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LACTOSE DIGESTION AND TOLERANCE AFTER WHOLE MILK AND LOW LACTOSE MILK PRODUCTS CONSUMPTION IN MIDDLE AGE PEOPLE

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Siriporn Wichito

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Milk is the major source of calcium, high quality protein and many other nutrients, including the unique disaccharide lactose that requires hydrolysis by a brush border enzyme, "lactase", before absorption. Populations with low lactase activity, which include Thai people, suffer discomfort from gastrointestinal symptoms when drinking milk. This in turn limits milk consumption among these lactose intolerants and most probably causes an undesirable effect to their bone health especially among the middle age and elderly. Previous studies in Thailand to study methods to overcome lactose intolerance, included having milk after meals, drinking milk with solid foods, reducing milk volume per serving, and having fermented milk products, were promising among Thai people. Before promoting milk consumption among middle age adults, who have a high risk of unhealthy bones, it is necessary to study their milk habit consumption, and status of lactose maldigestion and intolerance. In addition, Imitated low-lactose and lactose-free milk, which are formulated at the Institute of Nutrition, Mahidol University, are other choices for the lactose intolerants. Due to their higher cost, it is necessary to evaluate the effectiveness of the products for reducing lactose intolerance. In this study, data was collected among 322 Thai middle age adults, who were chosen by selective sampling, using a questionnaire. Seventy of these subjects were then tested for lactose maldigestion and intolerance.

The results showed that among 322 middle age adults (45-60 years), 81% consumed milk and about 1/3 of them drank milk regularly. Using breath hydrogen production as the indicator ($\geq$ 20 ppm) and 250-ml whole milk (containing 12 g lactose) as the test diet, 57% of the 70 studied subjects were found to be lactose maldigesters and 64% were lactose intorelants. Using the combined indicators of both hydrogen and methane production ($\geq$ 15 ppm), and the symptom of diarrhea, which directly indicates the presence of lactose in the colonic lumen, 83% of the subjects were identified the lactose maldigesters.

After replacing the 250-ml of whole milk in the 36 lactose maldigesters with 250-ml of low-lactose milk and lactose-free milk, all of them showed hydrogen production less than the cut-off point ($\geq$ 20 ppm), which indicated no lactose maldigestion. However, based on the combined indicators of hydrogen and methane production as well as the presence of diarrhea, 17 and 11 % of lactose maldigesters and 36% and 6% of lactose intolerants were found after low-lactose and lactose-free milk consumption, respectively. Mild gastrointestinal symptoms were found in lactose maldigesters after consumption of whole and low-lactose milk products. The symptom intensity scores were significantly different (whole milk>low-lactose milk>lactose-free milk). These results suggest that low-lactose products can reduce the degree of lactose maldigestion as well as the intensity of lactose intolerance. Due to the difference in the cost of the products, imitated low-lactose milk is, therefore, recommended for non-sensitive lactose intolerants and lactose-free milk for sensitive lactose intolerants.

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ศิริพร วิชิโต : ภาวะการย่อยน้ำตาลแลคโตสและอาการที่เกิดขึ้นภายหลังการดื่มนมและผลิตภัณฑ์นมแลคโตสต่ำในผู้ใหญ่วัยกลางคน (LACTOSE DIGESTION AND TOLERANCE AFTER WHOLE MILK AND LOW LACTOSE MILK PRODUCTS CONSUMPTION IN MIDDLE AGE PEOPLE) คณะกรรมการควบคุมวิทยานิพนธ์ : ประไพศรี ศิริจักรวาล, Ph.D. ประภาศรี ภูวเสถียร, Ph.D. วิสิฐ จะวะสิต, Ph.D. 105 หน้า. ISBN 974-04-0001-9

นมเป็นแหล่งที่ดีของสารอาหารต่างๆ รวมถึงแคลเซียมและโปรตีน แต่ในนมมีน้ำตาลแลคโตสซึ่งเป็นน้ำตาลโมเลกุลคู่ที่ต้องอาศัยเอนไซม์แลคเตสเพื่อทำการย่อยก่อนการดูดซึม คนที่มีเอ็นไซม์แลคเตสต่ำรวมทั้งคนไทยมักมีอาการไม่สบายท้องเกิดขึ้นหลังการดื่มนม ซึ่งเป็นข้อจำกัดในการดื่มนมโดยเฉพาะในผู้ใหญ่วัยกลางคนและผู้สูงอายุ ซึ่งอาจมีผลกระทบต่อภาวะและความแข็งแรงของกระดูกได้ การศึกษาที่ผ่านมา พบว่า มีวิธีที่สามารถช่วยลดอาการไม่สบายท้องหลังดื่มนม ได้แก่ การดื่มนมหลังอาหาร ดื่มนมพร้อมอาหารอื่น ลดปริมาณนมที่ดื่มในแต่ละครั้ง และการบริโภคผลิตภัณฑ์นมที่มีการย่อยสลายน้ำตาลแลคโตสโดยใช้จุลินทรีย์ ก่อนที่จะมีการส่งเสริมการดื่มนมในผู้ใหญ่วัยกลางคน ซึ่งมีความเสี่ยงต่อการเกิดภาวะกระดูกพรุน ควรที่จะต้องทำการศึกษาเกี่ยวกับบริโภคนิสัยในการดื่มนม ภาวะการย่อยน้ำตาลแลคโตส และอาการที่เกิดขึ้นภายหลังการดื่มนม สถาบันวิจัยโภชนาการมหาวิทยาลัยมหิดล ได้มีการผลิตผลิตภัณฑ์นมที่มีแลคโตสต่ำ เพื่อเป็นทางเลือกสำหรับผู้ที่มีอาการหลังดื่มนม เนื่องจากโดยวิธีนี้มีค่าใช้จ่ายสูงกว่าวิธีอื่น จึงจำเป็นจะต้องทำการศึกษาถึงการย่อยน้ำตาลแลคโตสและการเกิดอาการ หลังการบริโภคของผลิตภัณฑ์นมดังกล่าว

จากการศึกษาในผู้ใหญ่วัยกลางคน 322 คน อายุ 45-60 ปี พบว่า 81% มีการดื่มนมและมีเพียง 1/3 ที่ดื่มนมทุกวัน การศึกษาในอาสาสมัคร 70 คนโดยให้ดื่มนม 250 มิลลิลิตร แล้ววัดปริมาณก๊าซไฮโดรเจนที่มากกว่าและเท่ากับ 20 ส่วนในล้านส่วน พบว่า 57% ของอาสาสมัครมีภาวะบกพร่องในการย่อยน้ำตาลแลคโตส ซึ่ง 64% มีอาการไม่สบายท้อง เมื่อวิเคราะห์จากปริมาณก๊าซไฮโดรเจนร่วมกับก๊าซมีเธนที่มากกว่าและเท่ากับ 15 ส่วนในล้านส่วน และอาการท้องเสียที่เกิดขึ้นพบว่า 83% ของอาสาสมัครมีภาวะการย่อยน้ำตาลแลคโตสบกพร่อง การศึกษาในอาสาสมัคร 36 คนที่มีภาวะบกพร่องในการย่อยน้ำตาลแลคโตส โดยให้ดื่มนมที่มีน้ำตาลแลคโตสต่ำและนมที่มีการย่อยสลายน้ำตาลแลคโตสจนหมด ปริมาณ 250 มิลลิลิตร เมื่อวัดปริมาณก๊าซไฮโดรเจนหลังดื่มนม ปรากฏว่าไม่พบผู้ที่มีภาวะการย่อยน้ำตาลแลคโตสบกพร่องในอาสาสมัครดังกล่าวเลย อย่างไรก็ตามเมื่อวัดก๊าซไฮโดรเจนร่วมกับมีเธนและอาการท้องเสีย พบว่ามีผู้มีภาวะการย่อยน้ำตาลแลคโตสบกพร่อง 17% และ 11% ซึ่งมีอาการหลังการดื่มนม 36% และ 6% หลังจากดื่มนมน้ำตาลแลคโตสต่ำและนมที่ย่อยสลายน้ำตาลแลคโตสแล้วตามลำดับ อาการที่เกิดขึ้นหลังจากดื่มนมและผลิตภัณฑ์นมแลคโตสต่ำจัดอยู่ในระดับอาการเล็กน้อย ซึ่งความรุนแรงของอาการที่เกิดขึ้นหลังการดื่มนมแต่ละชนิดก็มีความแตกต่างกันอย่างมีนัยสำคัญทางสถิติ โดยอาการที่เกิดจากการดื่มนมปกติ > ดื่มนมที่มีน้ำตาลแลคโตสต่ำ > ดื่มนมที่ย่อยสลายน้ำตาลแลคโตสจนหมด ดังนั้นผลิตภัณฑ์นมแลคโตสต่ำ สามารถลดจำนวนผู้ที่มีภาวะการย่อยน้ำตาลแลคโตสบกพร่อง และผู้ที่มีอาการหลังการดื่มนมได้ เนื่องจากราคาของผลิตภัณฑ์นมมีความแตกต่างกัน ดังนั้นนมที่มีน้ำตาลแลคโตสต่ำจึงเป็นทางเลือกหนึ่งสำหรับผู้ที่มีอาการหลังการดื่มนมที่ไม่รุนแรง และนมที่มีการย่อยสลายน้ำตาลแลคโตสจนหมดเป็นทางเลือกของผู้ที่มีอาการรุนแรงหลังการดื่มนม

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CHAPTER I

INTRODUCTION

Calcium is the most biological essential mineral for bone health. Milk has been considered as the excellent source of many nutrients, especially calcium. Hence, milk consumption has been promoted to all age groups around the world. Milk contains the unique disaccharide, lactose, that requires hydrolysis by a brush border enzyme, "lactase" or "β-galactosidase" before absorption (1). The enzyme activity is high in nearly all infants, and its activity decreases after weaning in most racial group - the majority of the world's population - , except most whites, at approximately three to five years of age (2). Population with low lactase activity (is now termed lactose maldigesters, lactose malabsorbers, or lactase deficiency) maldigest lactose. Thus, lactose in milk is hydrolyzed incompletely and poorly absorbed. Lactose remains in the small intestine where it acts as an osmotic stimulus to net accumulation of sodium and water in the lumen (3). The undigested lactose transits into colon where it is fermented by the colonic bacteria; forming lactic acid, hydrogen, carbon dioxide and/or methane. This resulted in the discomfort gastrointestinal symptoms which possibly include abdominal pain, flatulence, diarrhea and sometime with abdominal cramps. Presumably, the presence of lactose in the colonic lumen holds water which increase osmotic pressure, resulting in an increase in motility, abdominal pain, loose stool and can induce diarrhea. Those who suffer with the undesirable gastrointestinal symptoms are called lactose intolerance (4, 5). Many people, therefore, avoid

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drinking milk which in turn may have an undesirable effect on their nutritional status and bone mass (2). Long-term dietary calcium inadequacy or deficiency is an important risk factor in the development of bone-related disorders, one of the most common is osteoporosis.

High prevalence of lactose maldigestion, ranged from 60 to 100% in African and Asian races population were reported (6, 7, 8, 9, 10). In Thailand, studies on lactose digestion in children and adults were performed in the last three decades (11, 12, 13, 14). High and non-physiological dose of lactose, 1g or 0.8g per kg body weight, was used as a test diet to determine lactose maldigestion. This resulted in the findings of the high prevalence of lactose maldigesters in Thai subjects. Recent studies used milk and physiological dose of lactose solution, equal to 12 g of lactose, as a test diet. The results showed decreasing in prevalence of lactose maldigesters (15, 16, 17).

Milk consumption promotion has been carry out in Thailand since 1985. At present, information on lactose maldigesters and intolerance in Thai middle age adults and elderly has not yet been available. Rao *et al* suggested that the prevalence of lactose maldigestion increase with age in blacks compared to whites (18). A study showed that lactose digestion and tolerance in adults and elderly did not differ in their metabolism and tolerance (19). In middle age adult, various physical function begin to deteriorate and being greater with advancing age (20). The decrease in skeletal mass in primarily caused by the age dependent decrease in sex hormones. The incidence rate of osteoporotic fracture is, therefore, expected to increase and still rising with older age (21). It begins in the third decade and cannot be arrested by adequate calcium intake alone (22). Therefore, adequate intake of calcium in childhood and

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adolescence is essential to attain the optimal bone mass and size (22). Adequate intake in women is party to slow bone calcium turnover to its programmed minimum (22). Previous reports showed associations of increasing dietary calcium, especially from dairy sources, and bone mass in middle-aged and elderly woman (23, 24, 25). The relationship between lactase deficiency and osteoporosis were also reported (26, 27).

