Lactase Persistence and Lactase Non-Persistence
To my family and friends
Lactase Persistence and Lactase Non-Persistence
Prevalence, influence on body fat, body height,
and relation to the metabolic syndrome
Abstract

A single nucleotide polymorphism (SNP) in chromosome 2 gives humans the capability to continue digesting lactose after weaning. Among individuals of European descent it is the SNP (LCT-13910 C>T), which is located 14 kb upstream from the start of transcription of the lactase gene (LCT). A C to T mutation enables the continued production of the enzyme lactase throughout life, and thereby the digestion of lactose throughout life without symptoms of lactose intolerance. The trait is called lactase persistence (LP). LP is an autosomal dominant trait. In contrast, lactase non-persistent (LNP) individuals show a decline of lactase production after weaning. LNP individuals habitually show symptoms of lactose intolerance after consumption of milk and some milk products.

Using the LCT -13910 C>T SNP we reassessed the prevalence of LP/LNP in Sweden. We increased the accuracy of our estimates by using Hardy-Weinberg’s formula for allelic frequencies. We found a prevalence of 14% for LNP. This is about 5.5 fold higher than the prevalence formerly assumed for Sweden (range: 1-5%).

Childhood milk consumption has become normative since the beginnings of the last century. Studies using milk supplements in schools, performed in the UK in the 1920s, showed that childhood milk consumption led to gains in body weight and height in children. We readdressed this question given the changed socioeconomic settings a century later. In today’s, nutritionally replete socioeconomic settings childhood obesity is, instead of stunting and undernourishment, the target of public health nutrition. We did not find evidence for higher measures of body fat coupled to consumption of milk and milk products or the lactase genotype in Swedish children and adolescents. We found, nevertheless, a positive association between milk intake as well as LP and body height in Swedish children and adolescents.

Finally, we studied if LP/LNP, using Mendelian randomization (MR), affects the development of the metabolic syndrome in adults. We chose an accessible population from the Canary Islands in Spain with a prevalence of 40% for LNP and 60% for LP. We found a positive association of LP with metabolic syndrome. LP subjects of the Canary Islands exhibited a 57% higher risk to develop metabolic syndrome compared to LNP subjects. Interestingly, LP women showed a 93% higher risk to develop MS compared to a 22% higher risk in LP men.

In conclusion, the previously not known relatively high prevalence of LNP for Sweden impacts health care and public health policy decisions. Childhood milk consumption affects longitudinal growth, but not body fat mass in nutritionally replete countries like Sweden. The long-term effects of childhood milk consumption need to be elucidated. Non-caloric milk constituents might influence the somatotropic axis of children, which can have both positive and negative long-term effects. LP status and milk consumption may increase the susceptibility to develop metabolic syndrome in some populations. Non-caloric constituents of milk might exert sex specific effects increasing the risk to develop metabolic syndrome in susceptible populations.

Keywords: LCT -13910 C>T polymorphism, prevalence, lactase persistence and lactase non-persistence, milk and milk product intake, body fat, body height, metabolic syndrome

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Sammanfattning
Orsaken till att vissa människor kan metabolisera mjölksocker efter avvänjning är en genmutation (single nucleotide polymorphism (SNP)) i kromosom 2. Bland europeer lokaliseras SNP (LCT-13910 C>T) 14 kb uppströms från starten av transkription av laktasgen (enzymet som bryter ned mjölksocker kallas laktas). Mutationen ger en livslångd bibehållen laktasproduktion. Tillståndet kallas laktaspersistens (LP). Däremot kallas tillståndet, när man tappar förmågan att producera laktas efter avvänjning, laktas non-persistens (LNP). Individer som är LNP får symtom efter konsumtion av mjölk och vissa mjölkprodukter och detta kallas för laktosintolerans.

Med hjälp av LCT-13910 C>T SNP skaffade vi nya estimat för LP/LNP prevalensen i Sverige. Vi höjde precisionen av estimat genom att använda oss av Hardy-Weinberg’s ekvation för allelfrekvenser. Vi hittade en prevalens på 14 % för LNP. Detta är ungefär 5,5 gånger högre än traditionella prevalensdata för Sverige (variationsvidd: 1-5 %).

Mjölkkonsumtion i barndomen har blivit normativ sedan begynnelsen av senaste seklet. Studier bland skolbarn i Storbritannien visade att de barn som fick mjölk tilllägg till skolmaten blev längre och ökade i kroppsvikt. I dagens nuläge är problemet barnobesitas i folkshälsofokus och inte växttardering eller malnutrition. Vi hittade ingen effekt av mjölkintaget på kroppsfetma eller vikt hos barn. Däremot hittade vi en positiv association mellan mjölkintag och kroppslängden hos svenska barn och ungdomar. LP statusen visade också en positiv association till kroppslängden.

Vi undersökte dessutom i en representativ vuxenpopulation från Kanarieöarna i Spanien om LP/LNP uppvisar en koppling till metabola syndrom. Populationen uppvisade en frekvens på 40 % på LNP och 60 % på LP. Vi hittade en positiv association mellan LP och metabola syndromet. LP individer på Kanarieöarna visade en 57 % högre risk att utveckla metabola syndromet jämfört med LNP-individer. LP kvinnor visade en 93 % högre risk att utveckla MS jämfört med 22 % högre risk för LP män.

List of publications


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<tr>
<td>AtH</td>
<td>Adult type Hypolactasia</td>
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<tr>
<td>ATP III</td>
<td>Adult Treatment Panel III</td>
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<td>BMI</td>
<td>body mass index</td>
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<td>CVD</td>
<td>cardio vascular disease</td>
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<td>EYHS</td>
<td>European Young Heart Study</td>
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<td>ENCA</td>
<td>Canarian Nutrition Survey</td>
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<td>IDF</td>
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<td>IGF-I</td>
<td>insulin-like growth factor I</td>
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<td>LCT</td>
<td>lactase</td>
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<td>MCM6</td>
<td>mini-chromosome maintenance gene</td>
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<td>MR</td>
<td>Mendelian randomization</td>
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1. Introduction

1.1. Lactase persistence (LP) and non-persistence (LNP)

1.1.1. Prevalence and Genetics

The consumption of milk and milk products has long traditions in human nutrition. Hippocrates first described lactose intolerance around 400 years BC. Differences in the ability of human adults to digest milk have also been remarked upon in Roman times. Milk and dairy products contain lactose, the sugar component of almost all milk. The disaccharide, lactose, is a unique carbohydrate present only in mammalian milk. Lactose is hydrolyzed to glucose and galactose in the brush border of the small intestine by the enzyme lactase phlorizin hydrolase (LPH), more commonly known as lactase (LCT). Lactase is essential for the nourishment of newborn mammals, whose sole source of nutrition is milk, in which lactose is the major carbohydrate component. In adult mammals other than in humans lactase production decreases significantly in quantity following weaning. The maturational decline in lactase activity renders most of the world’s adult population intolerant of excessive consumption of milk and other dairy products containing lactose. About 30% of the world population has continued lactase activity, i.e. is LP, beyond weaning and into adulthood.

