EFFECTS OF AGE AND MILK CONSUMPTION ON LACTOSE MALABSORPTION DETERMINED BY BREATH HYDROGEN ANALYSIS IN OKLAHOMA INDIANS

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1965

Submitted to the Faculty of the Graduate College
of the Oklahoma State University
in partial fulfillment of the requirements
for the Degree of
MASTER OF SCIENCE
May, 1976

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ACKNOWLEDGMENTS

The author wishes to express her appreciation to her major adviser, Dr. Donna Bose, for providing a graduate research assistantship which made this research possible. Also, her guidance and assistance throughout this study were invaluable. Gratitude is also expressed to the other committee members, Dr. Esther Winterfeldt, Dr. Harry Gearhart and Dr. Robert Morrison for their critique and encouragement in the preparation of this manuscript. A special word of thanks to Dr. Jack Welsh, Department of Medicine, The University of Oklahoma Health Sciences Center, for his consideration and valuable suggestions concerning this research.

A note of thanks is extended to Kathy Moffitt, Marlece Ebbesen and Paul James for their important contribution in helping gather the data. In addition, appreciation is given to the subjects for their cooperation.

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CHAPTER I

SIGNIFICANCE OF THE PROBLEM

Introduction

Lactose, the carbohydrate in milk, has gained the attention of many researchers across the world in recent years. When this disaccharide is consumed by some ethnic groups, physical discomfort may result due to the inability to properly hydrolyze this sugar, resulting in its malabsorption (3, 4, 49). The incidence of lactose malabsorption among Native North Americans has not been studied as extensively as in people of other origins (96). With the large American Indian population in Oklahoma, lactose malabsorption may be conveniently studied in this ethnic group. This study is the largest to be reported to date and the first to use breath hydrogen analysis to assess the occurrence of lactose malabsorption in Native Americans. Only two other studies of lactose malabsorption in Native North Americans have been reported; one by Leichter and Lee (65) with Canadian West Coast Indians and the other by Bose and Welsh (17) with Oklahoma Indians. These two studies used the lactose tolerance test (LTT), the most frequently used clinical method, to determine the incidence of lactose malabsorption.

The breath hydrogen test (BHT), a non-invasive method for determining lactose maldigestion by gas chromatography, is not extensively used at the present time as a clinical tool to evaluate lactase activity (16, 20, 34, 42, 75, 80). The production of hydrogen gas is one of

the by-products of bacterial fermentation of lactose in the intestinal tract (16, 19). When lactose, milk sugar, is ingested, if there is enough enzyme, lactase, present in the brush border of the small intestine, the lactose will be hydrolyzed to its constituent monosaccharides, glucose and galactose (49). The monosaccharides are then absorbed and subsequently gain access to the blood stream. However, when there is low activity of the enzyme, lactose will remain in the intestinal tract unhydrolyzed. The lactose then serves as a good host for intestinal microorganisms which generate hydrogen gas (19, 20). The hydrogen gas diffuses across the intestinal wall and is carried by the blood to the lungs. The amount of hydrogen excreted from the lungs correlates well with the total amount produced in the gut and hence can be used as a good indicator of low lactase activity (66). This is the basis for the breath hydrogen test in determining lactose malabsorption.

Purpose

The purpose of this study was to use the breath hydrogen test to determine the incidence of lactose malabsorption due to low lactase activity in 60 seven-eighths to full-blooded Oklahoma Indians. The subjects ranged in age from 3 to 64 years making it possible to study the effect of age on lactose malabsorption in this ethnic group. Another factor considered was the influence of routine milk consumption on lactose maldigestion in Native Americans.

CHAPTER II

REVIEW OF LITERATURE

Milk, the major food of most mammals during infancy, is known to be a complete food containing carbohydrate, fat and high-quality protein. In addition, it is an excellent source of calcium and riboflavin, as well as furnishing many other essential nutrients. Only in approximately the last 6000 years has milk been a constituent of the human diet beyond infancy. At the present time, milk constitutes a major dietary component throughout life for individuals in the Western Hemisphere, but in many other parts of the world milk is never consumed again following infancy (49).

A product of the mammary gland, milk, is believed by some ethnic groups not to be a proper food for man. They do not like its smell or taste and think that drinking milk is unclean because they experience diarrhea and other physical discomfort following its consumption (95). Early in this century diarrhea and excessive fermentation were associated with the ingestion of lactose, the sugar in milk. Hence, lactose malabsorption was recognized several decades ago, but only in the past 20 years has a more detailed knowledge led to the different types of lactose malabsorption (49).

Lactose Malabsorption

When speaking of an ethnic group's inability to absorb lactose,

several descriptive terms are used. The definitions are given here for clarity. Lactase deficiency is not a disease, for individuals throughout the world with low levels of lactase may have good health. Therefore, this is a condition that probably exists in the majority of the world's adult population and might better be called low lactase activity (99). Lactose malabsorption describes reduced absorption of lactose, a consequence of low lactase activity (86). With lactose intolerance, clinical signs (i.e., abdominal pain, diarrhea, bloating, flatulence) are evident following ingestion of a standard dose or less of lactose in an individual with proven lactose malabsorption (86).

Lactose malabsorption resulting from the decrease or absence of the enzyme, lactase, may be classified into one of three general categories: congenital, secondary or primary (13, 49). Congenital absence of lactase is a rare disorder that is exemplified immediately after birth with the ingestion of lactose. Fermentative diarrhea and failure to thrive result until lactose is removed from the diet. Lactose-free formulas are now readily available for adequate nutritional care of these infants.

Secondary lactose malabsorption results from a depression of lactase activity due to intestinal mucosal damage which may accompany disorders of the intestinal tract and other diseases such as celiac disease, cystic fibrosis, ulcerative colitis or malnutrition. This condition is more common and may result in only a temporary removal of lactose from the diet.