Physical changes in middle age adult and elderly have a definite effect on proper intake of foods that affect to nutritional status (20). Drinking of milk is one way to promote health status among these groups. Previous studies showed normal absorption of protein, calcium and fat from milk in adults with lactase deficiency (28, 29, 30, 31, 32). These results confirmed that nutrients in milk could be absorbed. Whole milk contains a high level of lactose (12 g/250 ml) which is likely to be the main reason in limiting milk consumption. This is due to the undesirable effects on abdominal symptoms after milk consumption. Asian populations, including Thai people, consumed dietary calcium approximately 400-600 mg/day (33, 34, 35), which lower than the Recommended Daily Allowance for Thais (800 mg/day). In the past, several studies in Thai people presented several ways to reduce the undesirable symptoms when milk is consumed. There are, for example, reducing of milk volume at a time (16), having milk during and after meal (16, 36), drinking milk with solid foods (37), and having fermented milk in the form of yogurt (15, 38, 39). Before milk consumption will be promoted among the middle age adults and elderly, milk drinking habit, status of lactose maldigestion and tolerant among these groups should be studied.

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Various low lactose milk products, such as 50%, 90% lactose hydrolyzed milk, have been developed and be available in the markets to reduce the signs and symptoms of lactose intolerance in lactase deficiency people (40, 41, 42, 43, 44, 45). In 1999, imitated low-lactose and hydrolyzed (lactose-free) milk were developed in Thailand by the Institute of Nutrition, Mahidol University and have been well accepted (46). These products can be other choices for the lactose intorelants. However, the effect of drinking of these milk on lactose digestion and tolerance has not yet been studied.

The objective of this study were therefore to:

1. study the milk consumption habit in Thai middle age adults
2. identify the lactose maldigesters and intolerants in middle age adults, using a physiological test diet of a 250-ml whole milk.
3. study the effects of imitated low-lactose and hydrolyzed (lactose-free) milk consumption on lactose digestion and tolerance

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CHAPTER II

LITERATURE REVIEW

Physiology of lactose digestion

Milk represents a rich source of several nutrients. However, many people cannot consume milk because of their problems in digestion and absorption of lactose in milk. Lactose, or milk sugar, is the principle carbohydrate found in milk of most mammals and is not found in other nature foodstuff. Human milk contains the highest concentration of lactose (7%), while whole cow milk contains about 5%. Lactose is a disaccharide, composed of one molecule of D-galactose bonded with a β(1-4) glycosidic link to a molecule of D-glucose (**Figure 1**). To digest lactose, a unique intestinal enzyme, lactase (a beta-galactosidase), is needed which breaks the chemical bond, freeing them for absorption (47). Lactose is hydrolyzed the most slowly of all the dietary sugars. Physiology of lactose digestion and absorption is shown in **Figure 2.** Lactase is one of five disaccharidase on the brush border of the intestinal epithelium. In the proximal ileum, activity of the lactase enzyme is highest and lowest in the first portion of duodenum and terminal ileum (48).

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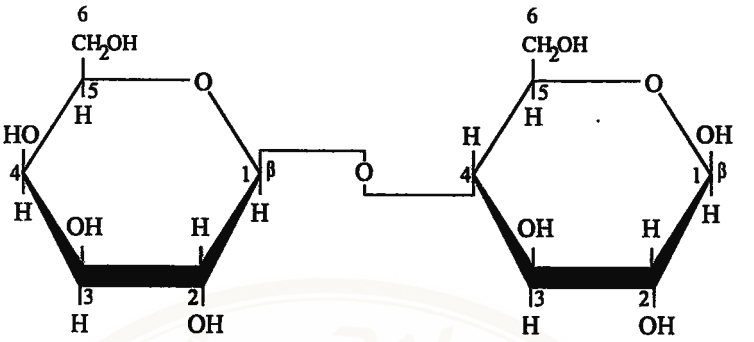

Figure 1. Chemical structure of lactose (β-form) O-β-D-galactopyranosyl-(1-4)-β-D-glucopyranose (49).

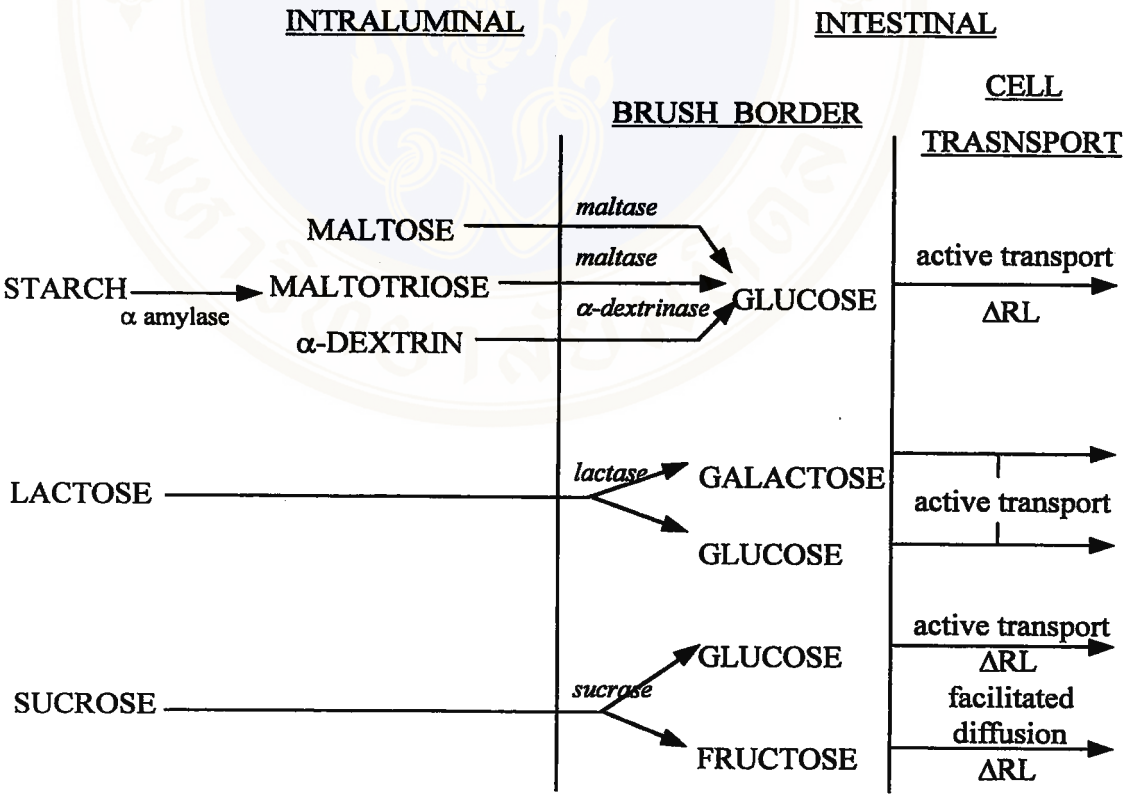

Figure 2. Schematic diagram of absorption of dietary carbohydrate at the intestinal brush border (50).

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Type of lactase deficiency

The absence or decline of intestinal lactase can be described as being congenital, primary or secondary.

1. Congenital lactase deficiency: Alactasia is an extremely rare condition in which detectable levels of lactase are absent at birth. In congenital lactase deficiency the histology of the small bowel is normal, as is the level of other disaccharidase. Persons with this disorder are unable to tolerate even small amount of lactose and will need to follow a lactose-free diet for the rest of their lives (51).

2. Primary lactase deficiency: In this type, lactase activity declines which occurs at variable periods after weaning depending on racial/ethnic background. It is genetically determined and is inherited as an autosomal recessive trait. In contrast, lactase persistence is inherited as an autosomal dominant characteristic (2, 47). Blacks have the earliest decline in intestinal lactase levels, starting at about three years of age (41). Several studies found that 70% of the American black population and 8% of American whites were lactose malabsorbers (52, 53). The ethnic mix of the population can affect the incidence of lactose maldigestion. The rate of maldigestion was fallen to 70% when Black Americans, have an expected rate of lactose maldigestion near 100%, intermixed with White Americans (54).

3. Secondary lactase deficiency: It can occur at any age by the intestinal mucosa where lactase is expressed, and any condition that injures which induce a decrease in lactase activity. The important causes of lactase deficiency are such as infectious diarrhea disease, the parasites giardia, gastrointestinal surgery and radiation treatment. Secondary lactase deficiency is reversed upon correction of the causative factors.

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Lactose maldigestion

If the amount of lactase produced is not enough to split all the lactose eaten, some undigested lactose will remain. Whereas 1% lactose is absorbed by passive diffusion into the bloodstream, which is then excreted into the urine unmetabolized (55). When undigested lactose reaches the jejunum, it increases the osmotic pressure and evokes an influx of water and sodium. Small intestinal volume may increase into fivefold, peristalsis is stimulated, and the lactose content is transported rapidly to the colon (56). Here, 65-100% of the undigested lactose is metabolized to short chain organic acid, hydrogen, methane, and carbon dioxide gases. Some of the organic acids are absorbed into bloodstream, while some are exerted in feces, resulting in acidic stools (**Figure 3**). The remainder of the H_2 is excreted in flatus or metabolized by colonic bacteria through methanogenesis, sulfate reduction or acetogenesis (**Figure 4**)(57). Several factors such as colonic pH and sulfate availability interact to determine which of these mechanisms predominates in an individual (57, 58). Methane formation is most active in the distal colon and then exhaled in addition to CO_2 and H_2 (59).

Relationship between maldigestion and intolerance

Lactose intolerance refers to gastrointestinal symptoms associated with the incomplete digestion of lactose. The presence of fermentation products in the colon may produce a variety of symptoms including abdominal discomfort, cramps, nausea, flatulence or diarrhea (47). Lactose maldigesters experience varying symptoms of lactose intolerance on lactose ingestion. Suarez and Levitt (1996) showed lactose load clearly include appreciable symptoms in the majority of lactose maldigesters (60). In principle, apparent lactose intolerances should not be found in lactose digesters but

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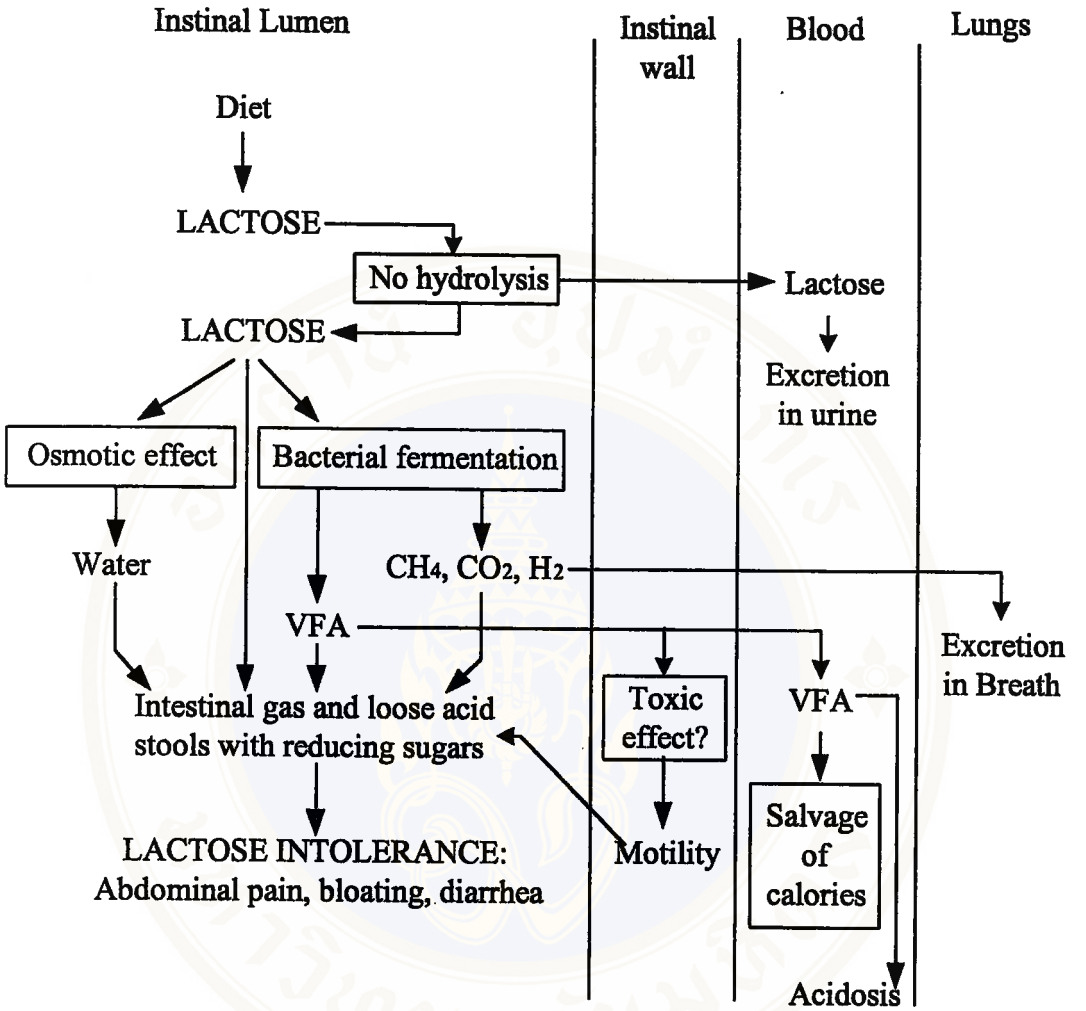

Figure 3. Palhophysiologic events following lactose maldigestion (47).