LP appeared in Eurasia about 10,000 years ago. Evidence for LP in Europeans, prior to the Neolithic, has not been found yet. Fully developed dairying-based farming economy emerges first during the late Neolithic in Southeast Europe and the Middle Neolithic cultures following the Linearbandkeramik in Central Europe and is mainly connected to cattle in Central Europe. The spread of the Neolithic lifestyle from Aegean to Central Europe goes hand in hand with the decline of the importance of sheep and goat and the rise in frequency of cattle bones in archaeological assemblages. Historic reports and archaeological findings point that in Southern Europe goat and sheep, and to lesser extent cattle, based economies used processed milk instead of fresh milk. The Romans, for example, used goat and sheep milk for the production of fermented milk products and cattle mainly as draught animal. In contrast, Germanic peoples and other habitants of Central and Northern Europe practiced cattle dairying and drank significant amounts of fresh milk. The Romans, on the other hand,
considered the consumption of fresh milk as barbaric and simple, instead processing it to yoghurt, cheese and other milk derived products.

The appearance of LP is most probably the result of a gene-culture co-evolution in contrast to, for example Malaria-associated haemoglobinopathies, which can be regarded as results of gene-environment interactions. Concerning LP, culture, economy and population history appear to be the driving force that led to the expansion of LP, and the frequencies of LP we observe today in Europe might be the result of a recent and strong natural selection 4-9. While not fully understood, the biological advantages of LP might include the continuous availability of an energy rich drink that enables farming communities to overcome poor harvests. Also, a combination of both theories is discussed as the driving force behind the expansion of LP. It also is possible that there exist additional unidentified selective advantages of milk consumption, which could explain natural selection.

Variation in expression of lactase was not established as genetically determined trait until the second half of the twentieth century. Before this, LP was considered by people of European descent to be the physiologically “normal” state, and widespread LNP in adults was only appreciated in the early 1960s 10-11. By the early 1970s it was established that the LP polymorphism in humans had a genetic cause, and is inherited in an autosomal dominant manner 12. In the 1980s, Ho et al reported a trimodal distribution of LCT. The trimodal distribution was interpreted as attributable to groups of individuals homozygous for LP (highest lactase activity), heterozygous for LP (mid-level activity) and LNP (low lactase activity) 13.

LP is inherited as dominant Mendelian trait in Europeans 14-15. This trait shows a high frequency in Northern Europeans (>90% in Swedes and Danes) and is also common in pastoralist populations in East-Africa (~90% in Tutsi, ~50% in Fulani). It decreases in frequency across Europe (~50% in Spanish, French and pastoralist Arab populations) and is low in non-pastoralist Asian and African populations (~1% in Chinese, ~5% - 20% in West African agriculturalists) 15.

The decline in lactase expression is usually complete during childhood, but the decline has also been reported to occur later in adolescence 16. The rate in which lactase activity declines can vary according to ethnicity. The reasons for this difference in timing are not known.
Postdecline, the level of lactase activity is 5-10% of childhood levels in most populations worldwide 17. In Northern Europeans it may take up to 18-20 years for lactase activity to reach lowest expression 1. However, some individuals, particularly descendents from populations that have traditionally practiced cattle domestication, maintain the ability to digest milk and other dairy products into adulthood 18.

A single nucleotide polymorphism (SNP) C>T -13910 located 14 kb upstream of the lactase gene (LCT) in the MCM6 (mini-chromosome-maintenance6), gene (Figure 1) has been shown to be associated with LP (LCT-13910 T allele) in individuals of European descent, but varies widely in frequency across Europe 4 (Figure 2). The allele appears to be dominant over the LCT-13910 C allele that represents LNP. Accumulating data imply the critical functional role of the LCT-13910 region 19. However, there are recent reports of other mutations at, or near this site: -13907, -13915, -13913, -13914, and -14010 variants that correlate with LP in different populations 18, 20. Furthermore, to test for LP status for people of East African or Arabian ancestry using only LCT-13910 C>T can be considered inappropriate 21. Data indicates the lack of the LCT -13910 T variant in most Sub-Saharan populations, known to show high prevalence of LP, implying thus that other LP mutations must exist globally. Recently this has been confirmed in Chinese populations. A variant at the locus LCT -22018 reflected LP in 6 different populations and the authors suggested that, taking together the results of previous studies, the origins of LP associated alleles are different in different pastoral populations 22. LP shows thus independent origins in varying populations worldwide which would imply thus convergent evolution.

Homozygosity for the C allele (LCT-13910 CC) shows for all practical purposes a complete association with LNP in most populations of European origin 14, 23-25. Homozygosty for the C allele is a contributing factor associated with the reduction of milk and some dairy products consumption to approximately 12 g lactose or two cups of milk daily in adults and some children and adolescents 26. Typical symptoms of higher consumption of these products can include pain, cramps, bloating, flatus, diarrhea and borborygmi 1, 27. Molecular epidemiologic studies have shown that the prevalence of LNP is consistent with previously published phenotypically determined epidemiologic data on LNP in more than 70 countries 28. The majority of heterozygotes, having intermediate levels of lactase activity in intestinal biopsies 29 are traditionally thought to produce sufficient lactase to be classified by the standard physiological tests as lactose digesters 30.
Figure 1: The physical map showing the analyzed genome region flanking the C/T-$13910$
associated with LP. The distance (in kb) from the first ATG of $LCT$ is shown. $A.$ Genes in the
region studied. $B.$ Expanded map of the 30-kb region in the $LCT$ and $MCM6$ genes, showing
SNPs 1–9 analyzed in the population samples.²⁰ (Courtesy of Enattah et al.)

1.1.2. Detection of LP and LNP

Genotyping for LNP and LP detects primary lactase deficiency (i.e. LNP) and the genetically
determined unrestricted capacity to digest lactose even in adulthood (i.e. LP). LNP can but not
always have to correlate with lactose maldigestion, and lactose maldigestion can, but not
always have to correlate with symptoms of ‘lactose intolerance’.

The term ‘lactose intolerance’ is commonly used to indicate symptoms experienced after
consumption of milk or milk products. The symptoms can be mild, moderate or severe. The
expression of symptoms depends on a number of factors. These factors include: quantity of
consumed milk, fat content of the milk, rate of stomach emptying, oro-cecal transit time,
individual sensitivity to abdominal discomfort, capacity of bacteria in the colon to digest
lactose not absorbed in the small intestine, psychological impact of anticipation of symptoms
in those who have had previous symptoms during drinking milk (nocebo-effect).

The detection of lactose maldigestion can usually be made on the basis of the history,
supported by dietary manipulation.³¹ Diagnostic tests range from biochemical (low fecal pH
indicates lactose maldigestion used primarily in infants) over functional tests (e.g. hydrogen
breath test or serum glucose levels after ingestion of standard doses of lactose) to biopsy of the small bowel and direct assay of intestinal enzymatic activity.