Primary lactose malabsorption describes the condition that becomes evident when an individual reaches an age when lactase activity decreases. Lactase production begins during the third month of gestation,

and in some ethnic groups lactase activity remains consistently high throughout life. In other races, low levels of lactase occur during infancy or early childhood.

Age of Onset

Primary lactose malabsorption appears to be very rare in healthy infants, even in populations where low lactase levels are prevalent after weaning. Studies of Ugandan Bantu and Australian aboriginal children indicate that at least 80 percent of those studied between the ages of three and five years had lactose maldigestion (22, 31). In Jamaican children tested, 70 percent under four years of age had flat lactose tolerance curves (100). Seventy-five percent of Peruvian children studied demonstrated an inability to digest a lactose load by the age of three years and 87 percent of 46 normal Thai children over two years of age were lactose intolerant (83, 54). Johnson and colleagues (49) state it is generally agreed that those people who are lactose non-digesters as adults actually lost their capacity to digest lactose effectively by three to five years of age. Other studies have shown the incidence of lactose malabsorption increases with age (4, 46, 60, 87).

Incidence of Lactose Malabsorption

The occurrence of primary lactose malabsorption has been studied in many ethnic groups and across the world more people are lactose malabsorbers than absorbers (4, 49). Table I shows a comparison in ethnic groups of the incidence of lactose malabsorption as determined by abnormal lactose tolerance tests or intestinal biopsies. When the geographical origins of ethnic groups are considered, the occurrence of lactose

TABLE I

LACTOSE MALABSORPTION AMONG ETHNIC GROUPS

Population	Locale of Study	Number of Subjects	Age Range	Percent Malabsorbers LTT	Percent Malabsorbers Biopsy	Reference
African Descendants						
African	Kenya	45	Children	62		70
American Negro	Maryland	98	22-72 yrs	81		7
American Negro	Maryland	41	14-78 yrs	73		25
American Negro	Illinois	24	Adults		75	69
American Negro	Oklahoma	22	Adults	77		103
American Negro	Maryland	20	18-54 yrs	75	70	6
American Negro	Maryland	20	11 mos-11 yrs	30		46
American Negro	Oklahoma	11	11-53 yrs	100		104
Baganda	Uganda	14	3-9 yrs	86		22
Baganda (Bantu)	Uganda	35	11-70 yrs	89		24
Bahima	Uganda	11	6-53 yrs	9		24
Bahutu and Nilotic	Uganda	11	8-60 yrs		64	24
Bantu	South Africa	22	Adults	91		47
Bantu	Uganda	22	5-70 yrs		95	24
Batutsi (Hamitic)	Uganda	7	10-50 yrs		14	24
Bush Negroes	Surinam	29	Adults	100		71
Fulani	Nigeria	33	Over 4 yrs	58		58
Hausa	Nigeria	17	Over 4 yrs	76		58
Hausa/Fulani	Nigeria	15	17-60 yrs	60		81
Ibos	Nigeria	11	13-38 yrs	82		81
Mixed Tribes	Nigeria	9	18-49 yrs	100		81
Sephardi (Morocco, Tripoli, Tunis)	Israel	32	19-65 yrs	63		35
Yoruba	Nigeria	48	13-70 yrs	83		81
Yoruba	Nigeria	41	Over 4 yrs	98		58

TABLE I (Continued)

Population	Locale of Study	Number of Subjects	Age Range	Percent Malabsorbers LTT	Percent Malabsorbers Biopsy	Reference
Asian Descendants						
Chinese	Australia	30	Adults	90		11
Chinese (Australian-born)	Australia	34	13-34 yrs	56		12
Chinese, Filipino	Maryland	20	23-38 yrs	95		45
Chinese, Japanese, Korean	Minnesota	11	23-39 yrs		100	21
Chinese, Malays, Indians	Singapore	11	1-3 yrs	36		14
Chinese, Malays, Indians	Singapore	14	3-5 yrs	21		14
Chinese, Malays, Indians	Singapore	14	5-7 yrs	50		14
Chinese, Malays, Indians	Singapore	17	7-9 yrs	70		14
Chinese, Malays, Indians	Singapore	14	9-15 yrs	93		14
Chinese, Malays, Indians	Singapore	22	15-42 yrs	100		14
Indian	India	54	7 mos-7 yrs	41		87
Indian	India	22	21-62 yrs		54	28
Indian	India	18	20-40 yrs	61		87
Indian	Australia	5	32-39 yrs	80		10, 11
Japanese	Japan	25	15-64 yrs		92	93
New Guinea Natives	Australia	8	14-30 yrs	100		10, 11
Oriental (Persia, Syria, Lebanon)	Israel	20	24-64 yrs	85		35
Thai	Thailand	140	Adults	97		55
Thai	Thailand	126	6 mos-2 yrs	44		54
Thai	Thailand	75	Adults	100		33
Thai	Thailand	46	30 mos-8 yrs	87		54
Thai	Thailand	39	13-72 yrs	100		101
Thai	Thailand	24	4-12 yrs	100		33
Thai	Thailand	13	Under 4 yrs	54		33

TABLE I (Continued)

Population	Locale of Study	Number of Subjects	Age Range	Percent Malabsorbers LTT	Percent Malabsorbers Biopsy	Reference
Australian Descendants						
Aborigines	Australia	44	1 mos-15 yrs	91		31
Eastern European Descendants						
Ashkenazi Jewish (Polish, Russian, Rumanian, et al)	Israel	53	20-70 yrs	79		35
Czechoslovakians	British Columbia	17	17-65 yrs	18		62
Finnish	Finland	159	21-65 yrs	17		50
Poles	British Columbia	21	17-65 yrs	29		62
Western European Descendants						
American Caucasian	Oklahoma	145	3-80 yrs	19		103
American Caucasian	Minnesota	100	20-63 yrs		6	79
American Caucasian	Illinois	93	Adults		19	69
American Caucasian	Maryland	59	19-66 yrs	12		7
American Caucasian	Thailand	58	Adults	24		55
American Caucasian	Washington, D. C.	50	19-42 yrs		16	94
American Caucasian	Maryland	20	11 mos-11 yrs	5		46
American Caucasian	Maryland	20	18-54 yrs	10	5	6
American Caucasian	Maryland	19	14-78 yrs	16		25
American Caucasian	Oklahoma	11	8-48 yrs	45		104
Australian Caucasian	Australia	100	18-40 yrs	6		15
Australian Caucasian	Australia	23	Adults	0		10, 11
Caucasian	Alaska	12	Adults	25		30
Caucasian	Thailand	9	Adults	0		33