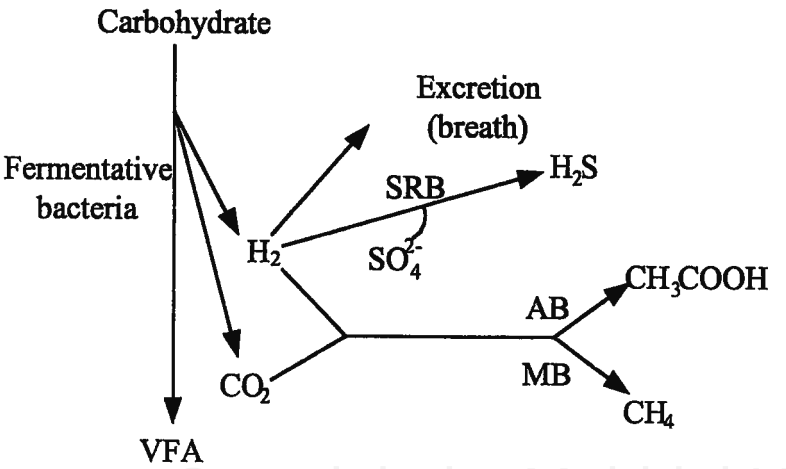

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Figure 4. Postulated mechanism for hydrogen disposal human colon (57).

many studies found lactose intolerance among lactose digesters (61, 62). In contrast, many lactose maldigesters are able to consume moderate amounts of milk without significant symptoms. Suarez *et al* (1995) showed the well-controlled trails of lactose maldigestion and intolerance, reported that lactose maldigesters had no significant difference in symptoms with ingestion of one cup of conventional milk dairy compared with one cup of lactose hydrolyzed milk (63).

The age of the subjects also appears to affect estimates of lactose intolerance. Newcomer *et al* (1978) indicated that children and teenagers with lactase deficiency are more tolerant to lactose than adults (64). The appearance of intolerance symptoms depend upon the amount of lactose ingested, the rate of gastric emptying, the motility of the small intestine (small bowel transit), the conditions of ingested (e.g., with or without food), the condition and activity of the large intestine microflora and evidently also upon the residual lactase activity throughout the small intestine (**Figure 5**) (65). These variables form the basis by which many individuals with lactose malabsorption attributable to "adult-onset" lactase deficiency tolerate significant amounts of milk without developing symptoms. In addition, social, cultural habits, attitudes toward milk, psychological factor and possibly intestinal adaptation to frequent drinking of milk are significant factors in the prevalence of such symptoms (66). Several reports of milk consumption by various population groups indicates that many confirmed lactose maldigesters continue to consume varying quantities of milk either without symptoms or despite mild symptoms (2). Double-blind studies are necessary for the evaluation of the role of lactose in causing symptoms reported after the consumption of milk and milk products (67). The double blind study of Liskeretal (68) showed that lactose–intolerant subjects are indeed milk-intolerant and that the frequency,

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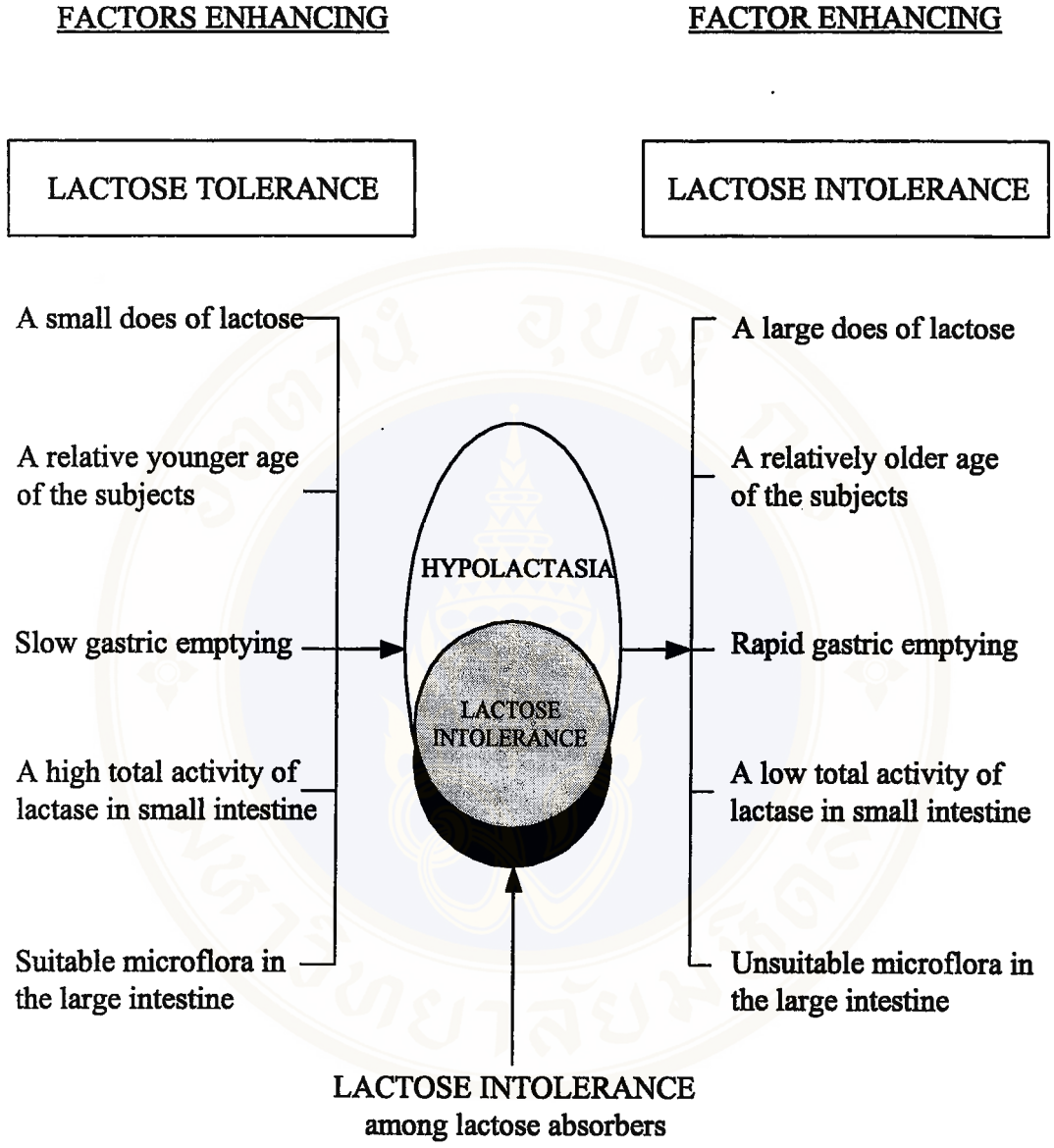

Figure 5. Factors influencing lactose intolerance symptoms in subjects with hypolactasia (65).

which symptoms occur in persons with lactose malabsorption increases in direct relation to the lactose content of milk. However, several studies have shown that both lactose digesters and maldigesters may report symptoms from consuming milk that is not because of its lactose content (66, 69, 70).

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Relationship of lactose maldigestion and lactose intolerance to calcium absorption, osteoporosis and other nutrients

Adult-onset lactase decline appears to be a risk factor for developing osteoporosis, owing to avoidance of dairy products or interference of undigested lactose with calcium absorption. Elderly with both adult-onset lactase decline and atrophic gastritis may have an increased risk of low calcium absorption owing to the lack of gastric acid to facilitate calcium uptake (71). Finkenstedt *et al* (1986) found that woman with osteoporosis had significantly higher prevalence of lactose malabsorption and milk intolerance and lower daily calcium intakes from milk than age-matched controls of similar ethnic origin (72). A recent study also demonstrated that both bone mineral density and calcium intake were significantly lower in women with both lactose malabsorption and intolerance than in those with lactose malabsorption alone (73). Honkanen *et al* (1996) studies in 2025 women aged 48-59, suggested that lactose intolerance slightly reduces perimenopausal bone mineral density, possibly through reduced calcium intake (74). This suggests that the discomforts of lactose intolerance are the main cause of dietary restriction of dairy product.

Osteoporosis is a systemic skeletal disease characterized by low bone mass and micro-architectural deterioration, with a consequent increase in bone fragility and susceptibility to fracture. Throughout life, bone undergoes a progress of remodeling, or formation and resorption (75). From birth until before the end of the second decade, bone grows linearly. After maximum height has reached, consolidation of bone continues until approximately ages 30-35 years when peak bone mass is attained. Thereafter, bone loss occurs in both sexes and continues throughout life. In women,

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bone loss is rapid during the first 5 to 10 years following the menopause, whereas in older women and in men, the rate of bone loss is slower. The level of bone mass achieved at skeletal maturity (peak bone mass) and the subsequent rate of post-menopaused and age associated bone loss greatly influence the risk of developing osteoporosis (75, 76, 77).

Prevention of osteoporosis is very important, one needs to increase peak bone mass and slow the rate of age-related bone loss. An adequate calcium intake in childhood and adolescence is essential to attain the optimal peak bone mass (22). In young adulthood, adequate calcium is important to support bone consolidation and increase peak bone mass (78). According to a three-year study, Baran *et al* (79) found that consuming more calcium in the form of dairy products increase vertebral bone mineral density in premenopausal women, age 37 year (30-42 years). Adequate calcium intake continues to be important to offset calcium losses and maintain bone health in the years after peak bone mass has been reached and before menopause (79, 80). In early postmenopausal, increased calcium intake has little effect in the rapid bone loss that occurs immediately after the menopause. Studies indicate that even early postmenopausal women benefit from calcium intakes at or greater than the current RDA (81, 82, 83). Elders *et al* suggested that calcium supplymentation during menopause may be more important for cortical than trabecular bone (84). In later postmenopausal years, about five or six years after menopause, calcium once again resumes relatively more importance in bone health than in the early postmenopausal years (82, 85, 86, 87). The difference in calcium's role in bone health between early and late menopause can best be explained by the effect of estrogen on the bone mass "set point". When estrogen decline at menopause, bone initially adjusts it mass

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downward releasing calcium. The result is that during the first few yesrs after menopause, there is little or no dependence on dietary calcium sources. However, after a period of adjustment, bone resorption declines and remains fairly constant and dependence on dietary calcium increase.

The Recommended Dietary Allowance (RDA) for calcium as established by many organizations, including the Thai RDA is based on the concept of calcium balance (88). Thai RDA for calcium is set at 800 mg/day for children aged 1-10 years and for non-pregnant, non-lactating persons aged 20 years and older. This allowance is increases to 1200 mg/day for adolescence aged 11-18 years, during pregnancy and lactation. Later, Thai Recommended Daily Intakes (Thai RDI) has set up the calcium requirement as 800 mg/day for Thai people aged 6 years or more to be used in nutrition labeling and guideline for formation of fortified food product. According to the Nation Institutes of Health (NIH), current Thai recommended levels of calcium are too low to protect against osteoporosis. It recommends that optimal calcium intakes a day are as follow: 1200 to 1500 mg for children and young adult ages 11 through 24, 1000 mg for women 25 to 49 years, 1000 mg for women 50 to 65 years on estrogen, 1500 mg for women of the same age not taking estrogen, 1500 mg for women 65 years up, 1000 mg for adult men 25 to 64 years and 1500 mg for men 65 years up (89).

The effect of lactose on calcium absorption remains controversial. Some studies indicate that calcium absorption is not affected by lactose (31, 90, 91). Although most studies suggested that calcium absorption parallels the degree of lactose hydrolysis in the small intestine (92, 93, 94). When calcium is administered with lactose or milk, calcium absorption is increased in lactase–persistent individuals but decreased in lactase–deficient adults (93, 95, 96). The products of lactose

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digestion, glucose and galactose were suggested as the enhanced factors in calcium absorption (97). Recent studies have demonstrated that a low calcium intake and/or low milk intake results in a low intake of other milk related nutrients both in adults and teenagers (98, 99, 100). Young adult women who had low calcium intakes were observed to have low intakes of protein and nine other vitamins and minerals as well (98). In addition, Debongnie *et al* showed that other nutrients in milk, specifically, protein, fat and minerals were recovered from the ileum in greater quantities in lactase deficiency (30). Therefore, it is important, from a nutritional standpoint, to encourage those with lactose intolerance to employ the dietary strategies available for including adequate amounts of dairy foods in the diet.

Hydrogen breath test

Hydrogen breath test has become the most popular method used for diagnosis of lactose maldigestion. It is sensitive, non-invasive, specificity, simplicity and sensitivity. The principle of breath hydrogen analysis was established by Leavitt and Colloway (101). Sensitivity and specificity in the diagnosis of lactose maldigestion are 100% (102). Rasado and Solomons (103) reported that sensitivity and specificity of the hydrogen breath test are 80% and 100% respectively. The test is based on the determination of hydrogen from expired air after oral lactose does. Hydrogen gas is a product of bacterial sugar fermentation and not of mammalian cellular metabolism, an increase in the pulmonary excretion of hydrogen after a carbohydrate meal presumes incomplete absorption in the small intestinal and the entry of sugar into the colon (104). The results of breath hydrogen analyses after lactose ingestion have correlated

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with measurements of lactase specific activity in intestinal mucosal biopsies and with lactose tolerance tests using blood sugar analysis (102).