Especially in children, genotyping for LNP/LP to detect ‘lactose intolerance’ appears inappropriate, also on account of the important differences in timing of decline of lactase expression in these age groups \(^1,16\). In this age group a good clinical history often reveals a relationship between ingestion of lactose and symptoms. When lactose maldigestion is suspected a lactose-free diet can be tried. It is important that all sources of lactose be eliminated, requiring the reading of food labels to identify ‘hidden’ sources of lactose. Generally, a 2-week trial of a strict lactose free diet with resolution of symptoms can be diagnostic. In more subtle cases, the hydrogen breath test is the least invasive and most helpful test to detect LNP \(^32\).

Genotyping for LNP/LP can be used as a screening test in adults \(^28\). Combinations of this technique with functional tests, including not only hydrogen but also methane measurement have been described \(^33\). Nevertheless, whatever result is gained by whatsoever technique, if lactose consumption are thought to produce symptoms, diagnosis must be confirmed by disappearance of symptoms following a lactose free diet. Thus, a major disadvantage of genotyping for LNP/LP is that it obviously does not indicate if a subject has or does not have symptoms of lactose maldigestion.

The advantages of genotyping for LNP/LP are that the test is non-invasive and that it saves both time and cost in healthcare. These circumstances, on the other hand, make genotyping for LNP/LP also to suitable test for large-scale screenings for LNP/LP in different populations or for studies with epidemiologic purpose.

1.1.3. Milk and milk product consumption across Europe

The present work studies two populations: one representative, randomly sampled population of 694 healthy children and adolescents belonging to Swedish section of the European Youth Heart Study (EYHS) and a representative sample of 551 healthy adults from the Canarian general population randomly sampled in the Canarian Nutrition Survey (ENCA). Further details about these studies can be found in the section Material and Methods. Between these
populations there exists a significant difference of the prevalence of LP/LNP. For Sweden the figures of prevalence of LP are one of the highest in the world with about 90%. In contrast, the prevalence of LP in Spain is about 50%.

Cow’s milk consumption is highest in Finland with 184 liters (l) per capita and year followed by Sweden with 146 l per capita and year. Ireland with 130 l and Netherlands with 123 l per capita and year are also among the countries with highest milk consumption. Spain, interestingly, with a consumption of 119 l milk per capita and year shows higher figures than countries like Norway (117 l), the United Kingdom (UK) (111 l), Germany (92 l) and Switzerland (113 l) (Source: International Dairy Federation, Bulletin 423/2007). The European Prospective Investigation into Cancer and Nutrition Study (EPIC), showed different dietary patterns among Europeans. The authors suggested two major dietary profiles among Europeans. The northern profile is characterized by low consumption of fruit and pasta/rice/other grain, vegetables, bread and wine, fish, legumes and vegetable oils, and high in consumption of butter, potatoes, dairy products and other cereals, such as flour, pastry and breakfast cereals, meat, non-alcoholic and alcoholic beverages except wine, sugar and cakes.

Based on the EPIC study, Hjärtåker et al could identify different patterns of milk and dairy consumption across Europe. The Spanish and Nordic countries reported a high consumption of milk; the Swedish (particularly Umeå) and but also Dutch centres reported high consumption of yoghurt and other fermented milk products, whereas the highest consumption of cheese is reported in French centers. The UK general population group and the Dutch centers showed the highest consumption of cream desserts and milk-based puddings. Italian and Nordic centers showed the highest consumption of ice-cream. As for butter, Germany, the UK and French reported highest intakes. The studied data interestingly suggested that countries consuming lower quantities of milk (e.g. Greece and Germany) tend to consume higher quantities of cheese.
1.1.4. Milk intake, body composition and growth among children and adolescents

Female mammals produce milk for the sole purpose to feed their offspring, a fact that lead us to hypothesize that milk might have some unique biological effects. The inclusion of milk and dairy from another mammalian order in human diets is evolutionary novel, compared to the time since mammals exist and to hominid evolution. But, humans are also the only species to wear skins of other animals and the only species to cook our food. These circumstances point to the adaptability of humans and are not always to inappropriate behavior. Nevertheless, milk is composed of an array of bioactive molecules that shape the development of nursing infants, who accrue body size (weight and linear dimensions) while organ systems mature. Specifically calves grow rapidly in skeletal size and weight, gaining in average 0.7-0.8 kg per day in the first year of life. In contrast, breastfeed infants gain in average about 0.02 kg per day in the first year of life (WHO, 2007). Cow’s milk supports thus biologically a much faster rate of increase in body size. Furthermore, formula-fed infants show greater gains in weight and length compared to breastfeed infants. The question we study in this work is, whether
routine consumption of cow’s milk generates gains in weight in children and adults, and in body height in adolescent and preadolescent children.

Human height is a highly complex trait. Within a population about 80-90% of the variation in body height is due to genetic factors 38. In modern western societies, about 10-20% of the variation in body height is due to environmental factors. The most important environmental factors affecting longitudinal growth are nutrition and diseases 39.

Milk consumption is one of the most studied nutritional factors in relation to height and growth rate in humans. Already in the early 1900s very well performed interventional studies, offering milk supplements to schoolchildren, showed that cow’s milk led to an increased longitudinal growth 40-41.

Today, almost a century later, the long-term effects of childhood milk consumption on growth is still debated. There is, however, considerable evidence that cow’s milk stimulates longitudinal growth 42-43. Non-caloric, bioactive growth-promoting constituents, which influence the insulin-like growth factor I (IGF-I) – axis leading to higher circulating levels of IGF-I, are considered to be responsible for the growth stimulating effect of cow’s milk in nutritionally replete, industrialized countries 44-50.

Milk and other dairy products are currently the primary source of calcium in western diets and milk avoidance has been linked to small stature 51-52. Swedish children and adolescents were of specific interest for our analysis of potential effects of milk consumption and longitudinal growth due to the high prevalence of lactase persistence (LP) 15, 53, and on account of the presence of one of the highest rates of milk consumption per capita in Europe and in the world 35.

As regards our study on potential effects of milk intake on body height in preadolescent and adolescent children we noticed with surprise that in similar studies no adjustments for parental height had been performed despite the large body of publications. We were able to adjust our statistical models, among other possible confounders, for birth weight and parental height, which are important covariates in relation to subsequent growth (as discussed above) 54.
1.1.5. Milk intake and the metabolic syndrome

The concept of the metabolic syndrome is a cluster of interrelated risk factors for cardiovascular disease (CVD) and type 2 diabetes mellitus. The factors include dysglycemia, raised blood pressure, elevated triglyceride (TG) levels, low high-density lipoprotein cholesterol (HDL) levels and obesity (particularly abdominal obesity). Several metabolic syndrome definitions exist \(^{55-58}\). Clinical definitions of the metabolic syndrome vary also in the priority given to obesity. The International Diabetes Federation (IDF) made abdominal obesity as necessary requirement in the diagnosis of the metabolic syndrome \(^{59}\). We have used the IDF definition of the metabolic syndrome in the present work. We consider obesity being central to the metabolic syndrome. Obesity is important years before the development of the other disorders associated to the metabolic syndrome become manifest. We believe that obesity, even seen separated, can be considered as an important risk factor for future metabolic derangement. The IDF definition of the metabolic syndrome is defined by central obesity (waist circumference in men ≥ 94 cm and in women ≥ 80 cm plus two of the following four criteria: fasting glucose ≥ 100 mg/dl, TG ≥ 150 mg/dl (or pharmacological treatment), HDL cholesterol in men < 40 mg/dl, in women < 50 mg/dl (or pharmacological treatment), systolic blood pressure > 130 mmHg and diastolic blood pressure > 85 mmHg (or pharmacological treatment).

