changes appear to have developed around 5000 – 10000 years ago in different locals and have been termed as examples of convergent evolution [3]. Lactase persistent dominant polymorphisms are likely to expand as additional populations are evaluated. As well, it is hypothesized that there is ongoing evolutionary pressures on these polymorphisms to this day [2].

Since different haplotypes have been recognized to be responsible for different SNPs the search for dominant genes also expanded to Africa and the Middle East [17, 23]. There populations (tribes) whose ancestors domesticated animals for milking generally were lactose digesters. Indeed 3 new different polymorphisms were described close to the European polymorphism. These included C/G-13907, T/G-13915, and G/C-14010 [3]. Of these the T/G -13915 SNP has been reported to be the principal polymorphism in Saudis [24]. Based on simulation models it is predicted that other polymorphisms will be found as different populations are systematically evaluated [25]. However, there is evidence that the leading LP polymorphisms in central Asia and northern India have so far retained the importance of the C/T – 13910 SNP [14, 15]. These findings support the contention that these world regions are populated by

migrants from northern Europe. The currently discovered genetic of dominant LP status are listed in Table 1.

## Symptoms Related to Lactose Consumption

If one "Googles" the title of this section there will be 1,330,000 sites displayed in 0.36 seconds. Such a volume attests to the interest and importance of this topic to the general public. However, there has been and there continues to be confusion about such symptoms and the meaning of different terms related to them. The main, often interchanged terms to denote symptoms are outlined in Table 2. It should be noted however, that each term has different meanings. For example lactase deficiency (LD) refers to a reduction in quantity/g of LPH in intestinal tissue for any reason. This LD in turn leads to the inability to digest lactose and is labeled as maldigestion (LM). This could be due to the rare congenital absence of LPH [26], a rare variant with non functional enzyme from birth described in a Japanese infant [27], secondary causes such as diseases which lead to villus small bowel damage (celiac disease, giardiasis, rotavirus infections, surgical resection, drugs and many others) and common primary adult LPH deficiency (lactase deficiency, LD) which leads to LNP status. With exception of serious consequences to nutritional deficiency in congenital absence or premature birth, the primary symptoms of LPH loss in adults are gastrointestinal. These include gas, abdominal bloating, cramps, and less often diarrhea and vomiting. These symptoms are termed lactose intolerance (LI). They are not specific to lactose and may occur with any food intolerance (especially carbohydrates from mono, other di, oligosaccharides and polyols like sorbitol). In addition other foods like coffee, fats and caffeine in other food scould reproduce some of the symptoms. Furthermore, diseases affecting the small or large bowel and psychological stressors could also aggravate such symptoms. More recently the term lactose sensitivity (LS) was coined by Mathews [28] and is also applied to the Inflammatory Bowel diseases (IBD). This LS refers to additional systemic symptoms and general fatigue, headaches and even pruritus [29]. The problem with these different terms is that they are not interchangeable and the relationship to lactose specifically may be lost as descriptions and tests of LM are deleted. Yet often these terms are used interchangeably. Partly because of these different terms and because LI is

often defined merely by self reported beliefs, there is no clear knowledge of the prevalence of LI in large Western societies like the USA and Canada. Furthermore development of LI in LNP persons can be modified by ingestion of lactose with different foods of different energy density and by low doses ingested more frequently. For example, lactose in water will precipitate symptoms more readily than lactose in high fat milk or chocolate milk [30].

The explanation for the development of symptoms with LM relates to the quantity of lactose that escapes digestion in the small bowel where the bulk of LPH is found (jejunum > duodenum>> ileum). It was pointed out above that in adult LNP at least some enzyme remains and that depending on dose, manner in which lactose is consumed and some physiologic conditions symptoms related to LM are modifiable. There are three non exclusive mechanisms by which symptoms could develop. In the first instance a very large dose of a lactose challenge in aqueous medium(like water), for example, 50 -100 mg (the lactose content of 1 - 2L of milk) will rapidly overwhelm whatever residual lactase is present in an LNP individual. As a result intestinal transit time will decrease. The residual lactose will rapidly reach the colon in large amounts and exert osmotic forces which will induce water to passively enter the colon. This will result in cramps, diarrhea and even on occasions vomiting. However, people who consider themselves to be LI will not likely consume such high levels in one session.

In the second scenario more modest doses of lactose in the range of 20-25 g (the content of about 2 cups of fluid milk) can also induce symptoms in LNP individuals. While the conditions mentioned in the first instance may also be at play here, intestinal (mainly colonic) microflora has been hypothesized to play an important role. In this scenario undigested lactose is metabolized by microbes which convert lactose into gases (hydrogen, carbon dioxide and methane) and the short chain fatty acid (SCFA) lactate and acetate [31]. In turn these substrates (especially lactate) stimulate other bacteria which can produce other SCFAs (propionate and butyrate) [32]. He et al postulate that in naïve LNP subjects it is an ab- normal production of SCFAs which lead to the development of symptoms [31]. In LNP persons who continue to consume lactose a microflora change occurs with the expansion of so called lactic acid producing bacteria. These include lactobacilli [33, 34] and bifidobacteria [35-37]. The expansion of these bacteria successfully compete for preferential metabolism of lactose [38] and are subsequently associated with less gas production and perhaps reduction of symptom [39]. This process is called colonic adaptation and will be discussed further below.