In normal exhalation, the breath sample can be collected by expiration into a bag (**Figure 6**). A special mouthpiece has been devised the first exhaled is collected in one compartment, and the remainder of alveolar volume in a second compartment. A variable volume of the first portion of exhaled breath is simply a washout of the airways that was filled with the last portion of room air inhaled with the preceding breath. Breath samples are collected in plastic syringes and introduce it directly into the analyzer. If the breath sample cannot be analyzed immediately, it can use be store for varying length time. Special gas tight glass syringe and oiled glass, or even dispossible plastic syringe can maintain constant hydrogen concentration for up to 12 hours (105). The use of Critocap® syringe closure can maintain breath sample at room temperature, refrigerator temperature and freezer temperature (-20 ˚C) for up to 72 hour (106). Storage over a period of days demonstrated deterioration of hydrogen concentration but storage over an 8-h period did not (107). Hydrogen concentration in breath samples is determined by gas chromatography. Quintron Microlyzer Model DP is based on the principle of gas chromatography, which used for measurement of H_2 and CH_4 (**Figure 7**).

Factors influence hydrogen breath test

Breath testing is used to evaluate breath of gasses that are normally produced in the body's metabolism, products of intestinal microflora or that are unique and found only under test conditions. Various conditions can alter the validity of hydrogen

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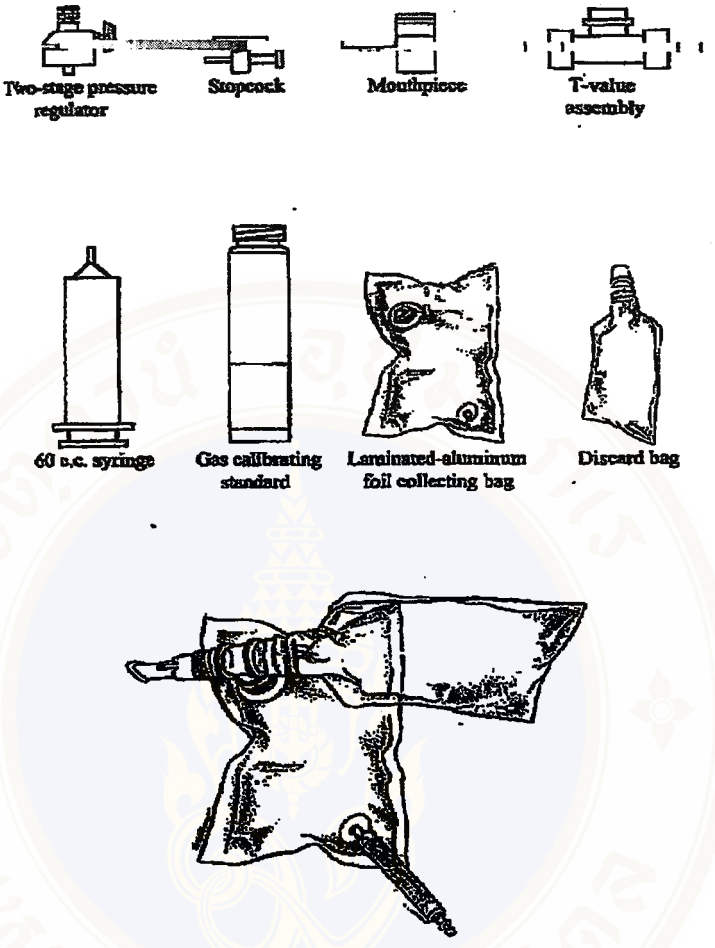

Figure 6. Accessory for the Microlizer

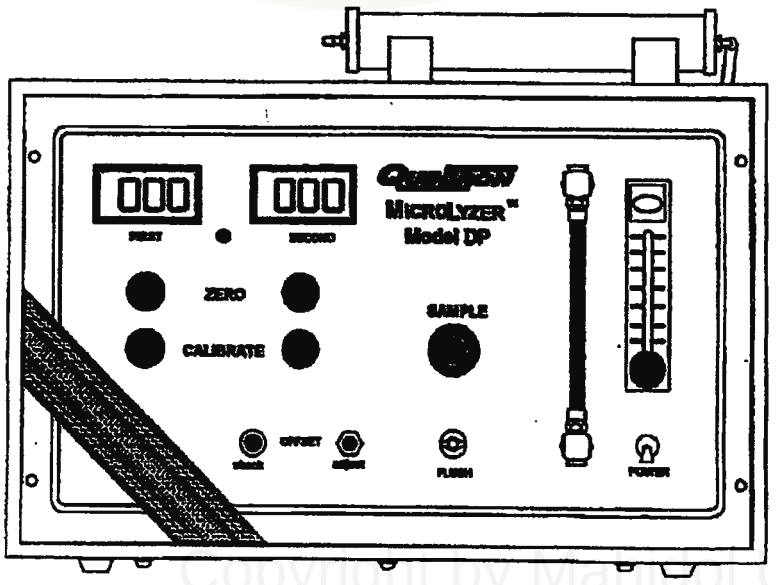

Figure 7. Qiuntron Model DP Microlizer™

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breath test or influence the interpretation of results. These cautions is essential to understand in hydrogen breath analysis:

1. Antibiotics: Broad-spectrum antibiotics will eliminate the H_2 producing species (108, 109) but Neomycin, penicillin and metronidazole may even increase hydrogen response (109, 110).
2. Cigarette smoking: Tobacco smoke contains high level of hydrogen, so smoking (by the patient or by anyone in the area) will produce high hydrogen levels and will cause extreme baseline instability of instrument (111, 112). Smoking by the patient should be avoided for least 15 minutes before any sample is taken, and there should be no smoking at any time in the vicinity of the instrument.
3. Laxative and enema: The hydrogen and methane was decreased to false negative by use of laxative or enema in malabsorber (113). The decrease may result from reducing the resident time for carbohydrate in colon, leading to reduced time for fermentation or may also result from a change in the concentration of the bacteria or from a change in the environment, such as increasing the acidity which could inhibit bacterial action (114). Rapid transit of carbohydrate through the colon due to hyper motility will also reduce the exposure time and decrease hydrogen production in the colon.
4. Sleeping: Sleeping during the test will cause increase in breath hydrogen and probably in methane. It also leads to false negative results. Hypoventilation, which slow down rate of hydrogen in turnover in lung and removal from the blood. Sleep also decreases intestinal motility, which

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slows down the movement of carbohydrate through the colon and allows a longer time for the accumulation of hydrogen (115).

5. Exercise: Effect of exercise reduces breath hydrogen by hyperventilation (116).
6. Colonic pH: A decrease in stool pH from 7.0 to 5.5 will cause a drop in hydrogen generation to ¼ the former rate (117). The rate is returned by increasing the pH. Thus, hyperacidic colon contents may inhibit the generation of hydrogen, or cause the generation of methane instead of hydrogen by colonic bacteria (113, 114, 118). This cause may be leads to false negative results.
7. Dinner meals: The meal on the day before the test day which containing high fiber diet, it could be influenced on accuracy of breath hydrogen testing (119). It is advisable to recommend a lower-fiber diet on the day before breath hydrogen test to lower the fasting breath hydrogen concentration (120).
8. Active diarrhea: Diarrhea during or before the test reduces the magnitude of breath hydrogen response. A washout of fermenting flora, a change in colonic pH, decreased contact time with the bacteria or preferential excretion of hydrogen by the rectal, all could be responsible for reduction of breath hydrogen during diarrhea (113).
9. Menstrual cycle: The luteal phase of menstrual cycle is associated with increased progesterone levels and prolongation of gastrointestinal transit time (121). Solomons *et al* (1997) documented the confounding influence gastric retention on the diagnosis lactose malabsorption in a child

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recovering from severe protein energy malnutrition (122). This condition produces false positive results.

10. Other diseases: Diseases such as pneumatosis cystoids intestinalis (123), cystic fibrosis (124), small bowel bacterial growth (125), celiac sprue (126) or sickle cell disease (127) may elevate fasting breath hydrogen excretion.

Advantage of hydrogen breath test

This method is that samples are easily obtained and non invasion, causes no appreciable discomfort, breath is much less complicated mixture than serum, no refrigeration of sample and can be reported as often as desired (128, 129). In addition the measurement of breath hydrogen excretion with the use of gas chromatography is simple and rapid. No preparation of the gas sample is required and the total time of a sample analysis is only 2 to 3 minutes (130). Newcomer *et al* (1975) found that the results of breath hydrogen analyses after lactose ingestion have correlated well with measurements of lactose-specific activity in intestinal mucosal biopsies and with lactose tolerance test using blood sugar analysis (102). In addition, the hydrogen breath test can detect the malabsorption of moderate or as small as 2 g of lactose intake (130, 131).

Interpretation of breath hydrogen test

Results of breath hydrogen concentrations are expressed in part per million (ppm) above baseline. The hydrogen concentration in an alveolar air sample from a healthy that has fasted for 12 h is normally less than 10 ppm. With patients who have elevated control value (10-20 ppm) due to residual fiber in colon, the hydrogen level might fall during the test period as the colon fiber-content is reduced through

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digestion. To identify a lactose maldigester, the change in trace-gas levels during the test should be related to the lowest point recorded for the hydrogen level prior to its increase. The baseline value can defined as the lowest value of hydrogen obtained at any sampling time (105). Parts per million above baselines are then calculated by subtraction of this value from the subsequent hydrogen concentration. There were various criteria for interpretion of breath hydrogen test. The concentration rised from basal level ≥20 ppm is generally regarded as consistent with lactose malabsotption by comparisons of the hydrogen concentration in the breath and the change in blood glucose and other correlative observation (102, 132, 133). Rasado and Solomons (1983) determined the sensitivity and specificity of various cut-off values for the maximum increase in breath hydrogen concentration (134). They found that, at least for a does of cow milk with a lactose content of 18 g (360 ml milk), the conventional criteria of ≥20 ppm is as efficient for detecting the same individuals as being lactose maldigesters as the more restrictive concentration of ≥25 ppm. At cut-off points with lower hydrogen concentration, the specificity in the diagnosis of lactose malabsorption is progressively lost. A simplified procedure suitable for field studies give 80-100% sensitivity and 100% specificity (133, 134). Veligati *et al* (1994) concluded that a rise of breath hydrogen concentration more than or equal to 20 ppm over the baseline appeared to give better correlation with subsequent symptoms development than did of more than or equal to 10 ppm (135).

The area under the discontinuous curve of the change in breath hydrogen concentration is often used to quantity response to oral carbohydrate (136). Rumessen *et al* (1990) suggested that both areas under the curve and peak hydrogen concentrations seem valid for comparison of various does of lactose ingested.

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Furthermore the magnitudes of areas under the curve and hydrogen peak were closely correlated (137).

Breath methane

Methane and hydrogen are produced during an aerobic bacterial activity in the large intestinal and are excreted in flatus and expired breath. There is a relationship between hydrogen and methane production in which methanogenic bacteria are able to convert the hydrogen to methane in the colon. Methane is rarely identified in the breath of subjects less than 3 years. Nevertheless, adult's patterns of excretion have been documented by age 11 (59, 138). In adults, methane producers comprise 30-65% evaluated by random breath analysis (59, 139, 140). The reasons for these age-related differences in methane production are unclear, but likely involve, at least in part, a late developmental acquisition of methanogenic flora (59). Recent studies in healthy about volunteers (141, 142) have shown that breath methane excretors have lower breath hydrogen production following ingestion to test dose of the nonabsorbable sugar lactulose than non methane producers. False negative results may occur in subjects unable to produce hydrogen (non producers). The reported incidence of non-producers has varied from 0 to 30% depending upon criteria used to lack of production (101, 130, 141, 143, 144, 145, 146). Furthermore, methane production has been identified in those who fail to produce hydrogen following ingestion of non-digestible sugars (113, 141). There are several criteria to use for detect methane producers. Cloarec *et al* (1990) indicated that the response to lactulose depend on breath methane producing status, using breath methane concentration greater than 2 ppm above ambient air to defined breath methane producing status (147). Linda *et al* (1985) used

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breath methane concentration >2 ppm in their studies (140). Criteria greater than 1 ppm and 4 ppm were used in other studies (148, 149).

Some studies suggested that breath methane measurement might enhance the accuracy of hydrogen breath testing in detecting carbohydrate malabsorption (150, 151). However, Montes *et al* (149) advised that methane-producing status is not necessary in the interpretation of the lactose breath hydrogen test. Rumessen and Myo-Khin (152, 153) concluded that the breath methane test for lactose malabsorption has a lower sensitivity and specificity than the breath hydrogen test and did not replace the lactose breath hydrogen test.