The metabolic syndrome appears almost as a ‘lifestyle disorder’. Otherwise, therapeutic lifestyle changes, such as ‘healthy eating patterns’ and increased physical activity, would not show the positive impact on the metabolic syndrome they overwhelmingly show. Overeating and sedentary lifestyles play an important role in the development of the metabolic syndrome. Also defined dietary patterns might influence the development of the metabolic syndrome in genetically susceptible subjects. The observation that elements of the diet beyond the caloric content can have an impact on the metabolic syndrome has led many investigators to question which specific food elements may influence the metabolic syndrome.

Research also indicates a substantial role for genetic susceptibility to the metabolic syndrome \(^{60}\). There are a growing number of studies investigating potential gene-nutrient interactions. Given that by definition the metabolic syndrome is coupled to different criteria, the analysis of potential gene-environment and gene-nutrient interactions is complex. To disentangle the relation of individual genes on the metabolic syndrome in the presence of multiple putative
SNPs and highly variable environmental factors may help to improve efficacy of dietary and other lifestyle recommendations. These circumstances led us to investigate if the LCT-13910 C>T polymorphism has an effect on the metabolic syndrome.

Due to the complexity of the studied matter we introduced the concept of Mendelian randomization (MR). By subdividing genotypes in LP and LNP we readdress the question of whether nutrigenetically defined factors, like milk and milk product intake, affect the metabolic syndrome. MR studies modifiable causes of disease in genetic epidemiology. A functional genetic variant, in our study LCT-13910 C>T polymorphism, acts as proxy for modifiable lifetime exposure patterns, i.e. milk consumption. The LCT-13910 C>T polymorphism is known to influence the exposure pattern, milk consumption 20, 26, 61-64. MR is described in more detail in section 4.2 (methodological considerations) below.

Some studies show that moderate increase of milk and milk product consumption have protective effects against the metabolic syndrome 65-67, high blood pressure 68-69, and influence positively unfavorable LDL and HDL profiles 70. Other studies suggest that milk and milk product consumption may help prevent weight gain and even promote weight loss 66, 71. Other studies in turn suggest that milk avoidance is associated with a reduced risk of MS 72 and in children there have been found a positive association between milk consumption and higher body mass indexes (BMI) 73-74. The role of milk consumption as regards MS and obesity is thus still controversially debated.

We chose for the present study concerning possible effects of milk intake on MS an available population of the Canary Islands in Spain, which we genotyped for LCT-13910 C>T polymorphism. The Canary Islands were of special interest because in contrast to the rest of Spain, there is a high prevalence of cardiovascular risk factors and one of the highest cardiovascular mortality rates of the country, particularly among women. In addition, the Canary Islands display one of the highest rates of milk consumption in Spain, comparable or even higher than in some Nordic countries.
2. Aims

2.1. Study 1: to determine the prevalence of LNP/LP in Sweden by genotyping for the LCT-13910 C>T SNP.

2.2. Study 2: to assess if LNP/LP, defined by genotyping for the LCT-13910 C>T SNP, is associated with differences in milk and milk product intake, and to evaluate if milk and milk product consumption is related to differences in measures of body fat in children and adolescents.

2.3. Study 3: to assess if milk intake and LNP/LP, defined by genotyping for the LCT-13910 C>T SNP, is associated to differences in body height in preadolescent and adolescent children.

2.4. Study 4: to assess if LNP/LP, defined by genotyping for the LCT-13910 C>T SNP, is related to the metabolic syndrome in the Canary Islands in Spain.
3. Participants, Material and Methods

3.1. Participants

3.1.1. The European Youth Heart Study (EYHS)

The EYHS is a school-based, cross-sectional study designed to examine the interactions between personal, environmental and lifestyle influences on risk factors for future CVD. A scientific Committee of senior researchers designed EYHS. Core original members of the committee were Drs. Chris Riddoch (UK), Michael Sjöström (Sweden), Karsten Froberg (Denmark) and Dawn Edwards (UK). EYHS data have been collected in five countries: Denmark (Odense, 1997/1998), Sweden (Örebro and Stockholm, 1998/1999), Estonia (Tartu, 1998/1999), Portugal (Madeira, 1998/1999) and Norway (Oslo, 1999/2000). All participating EYHS countries followed the protocol manual, and to attain comparable results between the countries meetings were held for new groups and one central laboratory was used for serum analysis.

The present work has been developed with data obtained from the Swedish section of the European Youth Heart Study (EYHS). The Swedish part of the study began with cross-sectional data collection in 1998-1999 (EYHS-I). Repeated observations of the key indicators addressing cardiovascular risk factors and their determinants were performed in 2004 - 2005 (EYHS-II). The present work is based on EYHS-I and thus cross-sectional data. The sampling frame in Sweden included the municipality of Örebro and the southern region of Stockholm. The municipality of Örebro had 160,000 inhabitants, of whom there were about 1,500 pupils in each of the EYHS age group (9 and 15 years). The south region of Stockholm, defined here by the municipalities of Botkyrka, Haninge, Huddinge, Nynäshamn, Salem, Södertalje and Tyresö, had about 370,000 inhabitants, of whom there were about 5,000 pupils in each of the two age groups.

A two-stage cluster procedure was employed in each of the study locations. In the first step all public schools within each local area were identified and stratified by target age groups and the mean income level (below or above the mean of their municipality) of the respective area. Subsequently, schools were randomly sampled within each stratified age group (9 and 15 years) and invited to partake. In a second step, groups of children and adolescents of the
predefined target age were randomly sampled from each school in proportion to school size and then invited to participate. The aim was to obtain 250 participants in each age or gender group (totally 1,000 participants). Fifty per cent oversampling was performed on the basis of experiences from a pilot study. The overall participation rate in Sweden was 50%. In total 1154 subjects agreed to participate in the EYHS. Twenty four per cent declined and 26% did not respond at all. Of those to agreed to partake in the study 17 (1%) failed to keep their appointment rendering finally 1137 participants. A comprehensive analysis of the non-participants was carried out including questionnaire to be filled out by the schoolchildren. The most common reason given for non-participation was ‘I didn’t want to have a blood sample taken’; 38 % of the preadolescent and 33 % of the adolescent children gave this answer. Participation in this study was quite demanding (eg blood sampling and maximal ergometer bicycle test). The socioeconomic background of participants was slightly above average compared to the general Swedish population. Study design, sampling procedure, participation rates and study protocol have been reported in detail elsewhere. We used in the present study a sub-set of 684 schoolchildren as they had been profiled for the LCT-19310 C>T polymorphism.