TABLE I (Continued)

Population	Locale of Study	Number of Subjects	Age Range	Percent Malabsorbers LTT	Percent Malabsorbers Biopsy	Reference
Western European Descendants (Continued)						
Danish	Denmark	700	Adults	3		38
Danish Eskimos	Greenland	7	13-75 yrs	14		39
Dutch	Surinam	14	Adults	36		71
English	England	67	Adults		22	74
Swiss	Switzerland	17	20-62 yrs		6	40
Mediterranean Descendants						
Cretans	Greece	50	15-78 yrs	56		51
Egyptian Arab	Egypt	14	13-65 yrs		93	41
Greek	Greece	600	15-78 yrs	45		51
Greek Cypriots	Greece	50	15-78 yrs	66		51
Greek Cypriots	England	17	Adults	88		74
Iraqis (Iraq)	Israel	38	17-65 yrs	84		35
Israeli Arab	Israel	67	20-80 yrs	81		36
Israeli Jewish	Israel	93	Adults	61		92
Sephardi (Turkey, Greece, Bulgaria)	Israel	36	17-69 yrs	72		35
Yemenites (Yemen)	Israel	36	20-70 yrs	44		35
Western Hemisphere Descendants						
American Indian	Oklahoma	36	18-57 yrs	81		17
American Indian	Oklahoma	5	3-22 mos	0		17
American Indian	Oklahoma	3	Adults	67		103

TABLE I (Continued)

Population	Locale of Study	Number of Subjects	Age Range	Percent Malabsorbers LTT	Percent Malabsorbers Biopsy	Reference
Western Hemisphere Descendants (Continued)						
Canadian Indians	British Columbia	30	14-24 yrs	63		65
Chami Indians	Columbia	24	15-55 yrs	100		2
Eskimos	Alaska	27	7-14 yrs	70		8
Eskimos	Greenland	25	13-75 yrs	88		39
Indians and Eskimos	Alaska	26	Adults	92		30
Jamaican	Jamaica	20	Under 4 yrs	70		100
Mexican	Mexico	193	13-17 yrs	77		68
Mexican	Mexico	108	13-21 yrs	69		68
Mexican	Mexico	100	18-72 yrs	74		68
Mexican-American	Oklahoma	33	9-60 yrs	48		97
Mexican-American	Texas	11	19-57 yrs		55	29
Peruvian	Peru	30	20-54 yrs	66		32

non-digestion can be readily summarized. Studies show a high incidence (50 to 100 percent) among populations of Asia, the Mediterranean, Africa, and native people of Australia and the Western Hemisphere. Low incidences of lactose malabsorption (30 percent or less) are most prevalent in people of European extraction.

Hypotheses of Lactose Malabsorption

To explain the regional and ethnic differences in primary lactose malabsorption, four hypotheses have been proposed: genetic, culture historical, induction or adaptive and inhibition. The genetic hypothesis is supported by more medical researchers than the other hypotheses (5, 21, 24, 33, 46, 47, 69, 104). It proposes that racial dissimilarities in incidence of lactose malabsorption reflect the genetic differences among ethnic groups. Such genetic contrasts have been brought about by some genetic factor or by some unknown selective process that is not directly related to milk consumption.

Several studies have been presented that gave supportive evidence to the genetic hypothesis. Two simultaneous studies by independent researchers, one in the United States and the other in Africa, reported the incidence of lactose malabsorption was 70 percent in black Americans and 72 percent in black Africans (5, 24). Black Americans descended from black Africans over 300 years ago and although they have experienced different cultural patterns, their incidence of lactose malabsorption has remained almost identical to the native African. Huang and Bayless (46) found 14 out of 20 Negro children in their study had a family history of milk intolerance. The children were 11 months to 11 years old and 30 percent were lactose malabsorbers. Lactose tolerance

tests obtained by Welsh and colleagues (104) on 22 members of two white and two black families revealed similar results. Sixteen individuals, representing two generations, were intolerant to lactose.

A variation of the genetic hypothesis is the culture historical hypothesis (49). It postulates that during the early stages of human evolution man may have had a developmental pattern of lactase activity similar to that of most other land mammals. Blaxter (9) has shown the developmental pattern of lactase activity to be high at birth in the rat, pig, calf, rabbit, cat and dog, and then decline sharply at weaning to a low level for the remainder of life. Hence, it appears the culture historical hypothesis does not explain why some ethnic groups such as white Americans continue to have high lactase activity during adulthood. Simoons (96) explained such a change in lactase activity by referring to the history of dairying and milk use in different cultures. Dairying was believed to have begun in Mesopotamia around the middle of the fourth millennium BC (57, 96). If the cultures of the world are categorized according to their historical use or omission of foods containing lactose, and if this classification is compared to the incidence of adult lactose malabsorption in ethnic groups, then the change in the evolutionary pattern of the culture historical hypothesis may be apparent. Three types of cultures have evolved since the domestication of dairy animals:

1. Some cultures have never engaged in dairying and thus, milk products have never been included in significant amounts in the adult diet. These groups include the aborigines of Australia, natives of New Guinea and the American Indian all of whom have high incidences of lactose malabsorption (72).