Prevalence of lactose maldigestion

Primary adult lactose malabsorption among most human groups usually comes on in the years immediately after weaning. The incidence of maldigestion has been described in various groups in several populations such as Negroes, Indians, Thais, New Guinea natives, Greek Cypriots, Australian Aborigines, Chinese and South American Indians. There are geographical, ethnic and age-related decline of intestinal mucosal lactase activity, which increases the prevalence of lactose maldigestion in healthy individuals of most population, has well documented (154, 155). More than 50 million Americans cannot adequately hydrolyzed lactose (51). Rao *et al* (18) suggest that prevalence of lactose maldigestion increase with age in blacks compared to whites and that the magnitude of the problem may be greater in black maldigesters than in white maldigesters. There were different frequencies of lactose maldigestion found in population of similar racial composition in different environments (156, 157). High prevalence of lactose maldigestion from about 60-100% the studied subjects, are

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typical of the overwhelming number of ethnic or racial groups around the world including (158):

Category A: Hunting and gathering people (prevalence of lactose maldigestion (LM) 86%)

Eskimo of Greenland, Alaskan Indians, Twa Pygmies of Rwanda

Category B: Agricultural people from the traditional zones of nonmilk and their relatively unmixed overseas descendants (Prevalence of LM 90%)

- In North and South America (Prevalence of LM 97%)
- In Sub-Saharan Africa (Prevalence of LM 87%)

Ibo, Yoruba, S. Nigerians

- In Southeast and East Asia (Prevalence of LM 90%)

Thai, Chinese, Malays, Japanese, Filipinos, Indonesians on Java, Vietnamese in U.S

- In the Pacific Region (Prevalence of LM 100%)

Fijians, New Guineas

Category C: Agricultural peoples in the traditional zones of nonmilk but who migrated into an adjacent zone, to become milk-users. (Prevalence of LM 88%)

Kenyans, Bantu of Zambia, Ganda, Shi

Category D: Peoples who have consumed amount of milk and lactose-rich dairy products for a long historical period and have lived under conditions of dietary stress. (Prevalence of LM 11%)

- In Africa and near East (Prevalence of LM 10%)

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Tussi, Bedouin Arabs, Nomadic Fulani

- Europeans and their overseas descendants (Prevalence of LM 11%)

Danes, Finns, Swedes, American whites, Anglo-Saxons

Category E: Peoples who have used milk since antiquity but who do not meet conditions of strong selective pressures against lactose maldigestion (Prevalence of LM 72%)

Jews in Britain, in Israel, Jordanian Arabs, Egyptian fellahin

There are some groups who have intermediate prevalence of LM (30-60%) but they are few, and almost all have a mixed digesters and maldigesters ancestry. Thus the relatively unmixed ethnic groups of the world seem to fall into two population categories: lactose digesters (0-30% prevalence of LM) and maldigesters (60-100% prevalence of LM).

Lactose maldigestion studies in Thailand

Studies on the lactose digestion status in Thai adults and children were performed in the last three decades. Many studies of lactose digestion in populations have focused only on adults or reported results over a wide age range without providing an age breakdown. Flatz *et al* (1969) conducted lactose tolerance test using 1 g lactose/kg body weight as the test diet in 114 healthy well nourished individuals in Northern Thailand, found that all 75 adults and 24 children aged 4-12 years were lactose maldigestion whereas 6 out of 13 children aged 2-4 years were lactose maldigestion (12). Similar cases in adults from Ayuthaya Province, using lactose

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tolerance test with change oral lactose does to 0.8 g/kg body weight; the degree of lactose maldigesters was 96% (13). Lactose tolerance tests were performed in malnourished Thai infants under 2 years of age. Lactose malabsorption was found in 60% of those with malnutrition and 69% of those with malnutrition and diarrhea (11).

Further studies prevalence of lactose maldigestion were reported by hydrogen breath test with the criterion of breath hydrogen production ≥20 ppm and used a 250-ml milk or lactose solution (12g equal to the amount in 250 ml milk) as a test diet are shown in **Table 1**. In addition, methane and hydrogen production was used in recently study to determined lactose maldigester.

Table 1. Summary of prevalence of lactose maldigestion by hydrogen breath test

Age	Lactose maldigesters	Lactose intolerance
Children (5-6 years)(36)	15%	-
Adult 20-45 years (16)	88%	75%
18-41 years (17)	77%	38.5%*
18-47 years (15)	67%	59%*
18-47 years (15)	48%(H_2≥ 25ppm)	59%*
Adolescence 14-16 years (38)	41%	30%*
13-15 years (37)	39%	28%*
14-16 years (39)	32%	29%*
14-16 years (39)	40% (add methane production)	29%*

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Note: * = lactose maldigester intolerance

Dietary management of lactose maldigesters and intolerance

Several factors have been found to influence an individual's tolerance to dairy food including: the amount of lactose, type of dairy food, whether the food has been fermented or hydrolyzed with an enzyme preparation and colonic adaptation.

Combination of lactose with a meal and the milk constituents

Milk is also better tolerated when it is consumed with a meal (159). It has been demonstrated that psyllium fiber, when added to aqueous lactose on milk, reduces hydrogen production and symptoms of intolerance (160). When lactose-containing foods are consumed with other solid foods, gastric emptying is delayed, both reducing and delaying peak hydrogen production. Martini and Savaiano (159) found that only 3 out of 12 subjects experienced symptoms following a 19-g lactose load consumed with breakfast meal. This amounted to a threefold reduction in both severity and incidence of symptoms when compared to aqueous lactose. Delay gastric emptying allows more time for any endogenous lactose enzyme present to digest dietary lactose. It also reduces the amount of undigested lactose, which reaches the colon at any one time. In study of Solomons *et al* (1985) found that a relative net reduction of 47% in lactose malabsorption was produced by adding food (a hard boiled egg, cornflakes and a medium banana) and the peak-rise in breath hydrogen was delay by 2 hours (161). Taveethavornsawat (1998) found significantly low-level breath hydrogen after consumed milk with cereal in adolescent lactose maldigesters (37).

Whole milk and chocolate milk appear to be the best tolerated milk types (162, 163). The fat content of milk influences milk tolerance by slowing gastric emptying. When 11 lactose-maldigesting and intolerant subjects were given 50g of lactose as

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whole milk, they experienced a significantly lower rise in blood glucose and decreased severity of symptoms, as compared to the same amount of lactose in either skin milk or an aqueous solution (162). The additions of cocoa to 250 ml of milk formula significantly reduce the breath hydrogen response and symptoms of bloating and cramping in 37 lactose maldigestion (163). Mechanism for cocoa's affect is not clear; sometime it may be delay gastric emptying.

Amount of Lactose

In principle low does of lactose can reduce symptoms, which occur in lactose digestion. Hertzler *et al* (1996) found that hydrogen production after consumption of the 0 and 2 g lactose was not significantly different, hydrogen production increased with the 6g lactose doses. Thus lactose maldigesters may be able to tolerate foods containing 6 g lactose or less per serving, such as hard cheese and small serving (120 ml or less) of milk (164). Paige *et al* (1975) showed that no difference between the blood sugar in lactose absorbing and malabsorbing subjects when consumed 50% lactose hydrolyzed milk (6g lactose) and 90% lactose hydrolyzed milk (1.2 g lactose) (44). Some studies found that the lactose-hydrolyzed milk did not reduce the symptoms of lactose intolerance in the milk intolerant population with double-blind control (165). In addition, Vesa *et al* (166) concluded that the gastrointestinal symptoms in most lactose maldigesters are not induced by lactose when small amount (0.5-7.0 g) of lactose. However, several studies found that low amount of lactose can reduced lactose intolerance in lactose maldigesters (45, 167, 168, 169, 170, 171).

Fermented milk products

Yogurt with active microbial culture improves the digestion of lactose. Improved lactose digestion with yogurt appears to be partly the results of its reduced

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lactose content, but is primarily due to autodigestion within the intestine by the microbial beta-galatosidease enzyme (172). Three related factors appear to be important to the survival and expression of microbial enzyme activity from yogurt: (2) the buffering of stomach acid by yogurt, (1) protection by the intact microbial cell to degradation by stomach acid or enzyme and (3) action of digestive enzyme and bile acids on the microbial cell which releases beta-galactosidase activity (173). Bear suggested that yogurt might help improve lactose intolerance, and it is now well established that yogurt improve both maldigestion and intolerance in primary and secondary lactase deficiency (174). Similarly, in several studies confirmed that hydrogen production with yogurt is significantly less than with milk (15, 172, 175, 176) and that symptoms are improved in both adults and children (15).

Enzyme preparations

Lactose-hydrolyzed milk or commercial oral enzyme replacement therapy has proven beneficial in adults and children and it recommended as a strategy for improving lactose tolerance (2, 47). The enzyme lactases generally used for lactose hydrolysis are extracted from yeasts such as *Kluyveromyces lactis* and *Kluy veromyces fragilis* or from fungus such as *Aspergillus Niger* and *Aspergillus oryzae.* Lactose-reduced milk is prepared at a processing plant by adding the liquid enzyme to previously pasteurized milk and holding for 24 hour. The milk is then pasteurized again to stop lactose hydrolysis when the appropriate level of reduction has been reached, usually 70% (177). Milk that has had 99.9% of its lactose hydrolyzed, labeled "lactose free". Milk labeled "lactose-reduced" must contain at least 70% less lactose than regular milk. Lisker *et al* (1980) found that lactose hydrolyzed milk is

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highly significant better tolerated than regular milk and lactose enriched milk in 240 rural and 101 urban children (178).

Colonic adaptation

Although human lactase has been described as a noninducible enzyme, there is evidence that adaptation to continued exposure to lactose occurs in the colon. Reddy and Pershad (1972) demonstrated that continued lactose intake increases the amount of lactose tolerated without symptoms and without change in intestinal lactase activity (179). In a recent study, 25 African-American adolescents and young adults who were confirmed lactose maldigesters and intolerant to the amount of lactose in 1 glass of milk (12 g), were given gradually increasing amounts of lactose in milk over a period of time until their maximum level of tolerance was determined (180). They found that the majority of African-American young adults who claim intolerance to moderate amounts of milk can ultimately adapt and tolerate ≥12g lactose in milk with minimal or no discomfort if milk is ingested in gradually increasing amounts. The mechanism of adaptation is assumed to be an increased tolerance to colonic lactose-fermentation products.

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CHAPTER III

MATERIALS AND METHODS

3.1 Questionnaire survey of milk consumption habit in middle age Thai people

Subjects

Three-hundred and twenty-two subjects were studied. They are teachers and instructors, age ranged 45-60 years old, from ten schools namely Yotinnukul, Watsakaew, Suranaree, Theadsaban 1 (เทศบาล 1), Theadsaban 3 (เทศบาล 3), Boonwattanawitayalai, Anuban Nakornratchasima (อนุบาลนครราชสีมา), Reummitwittaya, Nakornratchasima Vocational College and from Rajamangala Institute of Technology Northeastern Campus. The subjects were asked to fill a questionnaire **(Appendix A)** to review their habit of milk and milk products consumption and the experience on gastrointestinal symptoms.

3.2 Identification of lactose maldigestion and intolerance in Thai middle age Adults

Objective: To identify lactose maldigestion and intolerance among middle age adults using a 250-ml whole milk as the test diet.

3.2.1 Subjects

Seventy volunteers, 7 men and 63 women, age range of 45-60 years old (average 50.5 ± 4.2), from the above eight schools (except Boonwattanawitayalai and Reummitwittaya) participated in this study. The subjects signed a consent form after

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the risks and benefits of the study had been fully explained. They were healthy without history or symptoms of gastrointestinal diseases or diarrhea, had not recently used antibiotics or laxatives for at least two weeks before testing for lactose digestibility. Before the study, subjects were requested not to consume milk and milk products for a day and fast overnight (for at least 8 hours) before each test day. They were asked for not having eaten some slowly digesting fruits and vegetables, e.g., beans, dried legumes, banana, green mangoes, cabbage, collard and other high fiber foods; for one day before each test day, particularly at dinner, due to the significant amount of hydrogen produced after eating these foods. The foods that taken by subjects were recorded for 3 days. Twenty-four hour food record form is presented in Appendix B.

3.2.2 Test diets

Milk: ultra–heat treated (UHT) cow's milk, FOREMOST™ (Foremost Dairies Company (Bangkok) Co., Ltd.), containing 12.33 g of lactose/250 ml milk, was used as test diet for screening lactose maldigester and intolerance.

3.2.3 Experimental design

On the experiment day, the subjects were requested not to smoke, sleep and do excessive work or exercise but allowed normal daily activity and having lunch after breath collection for five hours.

After an overnight fast, subjects collected the first breath and drank a 250 ml cow's milk, then collected breath sample again at 30 min interval for 7 h. The lowest hydrogen concentration produced at the first 4 intervals during 1.5 h was used as individual baseline. The maximum absolute peak increment in breath hydrogen at the level of ≥ 20 ppm over baseline value during 7 h. collection period was the criteria for classification as a lactose maldigester. Subjects could had lunch when the peak

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hydrogen rised after 5 h. period because the carry-over effect of lunch were not counted for lactose maldigesters. The cumulative hydrogen production as an area under the consecutive hydrogen production curves was evaluated. The symptom after consumption of milk were recorded and used to indicate lactose intolerance.