3.1.2. Active Seniors

The second study population used to explore potential secular trends in the prevalence of LP/LNP in Sweden consisted of 392 elderly individuals (Active Seniors). This cohort constituted a non-randomly sampled cohort recruited from study circles for the elderly. The locations for the recruitment were selected to represent a broad range of socioeconomic levels and included rural as well as urban and sub-urban areas. Being retired (usual age \( \geq 65 \) years in Sweden) and living independently in their own homes in addition to the ability to participate in the study circles, were the sole inclusion criteria, not preset health criteria. However, this cohort of elderly subjects was not primarily recruited to partake in the present study. All were of European descent. The majority of the subjects of this study population were born between 1920 and 1932; the mean year of birth was 1928.
3.1.3. Nutrition Survey of the Canary Islands (ENCA)

The Canary Islands are a Spanish region formed by seven islands located in the Atlantic Ocean off the African coast of Morocco. The Canary Islands are a Spanish autonomous community and has about 2 million inhabitants and enjoy a sub-tropical climate. The name of the archipelago is probably derived from the latin, *Insula Canaria*, meaning “Island of the Dogs”. The original inhabitants were the “Guanches”; they worshiped dogs as holy animals. Also the ancient Greeks knew about these islands far to the west, whose inhabitants were the “dog-headed ones”. Phoenicians and Carthaginians have visited the islands. The history of the settlement of the archipelago is still unclear. Linguistic and genetic analyses point to possible common origin with the Berbers of northern Africa. When Europeans began to explore the islands they found several indigenous populations living at a Neolithic level of technology. In 1402 the Castilians began to conquer the archipelago, with the expedition of French adventurers, Jean de Béthencourt and Gadifer de la Salle, nobles and vassals of Henry III of Castile. Complete pacification of the resistance of the native Guanchos was achieved 1495. Thereafter the Canary Islands were incorporated in the Kingdom of Castile.

Today, and since establishment of a democratic constitutional monarchy after the end of Franco’s regime in 1975, important socioeconomic changes have had an impact on food consumption and health status. In contrast to what is found in the rest of Spain, in the Canary Islands a high prevalence of cardiovascular risk factors exists together with one of the highest cardiovascular mortality rates of the country, especially among women. ENCA showed several particular features for the population of the Canary Islands. The Canary Islands display on account of their history and geographic location nutritional singularities when compared to the rest of Spain. ENCA revealed an ongoing nutritional shift from a Mesoamerican to a more Mediterranean pattern of food consumption, nevertheless maintaining their sub-tropical Canarian characteristics. Furthermore, the “Tablas de Composición de Alimentos Españoles” (tables of composition of Spanish food items) of Mataix et al 79 used in the nutritional survey were revised and 55 food items typical for the Canary Island required to be added. In addition, the tables of composition of Spanish food items were completed by French tables of composition of food items 80.
In the Canarian Nutrition Survey (ENCA 1997-1998) a representative sample of the Canarian general population between 6- and 75-year-old. A total of 1747 individuals participated. Analysis of non-participation was performed. Details on data collection and methodology have been published in detail elsewhere \(^{81-84}\).

### 3.2.  Material and Methods

#### 3.2.1. Material and Methods EYHS

Blood samples were obtained from 684 children (334 girls and 350 boys) belonging to the Swedish part of the European Youth Heart Study (EYHS). Height, weight, hip and waist circumferences, as well as skinfold thickness were measured by standardized procedures \(^{85}\). Body mass index (BMI) was calculated as weight/height\(^2\) (kg/m\(^2\)). Body fat percentage was calculated from the equation by Slaughter, based on skinfold thickness measurements \(^{86}\). The skinfold measures have been shown to correlate highly with dual-energy X-ray absorptiometry-measured body fat percentages in children of similar ages \(^{87}\). The skinfold measurements were taken using a Harpenden caliper at the biceps, triceps, subscapular, suprailiac and triceps surae sites on the left side of the body. All measurements were taken twice and the mean calculated. If the difference between the 2 measurements differed by > 2 mm, a third measurement was taken and the 2 closest measurements were averaged.

The consumption of milk and milk products was assessed by an interviewer-mediated 24-hour recall. A qualitative food record completed the day before the interview with the help of parents served as a checklist for the data obtained during the recall in the nine-year-olds. A food atlas was used to estimate portion sizes. Dietary data were processed by StorMats (version 4.02, Rudans Lättdata, Sweden) and analysed using the Swedish National Food database (version 99.1). The milk products of interest were milk, soured milk (a traditional milk product, “filmjölk” in Sweden), yoghurt, and cheese, in both reduced-fat and full-fat varieties.

As regards dietary methodology it appears that two main concepts are often confounded or misinterpreted concerning the diet of an individual and a collective. These are the concepts of usual intakes and actual intakes. Observational nutritional epidemiology of large collectives is
primarily interested in an accurate estimation of actual intakes, eg 24-hour dietary recall. Analytic nutritional epidemiology requires an accurate estimate of the past to present, ie usual intakes. As regards the present work, based on genotyping for LP/LNP, which influences the life-time exposure pattern, milk consumption, usual intakes are of less interest than actual intakes. Genetic nutritional epidemiology using a functional genetic variant, already known to stand proxy for lifetime exposure patterns (milk consumption, eg), do not need to readdress the question of usual intakes of the food item in question for each and every individual. Intra-individual variance, which is regarded both as random error or systematic error (because it can differ between populations) as well as inter-individual variance is explained by the genotype of the single subject or, the prevalence of the functional genetic variant in a population. This is the reason why genetic nutritional epidemiology using a functional genetic variant (eg Mendelian randomization) primarily needs an accurate estimate of the actual intake of the food item in question.

3.2.2. Material and Methods ENCA

Sample size calculations resulted in a need of 1800 individuals, aged 6 to 75 years, necessary for robust estimations of events rates of 10% to 20% and a precision ranging between 8% and 10% ($\alpha = 0.05$) and to ensure a minimum of 100 individuals in every age group (10 years intervals). The sample size was increased by 40% to ensure representativeness for the Canary Islands. A stratified two-stage random sampling procedure was used weighted by the number of inhabitants of each of the 32 sampled municipalities. Municipalities were the first unit randomly sampled, and the last unit was the citizens registered in these municipalities. The response rate was 69%. Non-participation (31%) included 17% voluntary non-participation, 14% involuntary non-participation (errors in census, changed domicile: 9%, absence or impossibility to partake in the study: 6%).