In the third scenario symptoms are supposed to occur at low levels of lactose ingestion (< 15g of lactose). In practice symptoms incurred at this level are independent of LM [40-42]. In the case of LM subjects the development of symptoms will depend on the amount of lactose digested by residual intestinal LPH. There are a number of studies dealing with the minimal amount of lactose that can be digested by LNP subjects in a single session. In general the doses range from 6g to just over 10g of lactose [42-44]. In keeping with this notion a study examining symptoms incurred by ingestion of < 1g lactose , an amount which may be used in the production of medications did not find any problem at this dose [45]. In the usual scenario of symptoms incurred at these doses several studies examined in a blinded fashion the ability of persons believing themselves to be severely LI to distinguish between single ingestion of lactose free or regular lactose containing milk. In the study of Suarez et al such people were unable to distinguish lactose free from regular milk [46]. Later, a second study was published by the same group demonstrating the same phenomenon with 2 cups of milk separated in time [47].

**Table 2. Terms related to lactose and its digestion are shown. The terms should be distinct**

| | |
|---|---|
| Lactase Persistent LP | The dominant genetic trait in adults with continued ability to digets lactose |
| Lactase Non Persitent LNP | The natural decline in intestinal lactase to < 10u/g of tissue which leaves adults with minimal ability to digest lactose |
| Lactase Deficiency LD | Reduction of intestinal lactase enzyme from any Cause either genetic (LNP) or any secondary causes like diseases of the proximal small bowel mucosa |
| Lactose Maldigestion LM | Inability to digest lactose for any cause primary (LNP) or secondary causes |
| Lactose Intolerance LI | Symptoms resulting from the ingestion of lactose Including flatus, gas, bloating, cramps, diarrhea and rarely vomiting. LI may occur without LM |
| Lactose Sensitivity | Symptoms with or without symptoms of LI and systemic features depression, headache fatigue These features need to be further clarified and May occur with or without LM |

A meta –analysis of symptoms by self reported LI subjects demonstrated that there is a significant over statement of symptoms in this group [48]. Similarly Cassellas et al reported a significant discrepancy between symptoms reported for LI by subjects who subsequently underwent testing for LM [41]. Another group reported that rather than LM status, somatization as found on

psychologic testing was better correlated with self reported LI. Furthermore these persons also scored less well on measurements of quality of life. [40]

It is therefore noted that there is much variability in symptoms related to lactose ingestion. In general, symptoms tend to be worse in LM persons but LI symptoms are not limited to LM (LNP) subjects. The presence of symptoms in LP (as well as in some LNP) persons is governed by other parameters which may be related to additional psychological factors as well. As a result, self-reported LI is less specific to LM and the diagnosis of maldigestion is not made on history alone. However, under such uninvestigated conditions lactose induced symptoms are likely not specific and could apply to other offending foods as well. It should be also noted that lactose maldigestion is not more frequent in irritable bowel syndrome [49,50].

## Tests for Lactose Maldigestion

Adult LM leaves about <10u/g of lactase on the intestinal brush border. Direct measurement of this enzyme in biopsies could be the ideal test for diagnosing LM (of any type). However, this test is too invasive and not suited for population or individual clinical diagnosis of LM. Nevertheless, biopsies are not perfect because the decline in lactase in adults with LM may be spotty and so biopsies could be falsely negative [51]. Of note however, is that in the last 5 years the intestinal biopsy test for LPH deficiency has returned in the form of a "Quick $^{R}$ " test which allows rapid diagnosis of LPH deficiency during gastroscopy [52]. Again however, the test is suited to special situations (in particular it allows the exclusion of secondary causes of LM and LD), and is difficult to implement for widespread use. Instead prior to the description of genetic tests, indirect tests of lactose maldigestion were introduced. Although several tests were available, only two are still used clinically [53]. The more widespread test used is the Lactose Breath Hydrogen (LBHT) and occasionally the older lactose tolerance test (LTT) can be still used.These indirect tests have been correlated with intestinal biopsies.

Both tests have limitations and it should be clear that the tests are mirror "images" of each other. The LTT measures the host's ability to breakdown lactose into its monosaccharide components. Blood glucose is then measured in a fixed time interval (usually 2 hrs) after a baseline is measured. A lactose load is given and a rise above 1.-1.4 g/L is considered a positive test and establishes LP status [54]. A rise less than this, is a negative outcome supporting LNP status. On the other hand the breath test is interpreted in the

opposite way. A lactose load is given and the amount not digested by the host reaches the lower intestine. Here bacteria metabolize lactose and form gases and short chain fatty acids as discussed above. Hydrogen is a key element that diffuses across the colonic membrane, enters the circulation, traverses the lung where along with some other gases like carbon dioxide (and in a significant number, methane) are exchanged for oxygen. The exhaled hydrogen is measured, either by a electronic hydrogen sensor or as samples drawn from an airtight bag into which participants exhale at timed intervals. Samples are injected into a compact gas chromatograph and hydrogen concentration is measured. Thus the positive rise in hydrogen by 20 parts per million (ppm) above the baseline denotes a person with LNP status. A failure to raise hydrogen levels usually means LP status [53,55].

Both tests have limitations [56]. However, because the breath test is actually a bacterial function test dependent on the host, several caveats need to be mentioned. Firstly about 5-15 % of the population may not have bacteria which produce hydrogen. Although, if the limit of rise of hydrogen is lowered to 10ppm the likelihood of sensitivity is increased although specificity may be lowered [57]. Secondly an ethnically indecent portion of the population harbor methanogenic bacteria. These bacteria utilize hydrogen gas produced to form methane ($CH_4$), thus reducing the response to lactose. As a result some authors recommend including methane measurement to increase sensitivity [58]. The third variable which affects breath tests (but not yet studied and is not expected to affect LTT) is the notion of bacterial adaptation to lactose [39]. In this paradigm continued lactose consumption in LNP persons leads to altered bacterial flora. These include lactobacilli and bifidobacteria which produce less or very little hydrogen, possibly rendering the test less sensitive. For these and other reasons the correlation between the LBHT and LTT is modest to moderate at best [59].