3.2.4 Breath sample collection and analysis

3.2.4.1 Breath sample collection

Breath sample was collected by means of end-expiratory breath sampling technique using the QuinTron GaSampler system (**Figure 6**). The GaSampler consists of an alveolar air collection bag (750 ml), a discard bag (100 ml), a T-piece with one-way flap-valve and a mouthpiece. The collection bag is fitted with two ports, a large one with a one-way valve attached to T-piece and mouthpiece respectively, and a smaller port is used for sampling from the bag. The discard bag is assembled in-line with the mouthpiece on the other end of the T-piece.

After a normal inspiration, the subject was instructed to release a normal expiration through the mouth to empty the lung of as much as gas required to provide the alveolar gas sample. By this step, the discard bag was first filled with the first portion of the expired air, after that valves on the T-piece and in the collection bag were open from pressure in the discard bag and the remaining expired air will be diverted into the collection bag then removed the bag from the T-piece. A subsample of gas (40-50 ml) in the collection bag was aspirated into a 50-ml plastic syringe that fitted with a three-way stopcock and used for analysis. The collection bag can be emptied and reused.

Breath samples were taken after an overnight fast and at 30 min intervals for 7 h. after consumption of each test diet.

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3.2.4.2 Quantitative determination of hydrogen in expired air

Breath sample in expired air was analyzed by Microlyzer, Quintron Instrument Co., Inc. Milwaukee, Wisconsin, Model DP Microlyzer ™. It has been designed to measure small quantities of hydrogen gas in expired and alveolar air samples. The principle of the Model DP Microlyzer ™ is based on the technique of gas chromatography. Hydrogen in the breath is separated from other gases using an external chromatographic column, called a SivRite-10 cartridge which allows the hydrogen pass through freely, but traps all other interfering gases (those which can burn or react with oxygen). To operate the model DP Microlyzer ™, insert the male end of the SivRite-10 cartridge into the female lure fitting of the Flush port below the sample valve (**Figure 7**). The gas sample (20 ml) was injected through the SivRite-10 cartridge. Hydrogen and methane gases were carried passed the sensor by an inert carrier gas, which flowed continuously through the system. The carrier gas for the model DP Microlyzer ™ was room air, which had been conditioned with a large fore-column, which removed moisture and stabilized the baseline of the instrument. When the bolus of hydrogen and methane were carried into the sensor block, their presence produced electrical signals by interacting with the solid-state sensor. The values of the processed signals were directly related to the hydrogen and methane concentration in the sample. The circuit converted the signal to a decimal form which was displayed as part per million (ppm) of hydrogen on the first digital window and of methane on the second digital window. The breath sample in the 50-ml syringe was analyzed in duplicate, within 24 h after collection.

A compressed reference gas standard of known hydrogen and methane concentration (QuinGas-1) was used to calibrate the digital concentration (ppm)

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reading on the Microlyzer. The standard gas was 2-fold diluted with the room air and injected to the instrument. The concentration of hydrogen and methane were directly read. The calibration curve was plotted prior to the sample reading every day and standard checking was performed regularly during reading of the samples

3.2.4.3 Calculation of hydrogen concentration

Hydrogen concentration, above the baseline, after consumption of test diet was calculated by 2 methods. The lowest hydrogen concentration in the first 4 intervals was used as the baseline of individual.

1) The absolute concentration of hydrogen as peak height of magnitude hydrogen at 30-minute intervals expressed as ppm
2) Cumulative breath hydrogen excretion, computed as the area under the hydrogen production curve over the baseline which the following equation for the sum of the areas of consecutive trapezoids and were expressed as ppm.min (181).

$$A = (1/2\ H_1 + H_2 + H_3 + \ldots + H_{n-1} + 1/2\ H_n)\ t$$

where A = the area under the hydrogen production peak

H = absolute breath hydrogen concentration (ppm) between 0 and 7.0 h.

t = 30 minutes.

3.2.5 Gastrointestinal symptoms

Gastrointestinal symptoms which occurred after consumption of test diet during 7 hours were recorded in the form which given in **Appendix C**. The following set of symptoms, 1) abdominal pain, 2) abdominal bloating and flatulence, 3) nausea, 4) vomiting, 5) diarrhea and etc. were recorded as present or absent. Among these symptoms, the degree of severity was recorded as follows: 1 for mild, 2 for moderate

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and 3 for severe (182). For diarrheal symptoms, rate of intensity was classified in double rate, such as 2, 4, 6 for mild, moderate and severe respectively. The average intensities of each gastrointestinal symptoms were calculated.

3.2.6 Analysis of lactose content

Lactose in whole milk (UHT) was analyzed at Food Chemistry Division, Institute of Nutrition, Mahidol University. In the process, 20 ml of the milk sample was brought into a 50-ml volumetric flask and diluted with 10 ml distilled water. Protein in milk sample was precipitated by slowly adding of 5 ml of 10% $ZnSO_4$ while mixing, and 5 ml of 0.5 N NaOH. The solution was then mixed vigorously and left at room temperature for 15 min. The final volume was adjusted to 50 ml with distilled water and then filtered through Whatman No.1 filter paper. Lactose in the filtrate was analyzed by high performance liquid chromatography (HPLC) against lactose standard solution (1, 2 and 3 g/100g), prepared the same way as the samples.

Equipment: The chromatographic system consisted of a pump (Varian, Associated, Sienny Vale, USA; model 5000) completed with a stainless steel cartridges (LichroCART ® column (Lichrospher® 100 NH_2, particle size = 5 μm), dimension length = 250 mm, i.d. = 4 mm (Merek 516121) and a refractive index detector (Varian, Associated, Sienny Vale, USA; model series RI-3) connected to an integrator (Sputra Physic, SP 4270). Sample injection was done by means of a fixed loop Rheodyne injection valve (model 7120, 50 μl).

Separation and quantitation of lactose: The mobile phase used was acetonitrile-water (84:16,v/v), with a flow rate of 1.8 ml per min lactose peak on the chromatogram was identified by lactose standard with retention time of about 10 min.

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The amount of lactose in unknown sample was determined by comparing the peak area of the unknown to that of the standard lactose.

Lactose content in low-lactose and hydrolysed imitated milk were referenced from the values in previous products (46).

3.3 Effect of imitated milk products consumption on lactose digestion and tolerance in lactose maldigesters

Objective: to investigate the effect of low-lactose and lactose-free milk on lactose digestibility and tolerance in lactose maldigesters based on the breath hydrogen production and gastrointestinal symptoms.

3.3.1 Subjects

Thirty-six of lactose maldigesters who were identified by hydrogen production of more than 20 ppm above the based line after consumption of a 250-ml whole milk participated in this study.

3.3.2 Test diets: low-lactose imitated and lactose-free milk

1. Low-lactose imitated milk: a low lactose milk product containing 2.23 g lactose per 250 ml was prepared (46) by mixing pasteurized cow milk (FOREMOST™) with milk protein concentrate™ (MPC™80, DMV International, Netherlands). The product was then pasteurized and 250 ml was served cold to each lactose maldigester.

2. Lactose-free imitated milk: a lactose-free milk was prepared by adding lactase enzyme (Lactozym 3000L™, Type HP-G produced from Enzyme Process Division, Denmark) with a dosage of 3.5-5.4 ml per 1000 ml of pasteurized cow milk (FOREMOST™) (46). It was incubated in a refrigerator, 5°C, for 15 h. The lactose-

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free milk (0 g of lactose/250 ml milk), 250 ml, was served cold to each lactose maldigester.

3.3.3 Experimental design

Test diets 1 and 2 were given to the lactose maldigesters after an overnight fast. The washed out period between each diet was at least one week. The breath samples collection, analysis, calculation and the record for gastrointestinal symptom followed the protocol in Section 3.2.4 and 3.2.5.

Summary of all experiments in section 3.2 and 3.3 is shown in **Figure 8.**

3.4 Statistical analysis

The hydrogen concentration produced in each time interval were expressed as mean ± SD and were considered statistically significant if two-sided P values were < 0.05. One-way analysis of variance (ANOVA) was used to evaluate the difference of breath hydrogen production between treatments. Spearman correlation was used to evaluate the relationship of gastrointestinal symptoms and hydrogen production (as areas under the curve). The degree of gastrointestinal symptoms intensity between treatments was evaluated by Mann-Whitne U-test.

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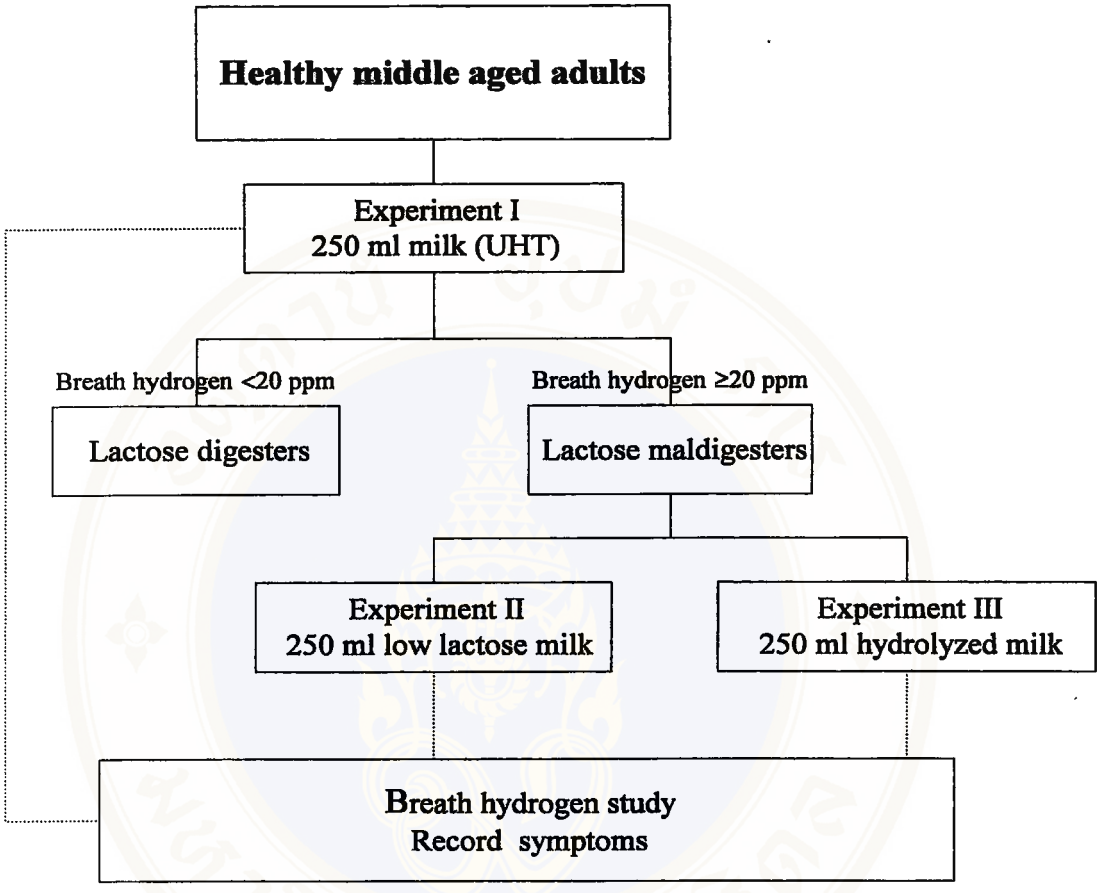

Figure 8. Experimental design

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CHAPTER IV

RESULTS

4.1 Questionnaire survey of milk consumption habit in middle age Thai people

Three hundred and twenty-two middle age adults, 39 males and 283 females, with age range of 45 to 60 years (Mean ±SD = 51±4.2) were surveyed. About 80 % of them consumed milk but only 1/3 drank milk (250 ml per time) regularly. Of 261 subjects who drank milk, 46 (18%) of them had self-recorded of gastrointestinal symptoms and most had the symptoms 1-2 h after consumed milk either after meal or with empty stomach. Milk consumption habit among this age group is summarized in **Table 2**

Table 2. Milk consumption habit in 322 middle age adults

Milk consumption habit	Middle age adults		
	Males	Females	Total (%)
1. Drinking milk normally			N=322
- no	9	52	61 (19)
- yes	30	231	261 (81)
2. Amount of milk consumed per day			N= 261
- <250 ml	1	6	7 (3)
- 250 ml	5	50	55 (21)
- 500 ml	3	18	21 (8)
- >500 ml	-	3	3 (1)
- Do not drink everyday	21	154	175 (67)
3. Amount of milk consumption per time			
- <250 ml	2	19	21 (8)
- 250 ml	28	211	239 (92)
- 500 ml	-	1	1(<1)

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Table 2. Milk consumption habit in 322 middle aged adults (continued)

Milk consumption habit	Middle age adults		
	Males	Females	Total (%)
4. Experience in gastrointestinal symptoms: abdominal bloating, abdominal pain, diarrhea and nausea after drinking milk			
- no	24	191	215 (82)
- every times	3	12	15 (6)
- sometimes	3	28	31 (12)
5. Milk is consumed			
- before meal	6	66	72 (28)
- after meal	9	88	97 (37)
- at empty stomach	15	77	92 (35)
6. Symptoms occurred after milk consumption			
- before meal			
: no	6	58	64 (25)
: yes	-	8	8 (3)
- after meal			
: no	8	74	82 (31)
: yes	1	14	15 (6)
- with empty stomach			
: no	10	59	69 (26)
: yes	5	18	23 (9)
7. After milk consumption, time period which the gastrointestinal symptoms occurred			N = 46
- suddenly	2	5	7 (15)
- 0.5 h.	-	4	4 (9)
- 1-2 h.	4	26	30 (65)
- 2-3 h	-	5	5 (11)

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Table 2. Milk consumption habit in 322 middle aged adults (continued)

Milk consumption habit	Middle age adult		
	Males	Females	Total (%)
8. Reason for not consuming milk			N=61
- having undesirable symptoms after milk consumption	2	13	15 (24)
- do not like flavor of milk	7	35	42 (69)
- others reasons: consume soymilk only	-	1	1 (2)
: high fat	-	3	3 (5)

4.2 Identification of lactose maldigestion and intolerance in Thai middle age adults

4.2.1 Lactose maldigesters based on breath hydrogen production

After drinking of 250-ml whole milk, hydrogen in expired air was measured over a 7-h. period in 70 Thai middle age adults. Individual absolute magnitude peak of breath hydrogen (maximum increment of breath hydrogen production over baseline) is shown in **Figure 9**, with 40 out 70 subjects (57%) whose absolute hydrogen production was ≥20 ppm. They were classified as lactose maldigesters **(Table 3)**.