To assess the nutritional status of the population two 24h-recall questionnaires were sent on non-consecutive days prior to a visit at domicile and interview. Nutritional assessment also included a food frequency questionnaire. We present in our sub-study data derived from the 24h-recall questionnaires. The “Tablas de Composición de Alimentos Españoles” (tables of composition of Spanish food items) of Mataix et al. and the French tables of composition of food items were used to transform data in energy intakes and single nutrients. ENCA also
included questionnaires about lifestyle and health status. Anthropometrics were determined according to standardized procedures and blood pressure was taken. Sociodemographic and lifestyle variables including age, sex, educational level, smoking, alcohol consumption and physical activity were registered. Details on data collection have been published elsewhere.  

Seven hundred eighty two subjects participated in the biochemical analysis. The present study is based on a sample of 551 adults aged 18 - 75 years (240 men and 311 women). Children were excluded. Genetic analysis as regards the present study was performed at the University hospital in Örebro, Sweden at the department of clinical chemistry.  

ENCA revealed several particularities of the Canarian population of interest for our study. The prevalence of obesity was in the Canary Islands 18 % higher than the national average and one of the highest in Europe. Trans fatty acid consumption was on average higher than estimates for the rest of Spain. With an intake of 6.8 g a day, trans fatty acid ingestion was at levels comparable to the USA. The prevalence of diabetes type 2 is with 8%, 3% higher than the national reference. Milk consumption in Spain is highest in the Canary Islands. The average milk consumption is 322 ml a day and thus comparable or even higher than in some countries in northern Europe. The same applies to the intakes potatoes and candy. Vegetable intake in Canary Islands is the lowest of all Spanish autonomies.  

3.2.3. Genomic Analysis  

Genomic DNA was isolated from the EDTA whole blood samples from the individuals with the QIAamp DNA Blood Mini Kit spin procedure. The DNA fragment spanning the -13910- C/T polymorphic site was amplified using a biotinylated forward-primer (5’ GGGCTGGCAATACAGATAAGATA-3’) and an unbiotinylated reverse-primer (5’ AGCAGGGCTCAAAGAACAATCTA-3’). PCR amplifications were carried out in a 50 µL volume containing the forward and reverse primer, 1,25 units of Taq polymerase, 2.0 mmol/L MgCl2 and 0.2 mmol/L each of dGTP, dATP, dTTP, and dCTP. As templates 100 ng of the extracted genomic DNA was added. The PCR products were prepared for Pyrosequencing according to a standard protocol (Pyrosequencing AB, Uppsala, Sweden) using 25 µL amplicon captured by streptavidin-coated Sepharose beads and using the
Pyrosequencing Sample Preparation Kit (Pyrosequencing AB, Uppsala, Sweden); the applied sequencing primer was: 5’-CTTTGAGGCCAGGG-3’. Sequencing was performed using a PSQ96 SNP reagent Kit and a PSQ 96MA system (Pyrosequencing AB) PSQ96MA 2.0.1 software. This protocol is based on direct sequencing and yields unambiguous genotyping results as well as sequence information beyond the SNP, which provides an internal control for each sample 89. Simultaneous genotyping for the three polymorphic loci linked to LP (LCT-13907 C>G, LCT-13910 C>T, LCT-13915 T>G) is routinely performed. Pyrosequencing is thus a suitable method to detect LP/LNP not only in individuals of European descent, but also of African or Middle East descent 30, 90.

4. Methodological Consideration

4.1. Hardy-Weinberg Equilibrium

Hardy-Weinberg equilibrium (HWE) describes a state in which genotype frequencies are constant from generation to generation and in which genotype frequencies are product of allele frequencies. This condition prevails in situations in which there are an absence of mutations, migrations, non-random mating and environmental factors favoring particular genotypes. In a paper published in 1908, Hardy wanted to disprove the Mendelian idea that dominant genetic traits would have a propensity to appear in the whole population, while recessive traits would tend to disappear. In the same year, Weinberg published a paper proving the same idea. He later went a step further to be the first to apply it to human populations. The original descriptions of HWE are an important landmark in the history of population genetics 91 and it is now common practice to check whether observed genotypes conform to Hardy-Weinberg expectations. Deviations from HWE can indicate inbreeding, population stratification, and even problems of genotyping 92.

The prevalence of a genetic recessive or dominant trait can, when studied by genotyping methods, be estimated directly as the prevalence of the homozygously mutated genotype. If this type of trait is rare, the number of homozygotes in the studied sample will not be very large, and the relative impact of sampling errors of various types can be expected to be rather large when a prevalence estimate is based on the simple division term CC/(TT+CT+CC) as is the case in the sub-study of this work. However, one can also arrive at an estimate of the
prevalence of the homozygous carriers of the mutated allele \(q^2\) by utilizing all the information that is contained in the Hardy-Weinberg Equilibrium, given the mathematical relation \(p^2 + 2pq + q^2 = 1.00\) between the prevalences of the mutated allele, eg \(q\), and the prevalence of the wildtype (unmutated) allele eg \(p\). The advantage would be that the estimate of \(q\) involves a much larger fraction of the population sample studied than the direct assay of subjects in the sample that happen to have the genotype \(q^2\), since not only these homozygous carriers of the mutated allele \((q^2)\) but also the heterozygotes \(2pq\) carry mutated alleles and therefore the random error in the estimation of \(q^2\) from a HWE calculation will be relatively smaller than that of the direct assay result by genotyping of the empirical prevalence of \(q^2\) in the particular sample studied.

4.2. Mendelian Randomization

Since the carriage of the LCT -13910 C>T polymorphism is subject to the random assortment of maternal and paternal alleles at the time of gamete formation (Mendel’s second law), associations of LCT genotype with MS should not be subject to reverse causality, and largely free from confounding by behavioral, physiological and socioeconomic factors related to both exposure (milk consumption) and outcome (MS). This is the basic assumption of MR 61-62. MR studies modifiable causes of disease in genetic epidemiology. A functional genetic variant, in our study LCT–13910 C>T polymorphism, acts as proxy for modifiable lifetime exposure patterns, i.e., milk consumption. The LCT–13910 C>T polymorphism is known to influence the exposure pattern, milk consumption 20, 26, 64. According to Mendel’s second law of independent assortment, the inheritance of one trait is independent of the inheritance of other traits. This law suggests that inheritance is independent of –that is, randomized with respect to –the inheritance of other traits. Thus, associations between genetic variants and outcome are not generally confounded by behavioral, physiological or environmental exposures. Hence, observational studies of genetic variants have similar properties to intention to treat analyses in randomized controlled trials 61-63.
5. Main Results and Discussion

5.1. Study 1

The historical prevalence data for LNP in Sweden based on functional tests range between 1% and at most 5% \(^{64}\). Using genotyping for LCT \(-13910\) C>T, we obtained an overall LNP estimate of 14% among young Swedish citizens born in the 1980s, a markedly higher value than the traditional figures ranging from 1 to 5%. In the elderly born in the 1920s we found a prevalence of 6.8% for LNP. We proposed the following interpretation of these findings. The frequency of the LCT \(-13910\) C allele has increased from 2 sources: long-range immigration from parts of the world with very high LNP prevalence, and a more long lasting immigration of Caucasians from neighbouring European countries with a less dramatic but still markedly higher LNP prevalence than the 6.8%. This higher LNP prevalence is seen in contrast to the prevalence that seemed to have prevailed earlier in Scandinavian population samples as found in studies from Norway, Finland, and Sweden (the elderly cohort in this study). This significant shift in LNP prevalence in Sweden may have public health implications and should therefore be monitored closely by health-planners and policy-makers \(^{53}\). We used initially, and in this sub-study, the term Adult type hypolactasia (AtH) to describe the status of LNP, which we changed in the following sub-studies, because we considered LNP more appropriate.