Most recently after the description of the North European C/T -13910 lactase promoter polymorphism RIA tests became available to genotype persons. This test however, is more difficult to use for daily clinical evaluation because the tests do not measure whether a person is LI. Even the notion of LM is age dependent so these tests are more relevant to epidemiological and anthropological genetic studies. The genetic tests have also been well correlated with intestinal enzyme levels [22]. In addition our group evaluated in a meta-analysis the accuracy of the two most frequently used indirect tests discussed above to predict genotype within the C/T-13910 polymorphism [60]. A literature search revealed 19 studies from worldwide centers of which 17 discussed the LBHT, and 2 the LTT uniquely. In 3 of 17 LBHT studies both

tests were evaluated. The overall sensitivity of the LBHT was 0.88 (CI, 0.85 – 0.90) and specificity was 0.85 (CI, 0.82 – 0.87). In sub-analyses, use of a 50g lactose load and the failure to include children (< 18 yrs), increased sensitivity and minimally changed specificity (lower with the high lactose load, and higher in the omission of children). Despite the poor correlation between the LBHT and LTT, the latter also performed well against the genetic tests albeit with very few studies (5 publications). The sensitivity was 0.94 (CI, 0.90 – 0.97) and specificity was 0.9 (CI, 0.84 – 0.95). This latter analysis was of strategic importance in order to allow comparisons of various disease rates to be correlated with population distributions of LP/LNP (to be discussed below). The study supports the contention that indirect tests individually do reflect genetic traits as long as the specific traits are measured for appropriate populations which may have different polymorphisms from the north European type.

## Epidemiology of Dairy Food Consumption, Lactase Phenotypes and Diseases

In the last few decades, two variables have been recognized that impact on geographic distributions on modern diseases. The first is the constant relationship of the position of the earth with respect to the sun. These polar/ equatorial directions are demarcated by latitudes in northern and southern hemispheres, diminishing toward the equator. Lower latitudes are also associated with increasing regional sunshine exposure. Sunshine is divided into visible light (400 – 700 nm) and ultraviolet light (UV). Of these UVC never reaches earth. UVA (320 - 400 nm) may be linked with melanoma [61] and UVB (280 – 315 nm) is responsible for skin synthesis of vitamin D from 7 – dehydrocholesterol [62].

With the discovery in the last two decades of important non skeletal functions for vitamin D3, the relationships between many diseases have been elaborated. A large number of diseases have been identified with modern day " western" cultures and lifestyles. These include at least 14 cancers, leukemia, multiple sclerosis and the inflammatory bowel diseases, [IBD; Crohn's disease and ulcerative colitis] [63 – 68]. In general all these diseases tend to follow diminishing rates from north toward the equator. However, these reductions in rates also occur eastward (if the reference points are highest in north America, western Europe toward Asia).Disease rates in both latitudinal and non

latitudinal directions are increasing with progressive “westernization” and/or industrialization.

It is observed, that especially in Europe there is a close relationship between latitude, UVB and LP/LNP distributions. These relationships were evaluated with both genetic models of LP [25] as well as phenotypic distributions derived from older observations [69] with concurrence between the outcomes. These relationships are much weaker outside of Europe. However, to date, national disease rates on a global level also show relationships to these three variables. The direction of change with LP/LNP distributions in disease rates seem to parallel directions achieved with latitude and UVB. That is decreasing disease rates with either increasing LNP fractions of populations or decreasing latitude associated with increasing UVB exposure.

However, because the leading hypothesis for emergence of LP is association with herding [3,7,9], LP/LNP distributions are thought to depend largely on gcc and population migrations. The independent development of LP status from latitude /UVB exposure suggests that disease patterns likely depend on interaction of both variables [70].

For some diseases like colon [71] and ovarian cancers [72] as well as the IBDs [73] it was recognized almost 2-4 decades ago that disease rates diminished with increasing rates of population LNP status. In a more encompassing study evaluating the relationship between the rates of seven “western” diseases and population LP/LNP distributions, two independent effects were noted [74]. The first effect on diseases was defined by the relationship of national per capita dairy food (DF) consumption (Fig 2). Relationships were more varied with DF consumption and the epidemiological data were also compared for outcome with existing meta-analyses. The second relationship was the effect of population distributions of LP/LNP, where LNP was inversely and significantly associated in each of the seven “western” diseases and directly with the one “eastern” disease (Table 3).

In this paradigm between dairy food, consumption and lactase distributions, six hypothetical non mutually exclusive interactions are possible; I. Dairy food and lactose dependent: a. risk decreased, b. risk increased, II. Dairy food dependent and lactose independent: a. risk decreased, b. risk increased, III. Dairy food independent (? Co – marker effect): a. risk decreased and b. risk increased. These relationships are discussed below and displayed in Table 4.