2. Some cultures have raised dairy animals but have never included significant quantities of dairy foods in their adult diets. Various reasons for not consuming milk products have been given, such as the cultural disdain that it was wrong to take milk that was intended for the young animal, or that one takes on the characteristics of the animal from which the milk was derived. In tropical regions, infestation of the tsetse fly-borne trypanosomiasis destroys dairy animals and is therefore a limiting factor for the production of milk products. Chinese, Thais, Filipinos and most African Negroes are some of the ethnic groups of this cultural pattern (72). As with the first cultural pattern, these groups have high incidences of lactose malabsorption.

3. Over several centuries some cultures have engaged in dairying, and foods containing lactose have been a significant component of their adult diets. Included in this pattern are pastoral tribes of Africa and most Europeans with their descendants all of whom have low incidences of lactose malabsorption (72).

Cook and Kajubi (24) interpreted their findings in two tribes in Africa to be the result of inherited differences and not due to cultural influences. The Baganda tribe with an incidence of 89 percent lactose malabsorption have a diet consisting mainly of vegetables and bananas. Their milk consumption is less than one-half cup per day. In contrast, the Bahima tribe consume four to fourteen cups of milk per day and little else. Only nine percent of the Bahima subjects were lactose malabsorbers. Other researchers interpret these results as support for the adaptive hypothesis (13).

In 1965, Cuatrecasas and colleagues (25) proposed the induction or adaptive hypothesis to account for differences in primary lactose

intolerance in adults. Bolin and Davis (13) also supported this hypothesis which stated that if an individual continued to consume dairy products after weaning, dietary lactose, the substrate for lactase, would induce the lactase activity in the small intestine to continue at the high levels prevalent in infancy. Therefore, this hypothesis implied lactose tolerance would result with continued milk drinking throughout life and in opposition intolerance would be associated with discontinuance of milk over a period of time.

A study of 60 patients, 41 of whom were Negro, showed a good correlation between milk consumption and lactose absorption (25). Eighty-seven percent of the milk drinkers were lactose absorbers and only 14 percent of non-milk drinkers were absorbers. However, this research indicated other factors must have an influence for the adaptive hypothesis was not inclusive for all people. An attempt was made to induce lactose absorption in seven of the malabsorbers by giving them 150 grams per day of lactose (equivalent to lactose in approximately three quarts of milk) for up to 45 days. The results showed there was no increase in lactose absorption or jejunal lactase activity. In comparison, two absorbers were deprived of lactose four to five months and showed a decline in enzyme activity.

In a similar study there was no significant change in the level of intestinal lactase in four persons on a lactose-free diet for two months and in another person who was fed a diet consisting of 30 percent lactose for 14 days (91). In Thailand, 50 lactase-deficient males were fed 50 grams of lactose for one month and showed no change in their ability to tolerate lactose (55). In contrast, Kogut and associates (56) determined that nine galactosemic children who had not consumed lactose for

7 to 17 years had normal levels of intestinal lactase. These studies indicate that the intestinal lactase level is independent of the amount of lactose consumed.

Another hypothesis, the inhibition of lactase by drugs or foods, was advanced in an attempt to help explain the ethnic differences in lactose absorption. This hypothesis is based on the observation that the drug colchicine inhibits lactase activity in the rat (43). Some foods and other drugs may contain similar lactase-inhibiting substances and the variation in ethnic food patterns may result in racial differences of lactose absorption. This hypothesis can only be considered speculative since very little supportive research has been reported.

Diagnostic Procedures

Several procedures are available to the clinician for assessing lactose malabsorption. The purpose of all diagnostic methods is to determine the amount of lactase activity in the intestinal tract that is available for the hydrolysis of lactose. Therefore it is necessary for a person to fast for eight to twelve hours before a dose of lactose is given in order for test results to indicate only lactose absorption or malabsorption. The oral lactose tolerance test (LTT) is a good screening procedure and the most frequently used to determine lactase activity. It is a biochemical test used to determine the rise in blood glucose following the ingestion of a test dose of lactose. The amount of lactose usually given is 2 gm/kg of body weight or 50 gm/square meter of body surface for children and 1 gm/kg of body weight for adults (49, 102). Capillary blood samples are taken at 0, 30, 60 and 120 minutes. Blood glucose is determined by one of the following methods:

Nelson-Somogyi, glucose oxidase or ferricyanide adapted to the Auto Analyzer (78, 73, 44). A rise in blood glucose over the fasting level of less than 20 mg/100 ml plus the presence of symptoms such as abdominal pain, flatulence or diarrhea are evidences of lactose malabsorption.

A modification of the lactose tolerance test is the administration of ethanol simultaneously with the lactose test dose (80). Ethanol inhibits the enzyme UDP galactose-4-epimerase which converts galactose to glucose (52). Therefore, blood galactose increases after lactose and ethanol ingestion if the lactose is hydrolyzed by lactase. An increase of blood galactose of less than 10 mg/100 ml indicates low lactase activity. The blood galactose reaches a peak in 45 to 60 minutes. It is proposed that a single blood galactose measurement at 45 minutes after a small lactose-ethanol test dose would accurately distinguish between absorbers and malabsorbers of lactose (52).

A noninvasive variation of the lactose tolerance test is the rating of the severity of symptoms such as diarrhea, flatulence, bloating and cramps that may occur following a lactose load. After a 50-gram dose of lactose, Stephenson and Latham (99) determined a subject's "lactose score" by the number and severity of symptoms that occurred within eight hours. Each symptom was rated by the subject as mild, moderate or severe. This procedure is not very reliable for it depends on the subjective estimate of the subject in determining the severity of symptoms.

The most reliable diagnostic procedure for determining lactase deficiency is the enzymatic analysis of a biopsy of the small intestine. A specimen of intestinal mucosa can be obtained using a peroral intestinal biopsy capsule attached to polyethylene tubing (90). The capsule

is swallowed by the subject and its position in the gastrointestinal tract can be determined by a fluoroscope. When the capsule reaches the ligament of Treitz at the junction of the duodenum and jejunum, a sample of intestinal mucosa is obtained (69). The specimen can be retrieved via the tubing so more than one biopsy can be taken while the capsule is in position. The lower limit for normal lactase activity ranges from 0.5 to 2.0 units per gram of mucosa (49). The sucrase to lactase ratio can also be determined from an intestinal biopsy to help interpret lactase activity. This ratio is 4:1 or less in normal individuals.