The average absolute peak hydrogen concentration of the lactose maldigesters was 37.5 ± 14.8 ppm (n = 40), whereas that of the lactose digesters was 13.2 ± 4.0 ppm (n=30). Average pattern of changes in absolute breath hydrogen concentration produced by lactose maldigesters and lactose digesters throughout the 7 h period was summarized in **Figure 10** and **Appendix F.** Absolute peak height of each subject

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appeared at different period of time. In the group of lactose maldigesters, it appeared between 3.5 to 5.5 h after drinking milk.

Table 3. Classification of adult subjects (n = 70) based on lactose digestibility

Subjects	Number		Total (%)
	Males	Females	
Lactose digesters (n = 30)	6 (8.6%)	24 (34.3%)	43
Lactose maldigesters (n = 40)	1 (1.4%)	39 (55.7%)	57

4.2.2 Lactose maldigesters based on breath methane production

Of the 70 middle age adults, 28 (40%) produced breath methane >2 ppm above the ambient air and the maximum value was 37 ppm. Fifteen lactose digesters (21%) and thirteen lactose maldigesters (19%) were classified as breath methane producers (**Table 4**). Both H_2 and CH_4 production were considered to estimate lactose maldigesters, the amount of gases produced was summed. Based on the criteria of breath $H_2+CH_4 \geq 15$ ppm (182), 10 out of 15 previously identified as lactose digester-CH_4 producers were finally classified as lactose maldigesters. Following both criteria (hydrogen and hydrogen + methane production), there were 50 lactose maldigesters (71%) among the middle age adults (**Table 5**).

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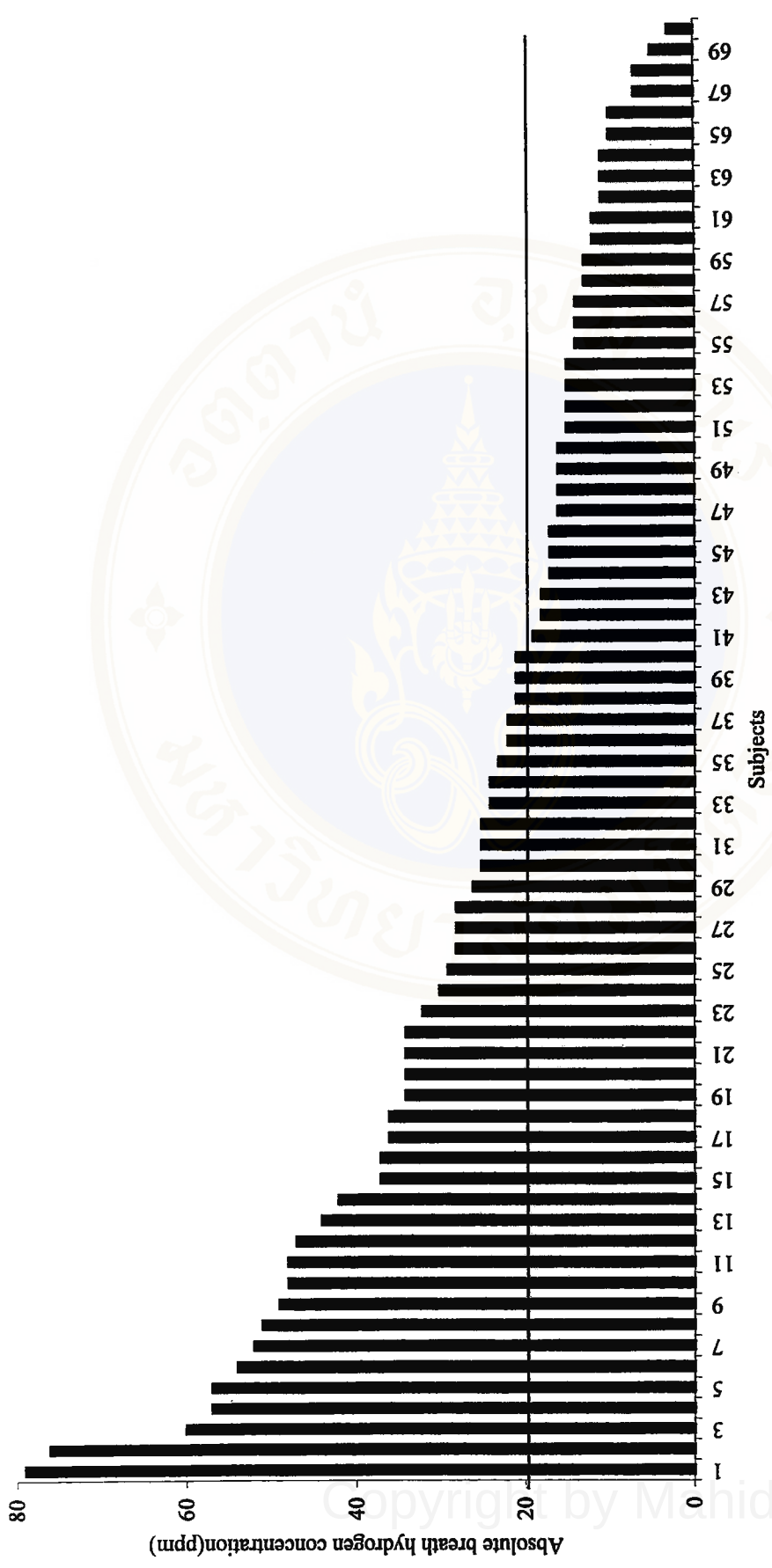

Figure 9. Absolute peak hydrogen concentration (ppm) in expired air of individuals after ingestion of 250-ml milk, n= 70. The line represents a rise in breath hydrogen concentration of 20 ppm above the baseline, taken as the cut-off criterion for classification of lactose maldigester

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Table 4. The number of breath methane producers after 250-ml whole milk consumption

Subjects	Number of subjects (%)	
	$CH_4 \leq 2$ ppm	$CH_4 > 2$ ppm
Lactose maldigester (n = 40)	27 (39%)	13 (19%)
Lactose digesters (n = 30)	15 (21%)	15 (21%)
Total (n = 70)	42 (60%)	28 (40%)

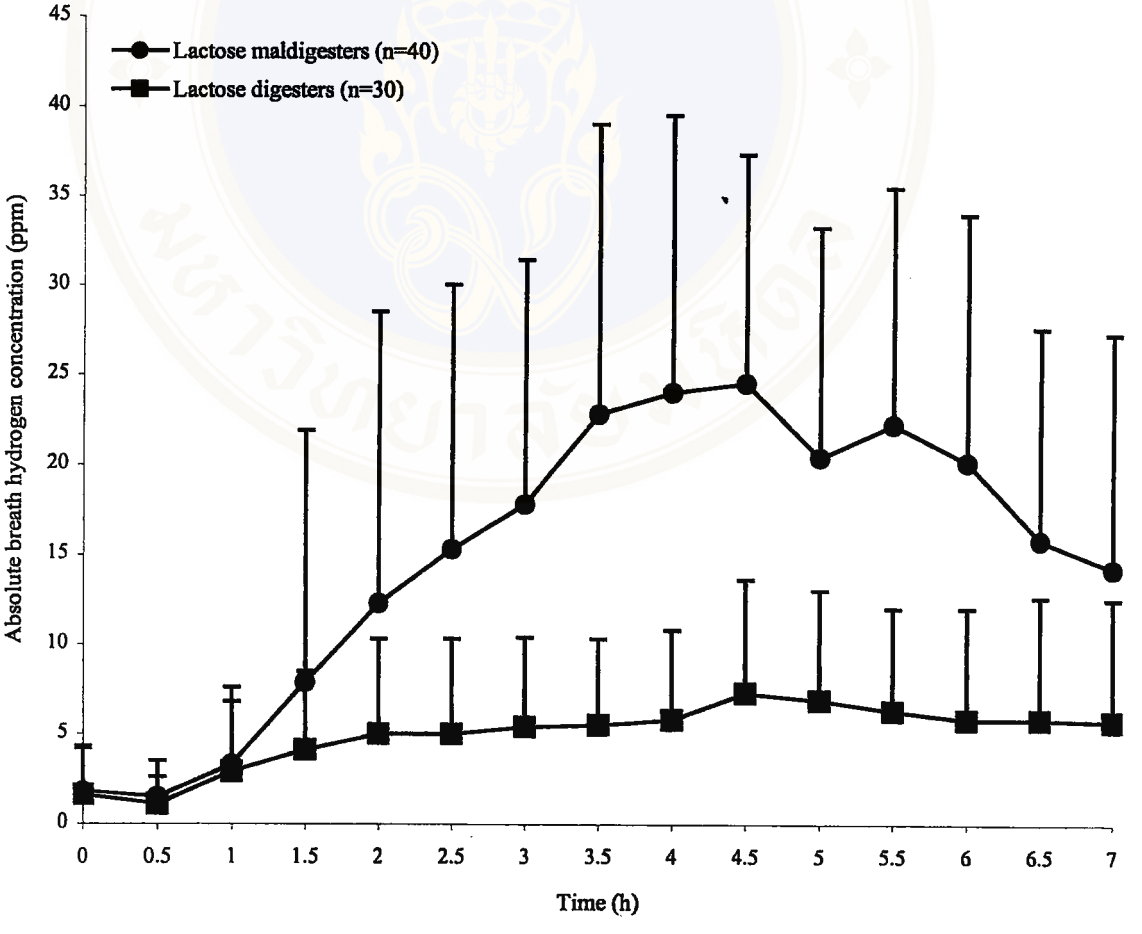

Figure 10. Average changes in absolute breath hydrogen concentration (ppm) after ingestion of 250 ml milk in lactose maldigesters (•)(n=40) and lactose digesters (■)(n=30).

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Table 5. Number of lactose maldigesters based on different criteria

Criteria	Number of lactose maldigesters (%)
Breath $H_2 \geq 20$ ppm	40 (57%)
Breath $H_2 + CH_4 \geq 15$ ppm	10 (14%)
Total (out of 70 subjects)	50 (71%)

4.2.3 Lactose intolerants based on the gastrointestinal symptoms

The subjects recorded gastrointestinal symptoms: abdominal pain, diarrhea, nausea, bloating and flatulence which occurred after test diet consumption. Gastrointestinal symptoms experiences were found in 28 out of 40 (70%) lactose maldigesters (identified by breath hydrogen production). They were classified as lactose maldigester intolerants. The rest of lactose maldigesters (30%) who had no symptoms were classified as lactose maldigester tolerants.

Mild diarrhea (8 subjects), flatulence and abdominal pain were recorded in 17 out of 30 previously classified as lactose digesters (57%) during the period of breath hydrogen collection. Of the 17 lactose intolerants, 8 subjects who had diarrhea and 5 who produced methane and hydrogen $\geq$ 15 ppm were, therefore, classified as additional lactose maldigesters-intolerants. The rest four subjects were lactose digesters (based on hydrogen and hydrogen-methane production), hence, they were called lactose digester-intolerants.

The subjects who reported no gastrointestinal symptoms were 13 out of 30 previously classified as lactose digesters based on hydrogen production. Five of them

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were subsequently classified as lactose maldigesters based on hydrogen and methane production, they were thus called lactose maldigester-tolerants. The rest of them were called lactose digester-tolerants. In total of 70 middle age adults, only 17 of lactose madigesters and 8 lactose digester were lactose tolerants (36%). The conclusion of the results is shown in **Table 6**.