**Table 3. Calculated Odds Ratios (OR) for Each Disease Either by per Capita Dairy Consumption or National Lactase Non Persistance Prevalence (LNP) Rates Using Negative Binomial Regression Reprinted with permission from Shrier et al Nutr Cancer 2008 [ref 74]**

| | Dairy Consumption | | | LNP Status | | |
|---|---|---|---|---|---|---|
| | Coefficient (SE) | OR per 10 | g/kg (CI) | Coefficient (SE) | OR per 10% | LNP (CI) |
| Incidence | | | | | | |
| Crohn's | .0079 (.0046) | 1.08 (0.98–1.18) | P=0.09 | –.018 (.0042) | 0.83 (0.76–0.90) | P<0.01 |
| UC | .0081 (.0025) | 1.08 (1.03–1.13) | P<0.01 | –.0087 (.003) | 0.91 (0.86–0.97) | P<0.01 |
| Mortality | | | | | | |
| Prostate | .0036 (.0016) | 1.03 (1.00–1.06) | P=0.03 | –.0115 (.0021) | 0.89 (0.85–0.92) | P<0.01 |
| Ovarian | .0022 (.0014) | 1.02 (0.99–1.05) | P = 0.10 | –.0075 (.0019) | 0.92 (0.89–0.96) | P<0.01 |
| Colorectal | .0041 (.0015) | 1.04 (1.01–1.07) | P <0.01 | –.0081 (.0022) | 0.92 (0.88–0.96) | P<0.01 |
| Lung | .0028 (.0014) | 1.02 (0.99–1.05) | P = 0.06 | –.0062 (.0023) | 0.93 (0.89–0.98) | P<0.01 |
| Stomach | −.0043 (.0015) | 0.95 (0.92–0.98) | P<0.01 | .0115 (.0027) | 1.12 (1.06–1.18) | P<0.01 |
| Breast | .0018 (.0016) | 1.01 (0.98–1.05) | P=0.29 | –.007 (.0026) | 0.93 (0.88–0.98) | P<0.01 |

[a] The coefficient represents the increase in log risk per 1 unit increase of the variable. Therefore, the OR per 10% increase in LNP is equal to "exp(coefficient$^{10}$)," and the OR per 50% increase in LNP is equal to "exp(coefficient$^{50}$)."

Abbreviations are as follows: CI, confidence interval; UC, ulcerative colitis.

**Table 4.**

A. Diseases linked with relationships to either dairy food consumption or disease rate change with lactase proportion distributions* See text for discussion of diseases

| Dairy Food Causes | Dairy Food Protects | Dairy Food Independent |
|---|---|---|
| Prostate Cancer | Colorectal Cancer | Breast Cancer |
| Ovarian Cancer | Stomach Cancer | Lung Cancer |
| | Osteoporosis | Crohn's disease |
| | Bone Fractures | Ulcerative colitis |

B. Diseases evaluated or not evaluated to be related to Lactase distributions (inverse with Lactase non persistence but have relations with Ultraviolet light (UVB). Potential mimicry of above

| Cause | Protective | Independent |
|---|---|---|
| Cataracts | | Multiple Sclerosis |
| Testicular Cancer | Hypertension | Lymphoma |
| | Diabetes II | Leukemia |

* The arbitrary divisions are based on best current information and may not be mutually exclusive.

## Specific Genes Evaluated in Relationship to Lactase Distributions

The evolution of lactase persistence in the Neolithic period allowed LP persons to consume fluid milk, increasing their nutritional intake. However, mortality associated with enteric pathogens through diarrhea increased. It is hypothesized that some genes co-evolved with LP in order to counter act mortality caused by enteric pathogens. To date all genes evaluated in relationship to milk drinking originated from Europe.

Genes thought to have evolved for protection against diarrhea include the Cystic fibrosis CFTR gene, A gene for Oxytocin receptor, HLA polymorphisms and NOD2/CARD15 microbial sensor system.

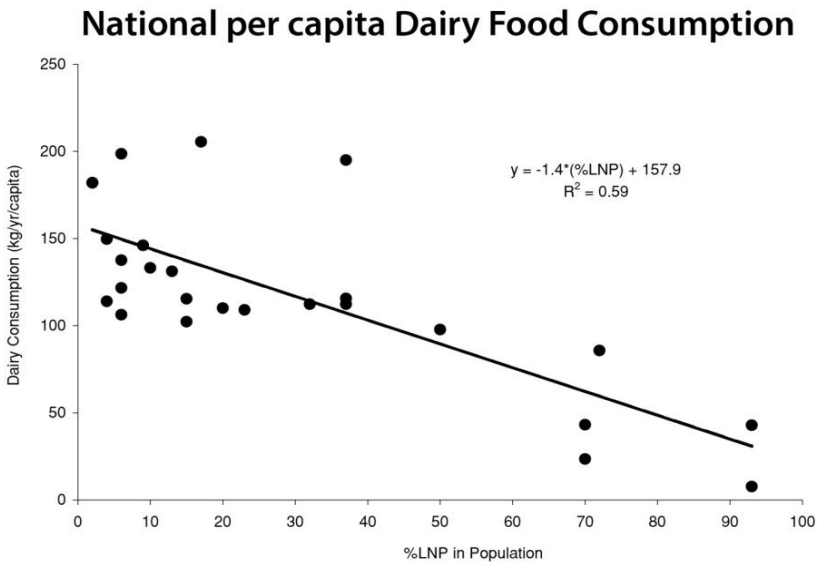


Figure 2. Dairy food consumption for each country is plotted against the percentage of the population that are lactase non persistent (LNP). Because LNP subjects experience side effects due to lactose if they are not adapted, they consume less dairy foods. Reprinted from Shrier et al Nutr Cancer 2008 [74] with permission.