![Image](image_url)
5.2. Study II

The underlying hypothesis of this study was that LP and LNP might influence the consumption of milk and milk products in children and adolescents, which could lead to differences in body fat mass, between the different genotypes.

There was a statistically significant difference between overall milk and milk product consumption in LP and LNP subjects. LNP was associated with a lower total consumption of milk, soured milk and yoghurt in children and adolescents. Children and adolescents with the TT and CT genotype consumed statistically significantly more reduced fat milk than LNP subjects. Concerning the key question of this study, whether LP and LNP influence the consumption of milk and milk products, which in turn could be associated with differences in body composition between LP and LNP, no differences between LP and LNP of body composition, especially in measures of body fat mass could be detected. In conclusion, LP was linked to an overall higher milk and dairy intake, but was not linked to higher measures of body fat mass in children and adolescents. Thus, an obesogenic effect of milk and milk product intake could not be detected in the studied population. During the revision process of this study a paper was published showing that a dietary pattern characterized by greater milk intake was associated with increased BMI among preschool children. We took this circumstance as an occasion to nurture the concept of adaptability in contrast to “one-size-fits-all” public policies or solutions as regards public health nutrition. We hypothesize that potential physiological effects of single food items, such as milk and milk products, may differ also between countries due to their particular food and farming culture, agricultural traditions and geographic as well as climatic singularities, among other potential factors.

The conception of this paper initially included the task to explore if an intermediate genotype (LCT-13910 CT) were detectable. Since the finding of the trimodal distribution of lactase in the small bowel traces back to the 1980s, we tested if the postulate of a mid-level-activity of the enzyme would have an impact on milk and milk product intake using LCT genotypes as proxy. We did not find mid-level intakes of milk and milk product in heterozygous individuals.
5.3. Study III

Cow’s milk consumption is one of the most studied nutritional factors in relation to height and growth rate in humans. Already in the early 1900s very well performed experimental studies showed that cow’s milk supplementation in schoolchildren led to an increased longitudinal growth \(^{40-41}\). Today, a century later, the long-term effect of childhood milk consumption on bone growth is still debated. There is, however, considerable evidence that cow’s milk stimulates longitudinal growth \(^{40-42, 46-47, 95}\). Of particular interest are the ages 9 and 15 years, thus, before and after initiation of the pubertal growth spurt. Cow’s milk and other dairy products are currently the primary source of calcium in Western diets and cow’s milk avoidance has been linked to small stature \(^{51-52}\). Swedish children and adolescents are of specific interest for this study of potential links between milk consumption and body height on account of the high prevalence of LP, and on account of the presence of one of the highest consumption of cow’s milk and cow’s milk products in Europe and in the world. We found that milk intake was a significant positive contributor to the observed variance in body height when adjusted for parental height (\(\beta = 0.46; 95\%\ CI: 0.040, 0.87 \) and \(p = 0.032\)). Also, the LCT C>T-13910 polymorphism (LP vs. LNP), remained significant in the final model (\(\beta = 2.05; 95\%\ CI: 0.18, 3.92 \) and \(p = 0.032\)), showing a positive association of LP with height. In addition, the statistical model we used was able to explain 90% of the observed variance in body height (adjusted \(R^2 = 0.885\)). We hypothesize that non-caloric constituents in milk such as IGF-I might influence the somatotropic axis in preadolescent and adolescent children. Neither LP nor LNP was associated to higher energy intakes and higher milk consumption did not translate in overall higher energy intakes. Figures 4 and 5 show the crude (without the other regressors) linear relationship between milk intake and body height stratified by Tanner score.
Figure 4: height (cm) by milk intake (g/d) in preadolescents (Tanner 1, 2), n=256

Figure 5: height (cm) by milk intake (g/d) in adolescents (Tanner 4, 5), n= 28
5.4. Study IV

In this study we examined an available population of the Canary Islands in Spain where, in contrast to rest of Spain, there is a high prevalence of cardiovascular risk factors and one of the highest cardiovascular mortality rates of the country. Furthermore, the highest milk consumption in Spain is found in the Canary Islands. LP subjects consumed in average about 17% more milk compared to LNP subjects. LP subjects of the Canary Islands exhibited a 57% higher risk to develop MS compared to LNP subjects. Interestingly, LP women showed a 93% higher risk to develop MS compared to a 22% higher risk in LP men, after adjustment for the main nutritional and environmental variables. We did not find, nevertheless, a significant difference in milk consumption between women and men in their respective LP or LNP group. This means that the quantity of milk consumed by women and men in their corresponding LP or LNP group cannot explain the higher risk of LP women to develop MS compared to LP men. We proposed the following rationale for this finding. Milk contains hormonal constituents that influence endogenous hormones in sufficient quantities to have biological effects in the consumer. One mechanism could be that milk consumption increases insulin-like growth factor-I plasma levels in consumers 44, 47, 96-97. Furthermore, because of contemporarily production methods, modern cow milk has a high content of sex steroids and precursors of sex steroids such as progesterone (also bovine recombinant growth hormone is used to increase milk production) 98. Progesterone and other steroid hormones have been associated with markers of insulin resistance 99. Together these factors could shed light on the observed higher risk of LP women to develop MS compared to LP men in the Canary Islands. In addition, other sex steroids present in milk, especially testosterone, can influence plasma levels of adiponectin plasma levels in women and men leading to changed insulin sensitivity.

![Figure 6: distribution LCT-13910 C>T (ENCA)](image)
6. General Discussion and Future Perspectives

Lactation has evolved gradually and a complex lactation system with elaborated milk secretion was already in place 200 millions of years in the ancestors of mammals. This said, it appears almost astonishing that LP emergence is dated only about 7,500 BC, thus during Neolithic transition, which suggests gene-culture coevolution.

Since 2002 we are able to genotype for the SNP giving rise to LP. This circumstance led us to investigate if the traditional prevalence figures for LNP in Sweden were up to date. We found a 5.5 fold higher prevalence (14%) for LNP than current literature and noticed a secular trend, which we interpreted as a result of the ongoing globalization leading to more heterogeneous populations in industrialized countries. This also means that we can expect a 5.5 fold or even higher prevalence of LNP individuals suffering symptoms of lactose intolerance, if milk and some less processed milk products are consumed. Noteworthy is that LNP often continues to be regarded by western scientific literature as a kind of disease that requires therapeutic measures. This is not especially surprising given the fact that LP has been considered the globally predominant condition and ‘normal’ status until the 1960s despite being 70% of the world population LNP. The concept of a particular disease can, according to this, just constitute a ‘social construct’ without anchoring in reality, evidence and last but not least science. The term ‘Milk drinking syndrome’ has been coined to describe the phenomenon when European experience is extrapolated to the other populations in the world.