The intestinal biopsy may present several undesirable circumstances while attempting to obtain a specimen. Sheehy and Anderson (94) listed the following: inability of the subject to swallow the capsule, failure to obtain tissue even though the capsule fired, broken tubing in an area chewed by the patient with non-retrieval of the capsule by mouth, failure of the capsule to fire, gastric or duodenal mucosa obtained instead of jejunum. Therefore, this procedure requires well-trained personnel.

The excretion of breath hydrogen as determined by gas chromatography is a very sensitive and reliable technique for determining lactose malabsorption (16, 20, 34, 42, 75, 80). It is a non-invasive method that lends itself to field work and to the study of subjects of all ages. The basis of this test was discussed in Chapter I and the test procedures are described in Chapter III.

Another breath analysis that may be used in diagnosing lactose malabsorption is the measurement of $^{14}CO_2$ by liquid scintillation spectroscopy after the ingestion of 1-^{14}C lactose (80). If the 1-^{14}C lactose is hydrolyzed, 1-^{14}C glucose is absorbed and then oxidized to $^{14}CO_2$ by

tissue enzymes. The rationale of this procedure is the decreased hydrolysis of lactose causes decreased glucose absorption and hence, decreased breath $^{14}CO_2$. One disadvantage of this method is that bacterial enzymes can also convert ^{14}C lactose to $^{14}CO_2$. Therefore, unabsorbed lactose in the colon will also generate $^{14}CO_2$ (80).

A less frequently used means in determining intestinal lactase activity is the interpretation of an abdominal radiograph taken one hour after the individual ingests a lactose-barium sulphate mixture (59). The radiograph of a lactase-deficient person indicates dilution of the barium and dilation of intestinal loops.

In infants a semi-quantitative test for lactose malabsorption is the determination of the pH and reducing substances in the feces after the consumption of a lactose test meal (53). Acid stools indicate the presence of lactic acid which was produced when unabsorbed lactose was fermented. Fecal-reducing substances result when unhydrolyzed lactose is excreted by infants with lactose malabsorption. This quick procedure is not of value in adults because the unabsorbed lactose is consumed by the bacteria in the large intestine (26).

CHAPTER III

METHODS AND PROCEDURE

The excretion of breath hydrogen resulting from nonabsorbed lactose was determined in 60 subjects who were seven-eighths to full-blooded Oklahoma Indians and ranged in age from 3 to 64 years. A brief family history was obtained to verify each subject's Indian heritage (see Appendix A, page 43). The subjects were classified into six age categories: preschool (3 to 5 years; mean 4.8 years), school age (6 to 12 years; mean 9.5 years), adolescence (13 to 19 years; mean 15.5 years), young adult (20 to 30 years; mean 24.5 years), adult (31 to 44 years; mean 36.2 years) and older adult (45 to 64 years; mean 54.7 years). The age of each individual was determined by his birthday in 1975. There were five males and five females in each group. No restriction was made as to tribe or kinship. A signed consent form was obtained from each subject prior to testing (see Appendix A, page 38). All participants were healthy and had no recent history of gastrointestinal disturbance or antibiotic usage. This was determined by a questionnaire taken on the day of testing (see Appendix A, page 40).

Two identical tests for each subject were given two to seven days apart resulting in a total of 120 tests for the 60 participants. After 12 hours of fasting, a basal breath sample was taken before the consumption of five milliliters of reconstituted nonfat dry milk (containing approximately 0.25 grams lactose) per kilogram of body weight. Nine

breath samples were obtained at 15-minute intervals from 60 to 180 minutes and two breath samples were collected at 30-minute intervals from 180 to 240 minutes following the test meal (see Appendix A, page 44). Breath samples were collected in multi-laminar bags and analyzed within 24 hours by a gas chromatograph equipped with a helium ionization detector (34). Each subject was instructed to inhale only through the nose and exhale slowly via the mouth into the collection apparatus. One-way valves were used for children five years of age and younger to prevent rebreathing of breath samples. A lactose-free snack of thin-sliced toasted French bread with half an ounce of jelly and decaffinated coffee, tea or water was given at 60 minutes. At 180 minutes, a snack consisting of a hard-cooked egg, whole wheat toast, one ounce of jelly and one of the aforementioned beverages was given. A limited amount of the same beverages was permitted upon request during the test period.

The average of the three highest consecutive hydrogen concentrations (ppm - parts per million) between 60 and 180 minutes which included the maximum reading was used as the response factor for each subject. These values were statistically analyzed in a split-plot design (98). The main plot treatments were in a 6 X 2 factorial having six age groups and two sexes. The mean response of the two identical tests for each subject was used to determine the incidence of malabsorption. This design made it possible to determine the effects of age and sex on lactose malabsorption.

A history of lactose consumption was obtained from each subject in order to study the effect of routine milk intake on lactose malabsorption (see Appendix A, page 42). The estimated daily lactose consumption was determined for each subject one month prior to testing.

CHAPTER IV

RESULTS

The basal breath sample taken before the test meal helped acquaint the subjects with the breath collection procedures and also served as a check that each subject had fasted for a sufficient period of time. The average basal breath hydrogen concentration (ppm) for participants was six ppm (S.D.$\pm$7.1), suggesting that subjects did fast.

Based on the results of a control population, 20 ppm of breath hydrogen was selected as the upper limit for the response in lactose absorbers (76). Thirty-six of the 60 subjects (60 percent) were classified as lactose malabsorbers since they had a response factor of 20 ppm or greater of breath hydrogen. Nineteen were females and 17 were males indicating that malabsorption, considering all age groups, was not related to sex.