### *Cystic Fibrosis and Mutations in the CFTR Gene*

The gene responsible for cystic fibrosis(CF) have been postulated to be more common in LP populations [75,76]. Cystic fibrosis is considered to be the most deadly gene affecting Europeans and their descendents. The autosomal recessive trait results in a loss of function in the Cystic Fibrosis Transmembrane conductance Regulator of Cl- channel and water excretion. It is also hypothesized that this gene in its heterozygous form counteracted diarrhea induced by milk drinking before the evolution of lactase persistence [76]. An evaluation however, of lactase mutations in patients with CF showed a higher frequency of the C allele of the European C/T-13910 polymorphism in homozygotes of severe CFTR mutations [77]. In cases of mild CFTR mutations there was a lower frequency of the C allele. The authors concluded that LNP was equally distributed between controls and patients. The expectation would be an increased rate of LP in CF patients since the ancestral risk was in milk drinkers. The fact that more severe cases had a higher rate may still link the evolution of these two genes. However, as noted earlier

latitude and lactase are closely linked in Europe, making distinction of evolutionary pressures and interpretation complex.

### *Non Parturition Effects of Oxytocin*

Recently the hormone oxytocin has been described as being important in pain modulation and gut, especially colonic motility. Genetic modification may play a role in response to enteric pathogens. To determine whether genetics of oxytocin (OXT) may be involved in pain modulation in IBS a study using DNA from 131 well characterized IBS patients 408 healthy controls and 299 asymptomatic Swedish blood donors were compared. As well the genotypes of CC and TT were simultaneously evaluated. There are 2 chromosomes involved in OXT control. One is a promoter and the other is an OXT receptor on different chromosomes both removed from the chromosome for lactase. It was found that an SNP in the promoter region of OXT was more frequent in LNP (CC) persons but did not differentiate patients with IBS [78]. Although neither of these polymorphisms are linked with the lactase gene, nevertheless are unequally distributed between lactase phenotypes.

### *Human Leucocyte Antigens and Immunity*

A third group of polymorphisms associated with resistance to pathogens are the HLA

(Human Leukocyte Antigens) system part of the Major Histocompatibility Complex located on chromosome 6 (6p21.31). A large proportion of genes are involved with the immune system and have a very high rate of heterozygosity (>80%). In an analysis of a large number of alleles from Europe a spatial pattern was observed in about 1 % but mostly with a latitudinal gradient. The authors concluded that selective pressures during the Neolithic period, most likely new epidemic diseases were responsible. A parallel emergence of herding, lactase persistence, and milk drinking with subsequent exposure to enteric pathogens could account for these patterns [75].

### *Elimination of Milk Transmitted Pathogens NOD2/CARD15*

A fourth genetically determined system, caspase-activation and recruitment domain gene 15 (part of the also called Nod 2 system) located on chromosome 16q12 was the first gene described to increase risk of Crohn's disease (CD). Three mutations increase the risk of CD about 40 fold if homozygous and about 4 fold if heterozygous [79]. The mutations SNP8 (containing R702W), SNP 12 (containing G908R) and SNP 13 (containing 1007fs) are responsible for 80 % of the risk and these are largely confined to

Caucasians from Europe and their decedents in the new worlds [80]. The NOD2 system through interactions with bacterial particle sensing Toll receptors [and other genes with autophagy function: IRGM, and ATG16L1, the latter located on chromosome 2q37 (the same chromosome as lactase)] are responsible for excluding bacteria from traversing the intestinal membrane and invading the human host [80, 81]. As with NOD2, A mutation at T300A confers an increased risk for CD [81]. The development of this system is hypothesized to have developed in response to the practice of milk drinking [82]. Specifically common bacteria which are passed from cattle through unpasteurized milk; Brucella, Listeria and Mycobacterium Bovis are targets of this system.

### *Latitudinal Changes of Taster / Non Taster Status*

Another polymorphism evaluated for co-evolution with lactase is the gene for taster status. Persons with the recessive gene for non taster status may consume more cruciferous vegetables because the associated bitter taste is reduced. The suggestion is that these vegetables may protect against cancer. Sacerdote et al evaluated taster status in northern against southern Italians and related this to LP/LNP status in the same population. They reported that non taster status was somewhat more common in the northern population where LP status was also higher than in the south [83]. The relationship of this polymorphism however, may be coincidental.

## Disease related to Dairy Food Consumption and the Lactase Dichotomy

Dairy foods contain a significant array of nutrients which could impact on disease modification [84].Although, calcium, fat, potassium, magnesium and vitamin D (added in North America) content are important elements in this function, lactose is also a key element toward disease modification. The lactose content of dairy foods has an important impact on quantities consumed by LNP as well as a prebiotic effect limited to LNP subjects [74]. These two qualities should affect any disease hypothesized to be related to dairy food consumption.

### Disease Related to Evolutionary Development of the Lactase Dichotomy

Evolution of lactase genotypes that resulted in improved nutrition in LP persons likely had additional impact on the evolution of other genotypes. Genes which conferred an advantage to ancestors may behave differently in a modern environment. The question of co evolution of other genes then poses the question whether genes evolved with LP or was independent of lactase but included those prevailing environmental pressures. These latter changes could be related to latitudinal effects as has been suggested for loss of pigmentation [85] and sodium homeostasis and hypertension [86,87].

In the next sections disorders linked with DFs consumption or lack thereof will be discussed. The classification outlined above for DF consumption dependency or independency is followed.