LNP individuals are on average able to consume around 12g of lactose without symptoms of lactose intolerance. This amounts to 250 ml fresh milk a day. However, consumed with meals or intakes of lactose containing food items distributed through the day elevates the threshold of tolerance to lactose. All together, this appears to be reasonable amounts of fresh milk consumption given the circumstance that most processed milk products only contain a fractional amount of lactose compared to milk, and can be consumed without symptoms of lactose intolerance.

If disambiguation succeeds among professionals and policy makers a significant amount of resources used to detect and treat LNP could be saved or utilized for diagnosis and treatments of real-existing pathologies. Having said this, the existing diagnostic measures to detect lactose intolerance in a broader sense are surely valuable and useful. A possible algorithm
about measures to be taken in cases of uncertainty or when differential diagnostics are required has been described in the section: 1.1.2. Detection of LP and LNP.

Sweden belongs together with Finland to the countries with the highest per capita cow’s milk intakes in the world. It was thus important for us to explore if milk consumption affects body weight and body fat mass in children. Childhood milk consumption has become normative in western countries surely influenced by two British scientific publications dated back to the beginnings of the 20th century (1928 & 1929, respectively) 40-41. Weight gain in children was regarded as beneficial at that time. In today’s completely different social settings, ‘high’ rates of milk consumption in childhood continue to be associated to a healthy lifestyle in many western countries. And, it is surely questionable if a ‘high’ consumption of whatever food item can be considered as health promoting. However, it kept to be elucidated if milk consumption affects measures of body weight, BMI and body fat mass in Swedish children; thus, in a nutritionally replete country. The issue, whether milk consumption affects negatively or positively children’s weight or body fat mass, constitutes an ongoing very controversial topic. On account of these circumstances we performed sub-study 2. We did not find any effect of milk intake on measures of body fat mass or body weight in preadolescent and adolescent children in Sweden. Milk consumption showed neither an obesogenic effect nor a weight reduction effect on the studied population. The same applies to the genotypes LP/LNP, which also did not correlate with measures of body fat mass. However, the drawbacks inherent to cross-sectional studies have to be kept in mind. We are not able to infer causality, and we would not too, if we would have found a positive or negative association. Interventional studies or studies with higher power will be necessary to confirm or rebut our findings. Since health policy should and can aspire to the most equitable and efficient delivery of preventive services, and following the time-honored principle of “first do no harm”, we cannot derive any public health recommendation from these findings.

Based on the studies of Leighton41 and Orr40 about a century ago, we decided to explore whether milk consumption continues to exert an effect on body height in children under today’s completely different socio-economic settings. We also become aware of Takahashi’s article published 1984, “Secular Trend in Milk Consumption and Growth in Japan” - Japan, as a country without appreciable cow’s milk consumption or dairy culture until the 1950s.
To our surprise we found a positive association between milk consumption and body height in children. Sweden with its long dairy tradition and high milk consumption together with one of the highest prevalence of LP could appear as the worst eligible scenario to detect an effect of milk on body height. This was also the case until the low figures for $R^2$ led us to reconsider our statistical model. Including adjustment for parental height in our model finally delivered not only an acceptable $R^2$ value but also showed a positive correlation of both milk intake and LP with body height. We also detected that nobody before us with exception of one study in adolescents had corrected for parental height when studying the relation of body height to milk consumption despite a large body of publications on this issue. In addition, even in many twin studies on longitudinal growth, the circumstance that 80-90% of body height is heritable, has been neglected. However, given the design of our study we are only able to generate hypotheses primarily. We hypothesize that milk consumption together with LP status affects positively body height in children. Given the paradigm “larger is healthier” which we today experience our results might a priori been perceived as a ‘positive’ message. This is however not the case. We conclude that our results should be interpreted with caution and should serve policy makers, professionals in public health and public health nutrition to await a more complete understanding of the complex effects of milk consumption on long-term health. This applies particularly to developing countries with complementary feeding practices, which are not based on dairy farming. Milk consumption in childhood might exert both negative and positive effects, since greater stature has been associated to higher risk for some cancers $^{102}$. Using the tenets of Mendelian randomization we explored in an available population of the Canary Islands in Spain if LP/LNP genotypes show an association with the metabolic syndrome (MS) defined according to the criteria of the International Diabetes Federation (IDF). The ENCA study revealed a prevalence of metabolic syndrome of about 24% in the Canary Islands, which is highest in Spain and similar to the figures in the USA. LP subjects from the Canary Islands displayed a 57% higher risk to develop metabolic syndrome than LNP subjects. Stratified for sex, LP women showed a significantly higher susceptibility to develop metabolic syndrome than men (93% vs 22%). This sex-specific difference might be in part attributable to the criteria we used to define metabolic syndrome. The IDF criteria are characterized by being abdominal obesity a necessary requirement to define metabolic syndrome and the most prevalent metabolic syndrome criteria in the Canary Island among women was abdominal obesity and low HDL-cholesterol. Given the panoply of different definitions of MS, the election of one definition might alter sex-specific differences in one or
the other direction. The same applies to the overall prevalence of metabolic syndrome in a defined population. Using the WHO criteria to detect the metabolic syndrome in the Canary Island showed a prevalence of metabolic syndrome of 28% and using the ‘Third Report of the National Cholesterol Education Program’ (NCEP) the figure was 23% \(^\text{103}\). However, as a construct that denotes risk factor clustering for cardio-vascular disease metabolic syndrome has been a useful paradigm.

We also applied the ATP III definition to explore if the LP/LNP genotypes showed an association with metabolic syndrome in the Canary Islands. We found a significant association of LP with metabolic syndrome and the same prevalence figure, which confirms our results using the IDF criteria. We conclude that the possibility of higher susceptibility of LP subjects towards the development of metabolic syndrome exists among the adult population of the Canary Islands. As LP individuals also showed higher intakes of milk we believe that this higher susceptibility might be modulated by higher milk intakes. The singularities of the diet of the Canary Islands has been described previously, and also other milk products than just fresh milk are consumed in higher amounts in the Canary Islands compared to the rest of Spain. The recommendation that can be derived out of this study has been already implemented in the Canary Islands by ENCA, which is a shift towards a more Mediterranean style diet.

In summary, it would be important to explore how LP evolved in other parts of the world, probably via convergent evolution and varying demographic dynamics in Middle and South America, Asia and Africa. This information would help international health and food aid organizations to meet the region’s nutritional requirements in a more differentiated way. It also is necessary to study in more detail and verticality the long-term effects of milk consumption. This applies not only to industrialized countries but also to developing countries. Studies with higher power than the power we were able to deliver or intervention studies might be promising prospects.
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