The incidence of malabsorption by age groups is shown in Table II. Only two of the ten females under 13 years, age five and twelve, were malabsorbers, and only one of the ten males under 13 years, age five, was a malabsorber. The breath hydrogen responses for subjects from 6 to 19 years of age indicate that lactose malabsorption becomes very evident during adolescence in Native North Americans. Approximately 82 percent (82.5) of subjects who were 13 years and older were lactose malabsorbers, and 90 percent of those who were 31 years and older were malabsorbers. Table IV (Appendix B, page 46) shows the breath hydrogen

TABLE II

INCIDENCE OF MALABSORPTION IN EACH AGE GROUP[+]

Age Group	Number of Subjects[#]	Mean Response Factor[*]			Number of Malabsorbers[**]		Percent of Malabsorption[++]
		Males	Females	Average	Males	Females	
3-5 years	10	12.8	11.5	12.1	1	1	20
6-12 years	10	5.0	16.7	10.9	0	1	10
13-19 years	10	38.6	23.8	31.2	5	2	70
20-30 years	10	36.7	50.5	43.6	3	5	80
31-44 years	10	43.6	68.0	55.8	4	5	90
45-64 years	10	53.7	47.5	50.6	4	5	90
		31.7[@]	36.3[@]	34.0[@@]			

[+] Average of test 1 and test 2.
[#] Half females and half males.
[*] Average of the three highest consecutive hydrogen concentrations (ppm) between 60 and 180 minutes which included the maximum reading.
[**] Malabsorption determined by a response factor of greater than 20 ppm of breath hydrogen.
[++] Indicates percent of malabsorption for all subjects in age group.
[@] Mean response factor for all subjects of that sex.
[@@] Mean response factor for all subjects.

response for each individual for test 1, test 2 and the mean of the two tests. Statistically there was no significant difference between the results of test 1 and test 2, that is the repeat testing of each individual. Only three subjects (5 percent) would have been classified differently if only one test had been administered to each subject.

Very few symptoms associated with lactose intolerance such as abdominal pain, flatulence and diarrhea were experienced by the participants during the breath hydrogen test. Only eight subjects had symptoms of flatulence and one had diarrhea. The remaining subjects were asymptomatic.

Twelve subjects (20 percent) related that after drinking milk they experienced symptoms associated with lactose intolerance. This was usually after one to two cups of milk. Fourteen subjects no longer drank milk, but consumed lactose in other dairy products. Table III shows a comparison of the estimated daily lactose ingested from all milk products one month prior to testing for absorbers and malabsorbers. Fifteen out of 36 (42 percent) malabsorbers consumed more lactose than some of the absorbers in their respective age categories.

TABLE III

ESTIMATED LACTOSE CONSUMPTION ONE MONTH PRIOR TO TESTING

Age Group	Number of Absorbers	Lactose Consumption Per Day (grams)			Number of Malabsorbers	Lactose Consumption Per Day (grams)		
		Range	Mean	Standard Deviation		Range	Mean	Standard Deviation
3-5 years	8	8.2-37.2	22.8	±10.1	2	4.0-20.9	12.5	±12.0
6-12 years	9	11.1-51.4	25.3	±12.0	1	0	40.9	0
13-19 years	3	2.0-12.7	6.8	± 5.4	7	8.1-38.7	22.9	±10.2
20-30 years	2	48.7-76.0	62.4	±19.3	8	0.6-46.6	15.5	±16.0
31-44 years	1	0	2.7	0	9	1.2-27.1	11.5	±11.2
45-64 years	1	0	92.0	0	9	1.9-53.2	19.5	±18.5

CHAPTER V

DISCUSSION

The results of this study show with increasing age from adolescence to adulthood the incidence of lactose malabsorption becomes more prevalent among Native Americans. Other researchers have presented evidence which indicates low lactase levels are the norm in the majority of adults in many populations (4, 26, 35, 49, 86). The findings in Native American children in this study did not support the statement that those people in ethnic groups who are lactose nondigesters as adults actually lose their capacity to digest lactose effectively by three to five years of age (49). Only three of twenty children (15 percent) under the age of 12 years were lactose malabsorbers.

Forty-two percent of the malabsorbers in this study consumed as much lactose or more per day than some of the absorbers in their respective age groups. Hence, it appears these Native Americans (malabsorbers) did not give support to the induction hypothesis which states if a person continued to consume dairy products after weaning, dietary lactose would induce lactase activity in the small intestine and this would continue at the high level typical of infancy (13, 25). The result therefore would be an adult lactose absorber.

On the basis of breath hydrogen tests in controls, 20 ppm was taken as the upper limit for lactose absorbers (76). In comparison, subjects known to be malabsorbers had a greater response following the

consumption of the same lactose test meal with little or no physical discomfort (34). Lactose in milk is more realistic than lactose in water and the small dose made it possible to distinguish lactose malabsorbers without inducing physical symptoms associated with nondigested lactose.

The breath hydrogen test has many advantages over other diagnostic procedures for lactose malabsorption. It is easy to administer, requires no sterile procedures (other than the mouthpiece used by the subject) or refrigeration of samples and causes little or no discomfort to the subject. For these reasons the breath hydrogen test was well suited for testing a large number of Native Americans. It can be used in the field as conveniently as in a laboratory. This was certainly true in this study in which over three-fourths of the samples were collected 25-30 miles from the laboratory. The equipment and necessary materials for conducting the tests were set up in a temporary lab in about 20 minutes. The breath samples were returned to the laboratory for analysis.

Other diagnostic tests that require blood sampling cause apprehension and fright in young children. Therefore, many Indian children were willing to participate in this study when they knew it was a noninvasive technique. Children as young as three years of age enjoyed "blowing up the bag."

Summary

Breath hydrogen excretion was used to determine lactose malabsorption in 30 healthy females and 30 healthy males between the ages of 3 and 64 years who were at least seven-eighths Native American. The test meal consisted of five milliliters reconstituted nonfat dry milk (0.25

grams lactose) per kilogram of body weight. A history of lactose consumption was obtained from each subject in order to study the effect of routine milk intake on lactose maldigestion.