## Dairy Food Dependent: Disease Risks Reduced by Lactose/Dairy Foods

A recent 2010 NIH consensus conference on lactose intolerance and health concluded that the main adverse outcome of LI is the avoidance of milk and other dairy foods (DFs) [88]. The independent panel noted that LI without proof could cross generation lines because of restriction of DFs in children of parents who believe themselves to be LI and thus believe that symptoms will develop in their offspring. Those avoiding DFs were found to be deficient in some nutrients, especially calcium and vitamin D [89].

The main effect of DFs avoidance is the possibility of osteopenia and osteoporosis. These then could lead to higher rates of bone fractures. At the 2010 NIH conference, Wilt et al reviewed the literature evidence for the effect of different categories of lactose intake (as a surrogate for dairy food intake) on bone mineral content (BMC), bone mineral density (BMD) and fracture risks [90].The analysis included four groups; general population, vegans (strict vegetarians), persons with self reported LI and or LM, and proven LNP persons. Fifty –two studies, including 9 randomized controlled trials were included, which dealt with lactose intake and bone health. The authors concluded that there was low level evidence, based on observational studies that milk avoidance may be associated with osteopenia, BMC, BMD and increased risk of bone fractures. They noted that in adults with LI, LM, the CC

genotype (LNP) or reduced lactose intake, may be associated with increased bone fractures. However, this was reported only from a single Finnish and a study dealing with vegans. Women were found to be at higher risk. There was also better evidence that increased lactose intake did improve BMC and BMD. A more recent report from two Dutch centers, not included at the NIH conference, found that while the CC genotype was associated with lower calcium intake and lower serum ionized calcium, BMD or fracture risk were unaffected in these elderly people [91]. In general however, the panel interpreting the presented data suggested that more studies are needed on the relationship between bone health, lactose intolerance and dairy food avoidance [88].

Other medical consequences relate to the metabolic syndrome. This includes obesity, hypertension, hyperlipidemia and type 2 diabetes mellitus. A large study including over 1800 Australian subjects evaluated 145 dietary items including regular fat dairy foods consumed over 10 years. The authors reported a 59% lower risk of metabolic syndrome in the highest quartile of regular fat DFs intake. Interestingly no association between total DFs intake obesity and diabetes was noted [92]. In an earlier, similarly large, long term study of subjects with self reported LI and DFs avoidance Nicklas et al reported an inverse association between total DFs and metabolic syndrome as well as diabetes [93].

The effect of DFs consumption specifically on obesity is controversial. A recent meta-analysis of 29 randomized controlled trials found only a modest benefit of DFs consumption on limiting obesity in the short term (94). In the absence of energy intake restriction no long term benefits to DFs consumption were noted.

The relationship with obesity and metabolic syndrome is further confused by a multicenter European study where a large number, over 31 thousand subjects were evaluated for lactase genotype and basal metabolic index (BMI). The report clearly linked an increased BMI to the lactase persistence status. The study did not evaluate DFs consumption in the test population [95]. However, as will be discussed below this variable is important because the relationship observed may be a false impression as is clearly the case with colorectal cancer (see below).

In conjunction with DFs restriction there is also a risk of inadequate vitamin D intake. The reason is that in the US and Canada milk is fortified with this vitamin. Vitamin D deficiency has been linked with an increased risk and an increased likelihood of worse outcome with a large number of diseases. Among these are at least 14 cancers [96,97], Lymphoma [98], Leukemia [99],

Inflammatory Bowel Diseases [100] and multiple sclerosis [101]. In the absence of vitamin D supplements therefore milk is the largest source of dietary intake of vitamin D. Ethnic groups such as African American and Hispanic groups may be at a higher risk for vitamin D deficiency also because of reduced skin production and perceived LI [102].

The other major area where dairy foods exert protective effects is on gastrointestinal cancers [103]. It is emphasized that in epidemiological studies countries with the highest national per capita DFs consumption have the highest rates of colorectal cancer. Paradoxically, DFs, calcium and vitamin D all exert protection against colon cancer [74,104]. The paradoxical relationship between a disease epidemiological link to a putative aggravating agent(s) and the actual findings from patient based studies is known as an ecological fallacy (Definition at: http://michaeljohnsonphilosophy.com/wp-content /uploads/2012/10/ecological-fallacy.pdf). A paradigm of false epidemiological connection may also apply to obesity as outlined above.

The primary mechanism of reduced cancer is the antiproliferative effect of calcium on aberrant foci and later advanced polyp formation. Initial reports on the effects of calcium suggested that at least 1200 g of calcium is needed for this effect [105,106]. However, the addition of vitamin D which has important antineoplastic cell signaling function may reduce the need for the level of calcium since the two may have additive effects [107]. However vitamin D added to milk is limited to western countries. It is of interest that DFs have been reported to protect against CRC in regions of the world with either relatively high frequency of LP persons (such as north western Europe, North America and Australia where large amounts of DFs are consumed) and high frequency of LNP (such as Japan and China where DFs consumption is about a third of western intakes). Protective effects in countries with more evenly distributed LP and LNP (such as southern Europe and South America) populations, DFs appear to have less of an effect [108].

The explanation for protection in LNP populations which consume less DFs is unclear but a prebiotic effect of lactose in such populations could substitute and compensate for lower calcium intake [37,109]. Prebiotics promote the preferred growth of probiotic type bacteria (lactobacilli and bofidobacteria). The anti-carcinogenic effects of such probiotic bacteria have been reported [110]. It should be noted that with lactulose (a disaccharide consisting of galactose and fructose) for example, bifidobacteria were significantly increased in stool after ingestion of 5g twice a day for 6 weeks [111]. This could be the equivalent of consuming <15 g lactose (content of 1

cup of milk where 3-5 g may reach the colon in LNP subjects) twice a day [47]. The effect of smaller doses over months or years has not been studied.

Whether people with self reported LI have higher rates of gastrointestinal cancers, especially colon cancer, has not been clarified. In the study of Nicklas et al referred to above no increased rates have been observed in self reported LI persons [93]. However, we do not know the actual frequency of LNP subjects who might compensate for diminished calcium intake with small amounts of lactose ingestion. The concept may be more direct as it applies to gastric cancer which is more common in LNP populations. In this paradigm exclusion of DFs may increase the risk of cancer. In the case of gastric cancer a meta-analysis from China [112] and a another large study from Japan [113] did find reduced rates in persons (especially in men in the latter study) who consumed more DFs. These findings have partly been supported by the study of Park alluded to above [103].

## Dairy Food Dependent: Increased Risk with Consumption of Lactose/Dairy Foods

A number of specific diseases will be highlighted in this section. One disease, malaria discussed above was hypothesized to derive protection from LNP status. The mechanism postulated was the formation of flavin deficient red cells which are then protected somewhat from invasion with M. Falciparum [11]. This hypothesis was not substantiated by an epidemiologic study from southern Italy. The refutation of the hypothesis rested on the parallel relationship of beta-thalassemia and glucose 6-phosphate dehydrogenase deficiency with regions located in high malaria endemicity. These hemoglobinopathies also have been postulated to protect against malaria. Alternatively, the distribution of lactase deficiency (LNP) status was similar in high and low endemicity regions [114]. In addition other studies suggested that cow's milk was actually protective against Falciparum Malaria [115].

One early link of lactose in LP persons was the predisposition to cataract formation, The pathogenesis is due to galactose absorption especially in diabetics [116]. It was suggested that the combination of high lactose intake and a presence of low galactokinase may increase the risk for cataracts by 4.1 fold [117].

Because greater quantities of milk drinking and DFs consumption occur more in countries with large LP population proportions, diseases in these societies may be linked. Hypercholesterolemia and cardiovascular diseases are one of the most common illnesses in "Western" societies. A number of studies have shown an increased rate of CVD and mortality to intakes of high- fat dairy products [118-120]. But a recent review of observational studies have failed to convincingly demonstrate an increased risk of cardiovascular disease or stroke regardless of fat content. The argument is that while low density lipoprotein is increased, so is high density lipoprotein which may counteract the increased risk. [121,122]

In evaluating relationships between dairy food consumption and a number of cancers discussed above Shrier et al found that ovarian, prostate and breast cancers had higher rates in countries with high dairy food consumption and high proportion of LP populations. At the time of publication of this observation, then available meta-analyses of the effects of dairy consumption on prostate, ovary and breast cancers appeared to have supported, the epidemiological findings, albeit not robustly [74]. The outcomes suggested that DFs consumption increased risks for both ovary and prostate but not breast cancer.

For prostate cancer the mechanism postulated is related to inhibition of conversion of vitamin D 25(OH) to 1, 25 $(OH)^2$ by calcium [123]. This in turn fails to inhibit cell proliferation. Since then, results of ongoing studies have still failed to settle conclusively whether dairy foods enhance risks or not. A recent US study reported that whole milk consumption did increase risk of the cancer [124]. Interestingly a European study examining a possible relationship of this cancer to the dominant genotype for lactase (LP status) did not find an association [125]. However, a study from India noted that lactose intolerance occurred less frequently in prostate cancer patients than controls, implying higher rates of LP [126]. An other study suggested that intake of milk, only in adolescence increased risk for advanced later life prostate cancer [127]. Following diagnosis of prostate cancer consumption of whole milk increased risk by 2.15 fold but no other DFs increased risk of metastases [128].

In the case of ovarian cancer, Cramer initially noted a marked decrease of cancer rates which correlated inversely with LNP status. He hypothesized that galactose was toxic to ovaries leading to malignant change [72]. Several reported meta-analyses did support a role for lactose and hence galactose in ovarian cancer [129-131] and a more recent Danish study did find a somewhat higher risk [132]. However, other studies failed to find any effect of dairy

products on ovarian cancer [133, 134] Therefore, DF consumption still remains a disputed pathogenic factor for ovarian cancer.

The putative use of additives like estrogen in dairy foods or galactose itself have also been reported to be pathogenic in testicular cancer [135,136]. The observation that LP persons consume more DFs in general and the fact that less galactose is available for host utilization may reduce risks for ovarian and testicular cancers.

In breast cancer initial studies suggested that risk may be increased by dairy foods [137], but as outlined above meta-analyses up to the end of the first decade of this millennium failed to substantiate an increased risk [138]. A more recent meta – analysis suggested a modest protective effect of total dairy foods but not milk [139]. The included studies however, proved to be heterogenous, and as such there is still no clear proof on the role of DFs in breast cancer.

Although smoking is the most important variable affecting lung cancer several studies suggested that DFs have a deleterious effect perhaps due to the fat content on lung [74,140]. There are no clear meta-analyses.

## Dairy Food Independent: Mechanisms of Effect Unclear, Evolutionary Co-marker

In the section on genetics of lactase it was pointed out that cis elements controlling target genes facilitate adaptation to environment by their amenability to soft environmental sweeps. In the review by Jones and Swallow at least 12 other genes are listed to be controlled by cis regulators [19]. Of those listed at least three interleukins (IL – 6, IL – 10, and IL – 13) are associated with immunity. They are involved with cell signaling in the inflammatory response.