Thirty-six of the 60 subjects (60 percent) were classified as lactose malabsorbers since they had response factors of 20 ppm or greater of breath hydrogen. Only 3 of 20 children (15 percent) who were under the age of 12 years were nondigesters of the small lactose dose used in this study. Approximately 82 percent (82.5) of subjects who were 13 years and older were lactose malabsorbers. Adolescence appears to be the period in which malabsorption of lactose becomes evident in Native North Americans.

Forty-two percent of the malabsorbers consumed as much lactose or more than some absorbers in their respective age categories. Hence, it appears the consumption of dairy products had little effect on the lactase activity of Native Americans in this study.

Recommendations for Future Research

1. Quantitate lactose not absorbed during breath hydrogen test in order to determine degree of lactase activity.

2. Test different dose levels with children to determine if 0.25 grams lactose per kilogram is an adequate dose to distinguish malabsorbers in young children.

3. Determine transit time of lactose in milk versus lactose in water with the breath hydrogen test.

4. Determine if age-related doses of lactose would be more realistic than weight-related doses.

5. Determine the dilutional effect of hyperventilation during the

breath hydrogen test.

6. With the use of the breath hydrogen test, test different foods to determine their dietary fiber content.

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APPENDIX A

STATEMENT OF INFORMED CONSENT

Development of Carbohydrate Malabsorption

OKLAHOMA STATE UNIVERSITY

I hereby acknowledge that I have been informed of the nature of the research for which I am to participate as part of the project, Development of Carbohydrate Malabsorption, as follows:

I. Statement of procedures and identification of those which are experimental:

You will arrive about 8:30 a.m. after having a good night of sleep and no food or drink (except water), since 9:00 p.m. the previous evening. Testing will start approximately 20 to 30 minutes after the arrival in the laboratory. You should become familiar with the surroundings and feel relaxed and comfortable in the lab and lounge area. Please feel free to ask the person or persons conducting the study any questions that may concern you or that may have aroused your curiosity.

The study will start by collecting a breath sample. This is done by blowing three breaths into a bag. Next you will eat or drink food or milk containing a known amount of sugar. One hour later another breath sample will be taken. Breath samples will be taken every 15 minutes after the first hour for the next three hours.

To avoid any discomfort from hunger during the time of the experiment a serving of toast and jelly will be given after the first hour, and a hard cooked egg and French bread after the third hour. A diet and family history will be taken sometime during the test period.

You will need to remain in the area of the lounge during the whole testing period unless other arrangements have been made previously.

II. Description of discomforts:

There should be few if any discomforts experienced. If you are intolerant to the sugar given you may experience mild stomach cramps, gas and diarrhea. These discomforts should last only a short time, $1\frac{1}{2}$ to 2 hours. With this new method to determine if a person is lactose intolerant, we give a very small amount of the lactose sugar as it occurs naturally in milk so that one will experience few or possibly no symptoms at all. If any of the above discomforts are experienced, please tell the person giving the test so she may record these symptoms.

INITIALS: _____

Statement of Informed Consent
Development of Carbohydrate Malabsorption
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III. Descriptions of benefits to be expected:

This test establishes whether or not the individual is tolerant to milk sugar. This should be valuable information to the person, as he will be able to avoid future discomfort which may arise due to lactose intolerance. The test may indicate the presence of diabetes (breath acetone).

IV. I have been given an opportunity to ask and receive answers to any questions concerning procedures.

V. I have been informed that I am free to withdraw my consent and to discontinue participation at any time. Furthermore, I agree that there has been no attempt, either written or oral, to get me to waive any of my legal rights or to hold any person or other entity blameless except as provided by law.

VI. I hereby give my informed consent to participate.

_____ _____

Date Signature

VII. I hereby give my informed consent for my child to participate.

_____ _____

Date Signature

QUESTIONNAIRE
(Ask before test)

NAME: _____ ID NUMBER: _____

ADDRESS: _____ HOME PHONE: _____

DATE: _____ OFFICE PHONE: _____

SEX: _____ BIRTH YEAR: _____

Some of these questions may be of a personal nature, please answer them truthfully and to the best of your ability because the validity of our data depends on how you answer the following questions. All information on this sheet will be confidential.

Have you eaten any food or had any drink with the exception of water in the last 12 hours? If so when? _____

What time did you get out of bed this morning? _____

Are you a diabetic? If yes, what medication do you take? _____

Have you been ill during the past seven days? _____

If yes, how many days since you have been feeling better? _____

Have you taken any of these drugs in the last ten days? If yes, name drug please.

Antibiotics: _____ When last taken? _____

Sulfa drug: _____ When last taken? _____

Other: _____ When last taken? _____

Have you had an emotional upset recently? For example a death in the family, fight with a spouse or boy friend (girl friend), car accident, failed a test, unwanted pregnancy, or lost a large amount of money? _____

Are you taking any unprescribed drugs? If yes, what kind. _____

Are you taking cold capsules? If yes, please name them. _____

Are you taking antihistamines? If yes, please name them. _____

QUESTIONNAIRE
Page 2

ID NUMBER: _____

DATE: _____

STUDY: _____

Have you taken any of the following in the last 48 hours:

1) Aspirin _____ When? _____
2) Cope _____ When? _____
3) Mydol _____ When? _____
4) Vanquish _____ When? _____
5) Darvon _____ When? _____

Do you use any of the following tobaccos:

Cigarette: _____ How many packs/week? _____ How long? _____

Cigar: _____ How many cigars/week? _____ How long? _____

Chewing: _____ How many plugs/week? _____ How long? _____

Snuff: _____ How many tins/week? _____ How long? _____

Pipe: _____ How many pouches/week? _____ How long? _____

Have you had any of the following gastrointestinal problems during the last two weeks?

Diarrhea: _____ Severity? _____ When? ____(days ago)

Constipation: _____ Severity? _____ When? ____(days ago)

Other: _____ Severity? _____ When? ____(days ago)

Are you having your monthly period at this time? _____

What day of your period is it? _____

Do you notice any discomfort during your period having to do with the:

Stomach? _____ When? _____

Intestine? _____ When? _____

Have you ever had any pulmonary diseases such as emphysema or TB? _____

Are you taking birth control pills? _____

How long have you taken the birth control pills? _____

Comments: _____

_____

LACTOSE AND FAMILY HISTORY

NAME: _____ ID NUMBER: _____

DATE: _____ SEX: _____

1. How much milk do you drink each day?

   _____ Less than 1 cup of milk
   _____ 1 cup of milk
   _____ 2 cups of milk
   _____ 3 cups of milk
   _____ More than 3 cups of milk

2. Did you drink milk when you were in high school? _____

3. Did you drink milk when you were in grade school? _____

4. How old were you when you stopped drinking milk? _____

5. When you drink milk does it cause any symptoms? _____

   _____

6. Approximately how many cups of milk would cause you to experience any discomfort after drinking milk? _____

7. Which of the following foods do you eat:

		How Many Times In The Past Month?	Size of 1 Serving
_____	Buttermilk	_____	_____
_____	Cottage Cheese	_____	_____
_____	Other Cheese	_____	_____
_____	Cream Soups	_____	_____
_____	Ice Cream or Ice Milk . . .	_____	_____
_____	Gravy made with milk . . .	_____	_____
_____	Malts or Milkshakes	_____	_____
_____	Milk on Cereals	_____	_____
_____	Milk-Based Diet Drinks . .	_____	_____
_____	Puddings or Custards . . .	_____	_____
_____	Snack Dips	_____	_____
_____	Sour Cream	_____	_____
_____	Yogurt	_____	_____

LACTOSE AND FAMILY HISTORY
Page 2

ID NUMBER: _____

DATE: _____

STUDY: _____

8. Are you allergic to any foods? Please list. _____

_____

_____

FAMILY HISTORY

Trace your family history back two generations. Please list the name, tribe and Native American percentage of each family member. Include adopted and half brothers and sisters. Example: Mary Wolfe--Creek 4/4.

MATERNAL GRANDPARENTS

_____

_____

PATERNAL GRANDPARENTS

_____

_____

PARENTS

_____

_____

CHILDREN

_____

_____

_____

_____

_____

_____

DATA SHEET

NAME: _____ BAG COLOR CODE: _____

ID NUMBER: _____ HEIGHT: _____

DATE: _____ WEIGHT: _____

ARRIVAL TIME: _____ AGE: _____

TIME MILK INGESTED: _____ SEX: _____

ML. OF MILK GIVEN: _____ PERCENT INDIAN: _____

DOSAGE AND SUGAR: _____ TRIBE: _____

STUDY: _____ BIRTH YEAR: _____

BAG #	TIME	MINUTES	ppm H_2	COMMENTS
		00		
		60		
		75		
		90		
		105		
		120		
		135		
		150		
		165		
		180		
		210		
		240		

How did you feel during the test? _____

_____

_____

APPENDIX B

TABLE IV

TEST RESULTS FOR EACH SUBJECT

Subject Number	Age	Response Factor* Test 1	Response Factor* Test 2	Average Response Factor Tests 1 and 2
35	3	4.0	4.0	4.0
13	5	38.0	7.7	22.8
26	5	5.7	2.7	4.2
34	5	5.3	10.7	8.0
46	5	9.3	8.3	8.8
60	5	3.7	15.3	9.5
61	5	14.0	5.0	9.5
62	5	35.7	28.7	32.2
67	5	6.0	9.3	7.7
69	5	16.0	14.0	15.0
50	6	4.3	6.7	5.5
23	7	13.0	8.3	10.7
18	8	1.3	1.7	1.5
22	9	7.7	6.0	6.8
19	10	3.3	1.3	2.3
28	10	16.0	13.3	14.7
16	11	3.0	7.0	5.0
38	11	10.7	8.3	9.5
41	11	6.7	17.3	12.0
48	12	50.7	30.3	40.5
30	13	11.3	17.0	14.2
37	14	34.7	68.0	51.3
47	14	41.0	35.7	38.3
57	14	20.3	29.3	24.8
31	15	5.3	4.0	4.7
33	16	35.3	33.3	34.3
58	16	49.3	31.7	40.5
68	16	27.7	48.3	38.0
63	18	41.7	50.7	46.2
64	19	25.0	14.3	19.7
36	20	33.7	34.0	33.8
53	20	80.0	55.0	67.5
65	21	70.3	87.0	78.7
10	22	12.7	8.3	10.5
24	24	73.0	59.0	66.0
59	24	5.7	5.7	5.7
12	26	7.3	43.0	25.2
49	28	34.3	20.3	27.3
14	30	51.3	65.3	58.3
43	30	61.0	64.7	62.8
42	31	34.3	52.3	43.3
25	32	97.3	80.3	88.8
40	33	82.3	108.0	95.2
39	34	53.3	62.7	58.0

TABLE IV (Continued)

Subject Number	Age	Response Factor* Test 1	Response Factor* Test 2	Average Response Factor Tests 1 and 2
27	35	74.3	35.0	54.7
20	37	14.0	3.0	8.5
11	38	48.3	21.0	34.7
44	39	62.0	49.0	55.5
51	41	55.7	49.7	52.7
21	42	53.7	79.3	66.5
15	45	42.3	84.3	63.3
29	47	5.0	9.7	7.3
52	48	87.0	103.7	95.3
66	48	48.7	31.0	39.8
17	56	37.7	68.7	53.2
55	57	71.3	70.0	70.7
32	60	15.3	24.7	20.5
45	61	24.7	35.3	30.0
54	61	61.3	73.3	67.3
56	64	59.3	58.3	58.8

*Response Factor: average of the three highest consecutive hydrogen concentrations (ppm) between 60 and 180 minutes which included the maximum reading.

VITA

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