In most of the diseases described the exact mechanisms are not clearly delineated but are hypothesized. Subsequent studies then either support or refute these hypotheses. Such is the case with breast, prostate and ovaries. The reasons for the vacillator outcomes may be methodological differences ar introduction of subtle biases. Nevertheless, epidemiological correlations point to differences between intake and disease and reductions with increasing LNP status.

Among disease risks apparently modified by latitude/UVB/vitamin D [68,141,142] and LP/LNP distributions [74, 143] IBDs figure prominently. In

fact, there are numerous observational studies and a few interventional studies which support the benefit of vitamin D in reducing risks and improving outcome of IBD, (especially CD) [100, 144– 147]. The specific etiology of IBD is still unknown but environmental factor(s) and an abnormal immune response to microbial flora are thought to be key in pathogenesis [80]. Importantly IBD is the sine-qua-non of a complex disease in which a multitude of genetic interactive predisposing factors contribute [80]. As outlined above the first gene predisposing to CD was described at genetic locus on chromosome 16 [79]. This locus controls the interactive NOD2/CARD15 system in concert with other gene loci for Toll 2,4, 9 and autophagy sites to protect the host from luminal bacterial invasion [80]. Among pathogens which can be ingested through unpasteurized milk Brucella, Mycobacterium Tuberculosis and Listeria Monocytogenes are the most common [82].

A disease resembling CD exists in cattle, camels and goats, Johne's disease is caused by Mycobacterium Avium Paratuberculosis (MAP) [148]. This MAP organism possibly passed to humans through milk (even when pasteurized) has been sought as an etiological agent in CD and still debated [149]. The NOD2 system may have evolved in parallel to herding and milking practices and mutations described above are strongly correlated with CD [72]. However, the relationship appears to be limited largely to Caucasians, at least for the NOD2 mutations [150-152]. Most recently the CD predisposing mutation of ATG16L1 also appears to be restricted to Caucasians compared with aboriginal first nations in Canada [152]. These evolutionary changes are one potential mechanism linking IBD with lactase distributions.

This specific hypothesis of evolutionary relationship of IBD to milk drinking raises the question whether the dominant LP status itself increases IBD risk, The correlation of Crohn's disease is particularly close in geographic distributions with LP population distributions [153]. However, the answer to this question is unknown. There are only four studies which examined IBD relationships to LP/LNP status and report conflicting results. A study from Germany could not establish a protective link of CD with LNP genotype [154]. Two studies, one from north Spain [155] and one from New Zealand [156] did find that the dominant TT genotype was more represented in CD patients. However, another study from Spain failed to confirm the relationship of CD with TT genotype but did find a lower frequency in UC [157].

Evolutionary process could also be independent of either lactase genotype. An examination of genetic links between IBD and 23 other autoimmune diseases showed common predisposing genotypes [158]. These links and other

yet unidentified diseases support possible associations of genes hypothesized to have evolved along with lactase.

## Conclusion

Overall lactose digestion remains a great interest from three perspectives. First the symptoms related to lactose ingestion continue to occupy research efforts. With the discovery of the enzyme LPH and symptoms observed in LM subjects it was hoped that a biologically plausible explanation for irritable bowel syndrome was at hand. However, with reports that LNP persons were not the ones predominantly representing IBS cases, the LM hypothesis of IBS quickly dissipated. In the last year the geographic pattern of irritable bowel syndrome has been published and is very different from those diseases related to LP/LNP distributions [159]. In the last decade LI and LM were clearly separated and in fact the emphasis shifted from IBS symptoms to the medical hazards of DFs exclusion. Although still not clear on the best management of LI it has been shown that relatively low doses, especially consumed with food, even twice a day is readily acceptable to LM persons. Those with lactose sensitivity or LI without LM may need further assessment for other food component intolerances.

Second, the diseases and conditions associated with dairy food consumption display variable outcomes. In some cases risk of disease is increased and in others it is decreased while in still others a clear effect is difficult to prove. While the multitude of compounds found in milk naturally or added in the production (for cattle benefit) surely play some etiological roles, there appears to be less emphasis in research toward the role of lactose.

However, the lactase population dichotomy also plays a role. On a population level LNP consume less dairy foods. Any disease putatively caused by dairy would be less often observed in LNP populations. If LNP populations consume enough daily lactose which ends up being metabolized by bacteria, over time regular consumption could lead to altered and more beneficial "probiotic" type bacteria.

Finally genetic changes which in ancient times led to the emergence of LP dominance likely were accompanied by changes to protect early man against infections passed through consumption of unpasteurized milk. As well soft evolutionary sweeps which may have been responsible for LP emergence may have had effects of other similarly controlled polymorphisms.

This lactose digestive divide offers interesting teleogical (the why so) questions. In the early days of lactase/digestion of lactose, research the question was why would the world's population be divided into those who can and those who can't digest lactose? It seems this question was largely answered: because those of our ancestors who began herding cattle, camels or goats and started to milk and consume it, developed dominant genes to digest lactose. As a result it is thought that they derived increased nutritional benefits. In current times a new and important teliological question arises. Why is it that a gene for "better" nutrition which evolved 7.5 x $10^3$ -$10^4$ years ago affects geographic distributions of so many current diseases; most having reached alarming incidence rates in the last 50-100 years. An answer may lie in altered nutrition and modernization. The evolution of protective genes against infections so that man could improve nutrition in times of scarcity may be counterproductive or altered in response in times of plenty [160].

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