Table 6. Lactose intolerance and tolerance after drinking 250-ml whole milk in 70 middle age adults

GI symptoms	Number of subjects (%)		Total
	Lactose digesters (Breath $H_2 \geq 20$ ppm) 30 (43%)	Lactose maldigesters (Breath $H_2 \geq 20$ ppm) 40 (57%)	100%
Intolerance	17 (24%) (*8* with diarrhea) → *8*; (*5* with methane and hydrogen ≥15 ppm) → *5*	28 (40%)	64%
Tolerance	13 (19%) (*5* with methane and hydrogen ≥15 ppm) → *5*	12 (17%)	36%

The gastrointestinal symptoms experienced by the adult subjects were considered minimal, the symptom intensity scores in the final of 12 lactose digesters (based on breath hydrogen, hydrogen plus methane production, and diarrheal symptom) and 58 lactose maldigesters were 0.01 and 0.12, respectively **(Appendix H).** The individual gastrointestinal symptoms intensity scores, occurred in the lactose maldigesters

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including subjects with diarrhea (n=58), and their accumulative breath hydrogen production had no statistically correlation (P=0.979). If subjects with diarrhea were excluded (n=43), there was a significant correlation between symptom intensity of individuals and their cumulative breath hydrogen production (P=0.015).

4.2.4 Milk consumption habit of the subjects

Of 70 adults who participated in this study, 90% of them consumed milk but only 29% drank milk regularly **(Table 7).** The volume of milk normally consumed was 250-ml. About 20% of the subjects who were identified as lactose maldigesters based on the hydrogen and methane production and with diarrhea, and about 17% of lactose digesters gave self-recorded of gastrointestinal experience and most occurred 1-2 h after drinking milk.

Average calcium intake of the studied middle age adults (from 3-day record) was 505 $\pm$ 226 mg/day.

Table 7. Characteristics of the subjects: questionnaire survey

Frequency of milk consumption	Total subjects		
	Lactose digesters	Lactose maldigesters	Total (%)
			N=70
Do not consume milk at all	0	7	7 (10)
Every day	4	16	20 (29)
2-3 times/week	8	29	37 (53)
2-3 times/month	-	6	6 (8)

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Table 7. Characteristics of the subjects: questionnaire survey (continued)

Frequency of milk consumption	Total subjects		
	Lactose digesters	Lactose maldigesters	Total (%)
Amount of milk consumed Per day :			N=63
- <250 ml	-	3	3 (5)
- 250 ml	10	46	56 (89)
- 500 ml	2	2	4 (6)
Experience in gastrointestinal symptoms:			N=63
- yes	2	10	12 (19)
- no	10	41	51 (81)
After milk consumption, time period which the gastrointestinal symptoms occurred :			N=12
- suddenly	-	1	1 (8)
- 0.5 h.	-	2	2 (17)
- 1-2 h.	2	7	9 (75)

4.3 Effect of imitated milk products consumption on lactose digestion and tolerance in lactose maldigesters

Of 40 lactose maldigesters, 36 agreed to participate in this study. Breath hydrogen production and gastrointestinal symptoms experienced in the subjects were used to evaluate the effect of drinking low and lactose-free imitated milk.

4.3.1 Production of breath hydrogen

The maximum absolute peak height of breath hydrogen produced in individuals after consumption of whole milk, low-lactose and lactose-free milk ranged from 21-79,

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1-20 and 1-19 ppm, respectively. All lactose maldigesters showed lower absolute peak height and cumulative breath hydrogen concentration after drinking low-lactose and lactose-free milk compared to whole milk (**Figure 11** and **Figure 12**). The mean ± SD of absolute peak height production of individuals after whole milk, low lactose and lactose-free milk consumption were 37.9 ± 15.6, 5.9 ± 4.7, 4.8 ± 3.5 ppm, respectively (**Table 8**). The mean ± SD of cumulative breath hydrogen excretion after whole milk, low-lactose and lactose-free milk consumption were 6,495 ± 3,261, 626 ± 629, and 687 ± 925 ppm.min, respectively (**Table 8**).

The average pattern of change in absolute breath hydrogen concentration throughout 7-h period was shown in **Figure 13** and **Appendix G.**

The level of absolute peak hydrogen concentration and cumulative breath hydrogen production by the subjects after low-lactose and lactose-free milk consumption were significantly lower ($p < 0.05$) than whole milk consumption. No differences in these parameters were found in the subjects after low-lactose and lactose-free milk consumption.

4.3.2 Production of breath methane

Twenty-two subjects out of 36 produced methane > 2 ppm after having the imitated low-lactose and lactose-free milk **(Appendix D).** Of these, the same number of subjects, 4 (11%), produced hydrogen and methane at the level ≥ 15 ppm. They were still being lactose maldigesters.

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Table 8. Absolute peak hydrogen concentration and cumulative breath hydrogen production (area under hydrogen production curve) as mean ±SD after consumption of whole milk, low lactose milk and lactose-free milk

Test diet	Absolute peak hydrogen (ppm)	Cumulative breath hydrogen (ppm.min)
Milk	37.9 ± 15.6 [a]	6495 ±3261 [a]
Low-lactose milk	5.9 ± 4.7 [b]	626 ±629 [b]
Lactose-free milk	4.8 ± 3.5 [b]	687 ± 925 [b]

Note: Numbers with different superscripts in the same column are significantly different, $p < 0.05$

4.3.2 Gastrointestinal symptoms

Gastrointestinal symptoms are shown in **Appendix H.** Mild symptoms were reported in 13 and 2 out of 36 (36 and 6%) after low-lactose and lactose-free milk consumption, respectively. The symptom intensity scores were significant different, 0.04 and 0.005, which were classified as mild symptom intensity (mild = 0-0.33, moderate = 0.34-0.67 and severe = 0.68-1). Marked decreasing in the gastrointestinal symptoms between individuals after consumption of low-lactose and lactose-free milk which showed significant differences ($P<0.05$) in gastrointestinal symptom intensity scores compared to that of the whole milk.

However, 2 subjects out of 36 had mild diarrhea after drinking low-lactose milk, they were counted as lactose maldigesters based on the presence of diarrhea. None was found with diarrhea after lactose-free milk.

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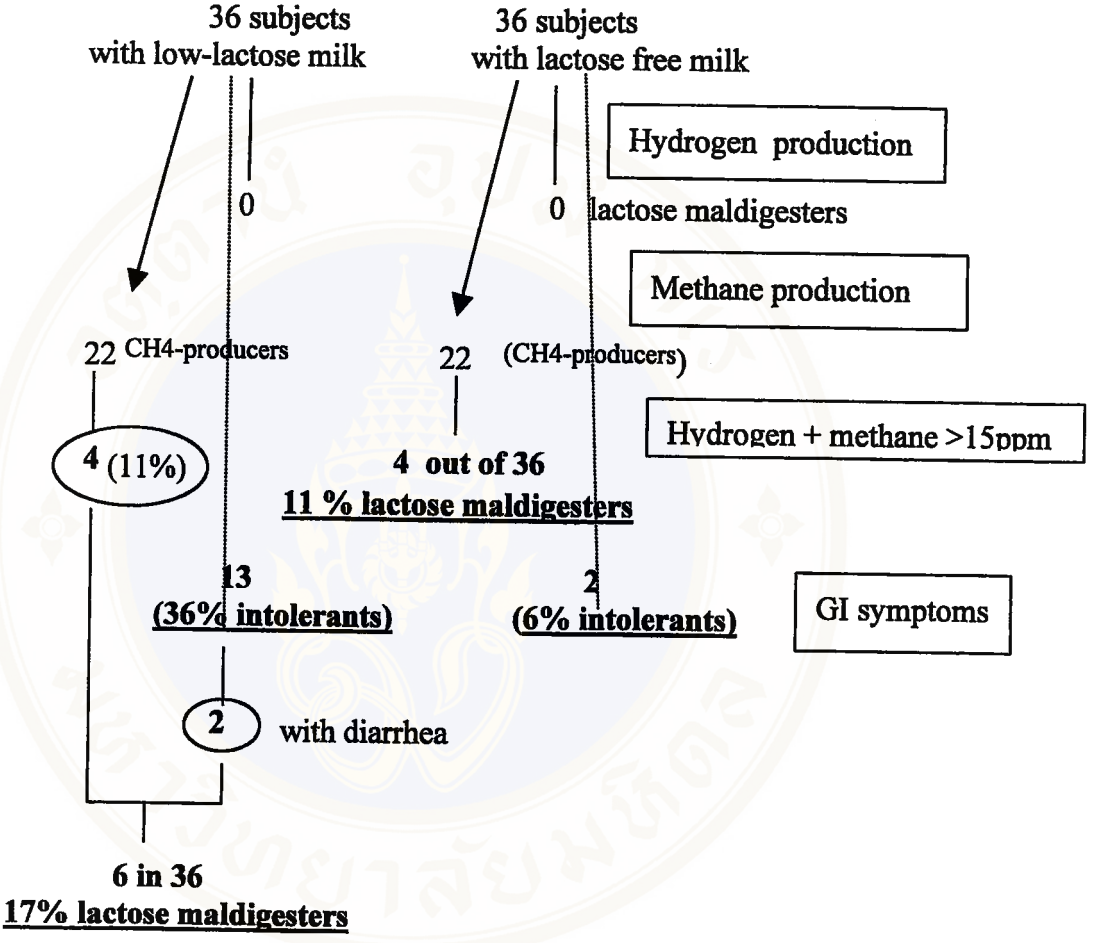
Lactose maldigesters and intolerants after drinking of low-lactose and lactose-free milk



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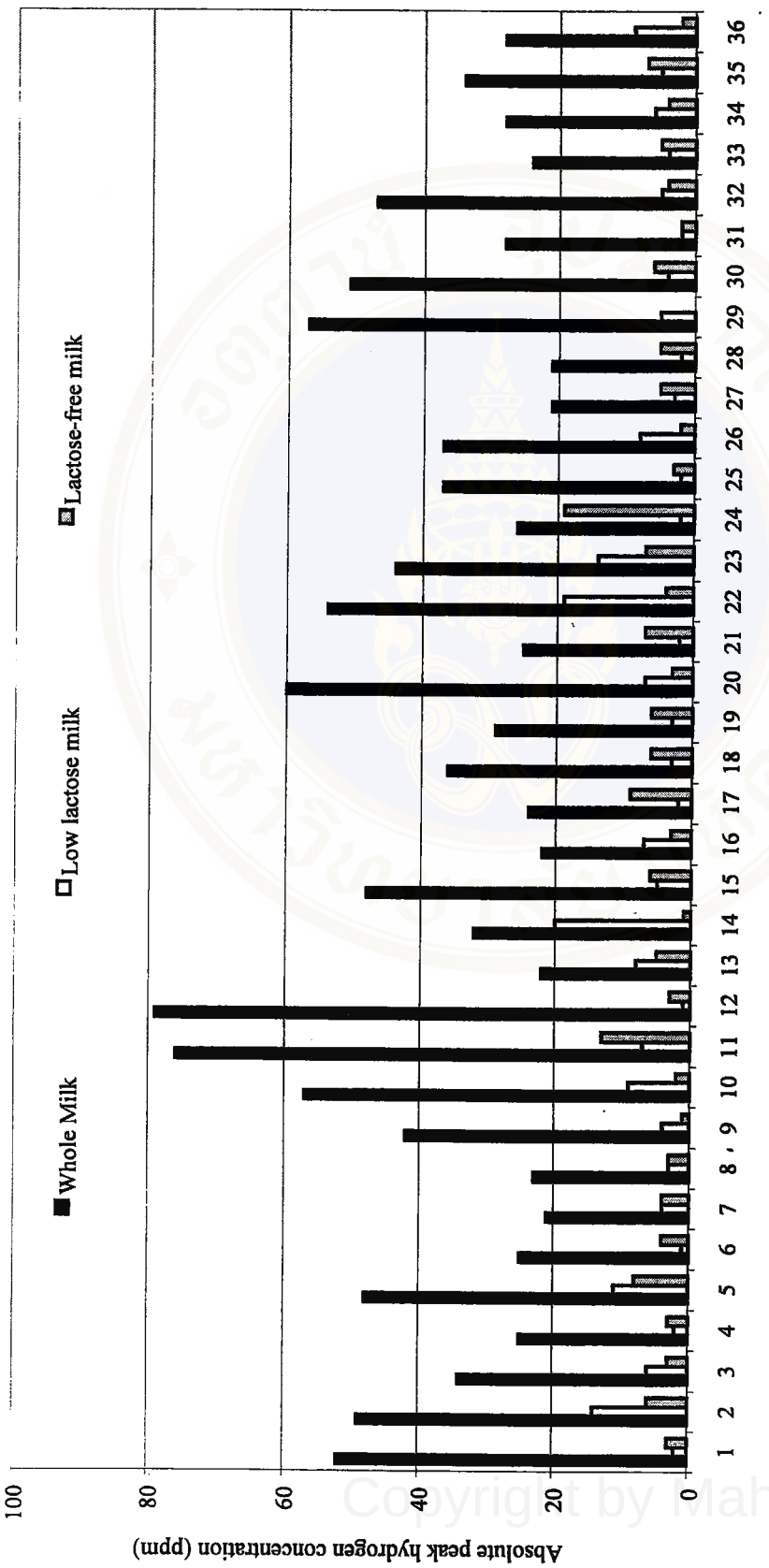

Figure 11. Individual absolute peak hydrogen concentration (ppm) over the 7 h period in lactose maldigesters (n=36) after ingestion of 250- ml whole milk, low lactose milk and lactose free milk

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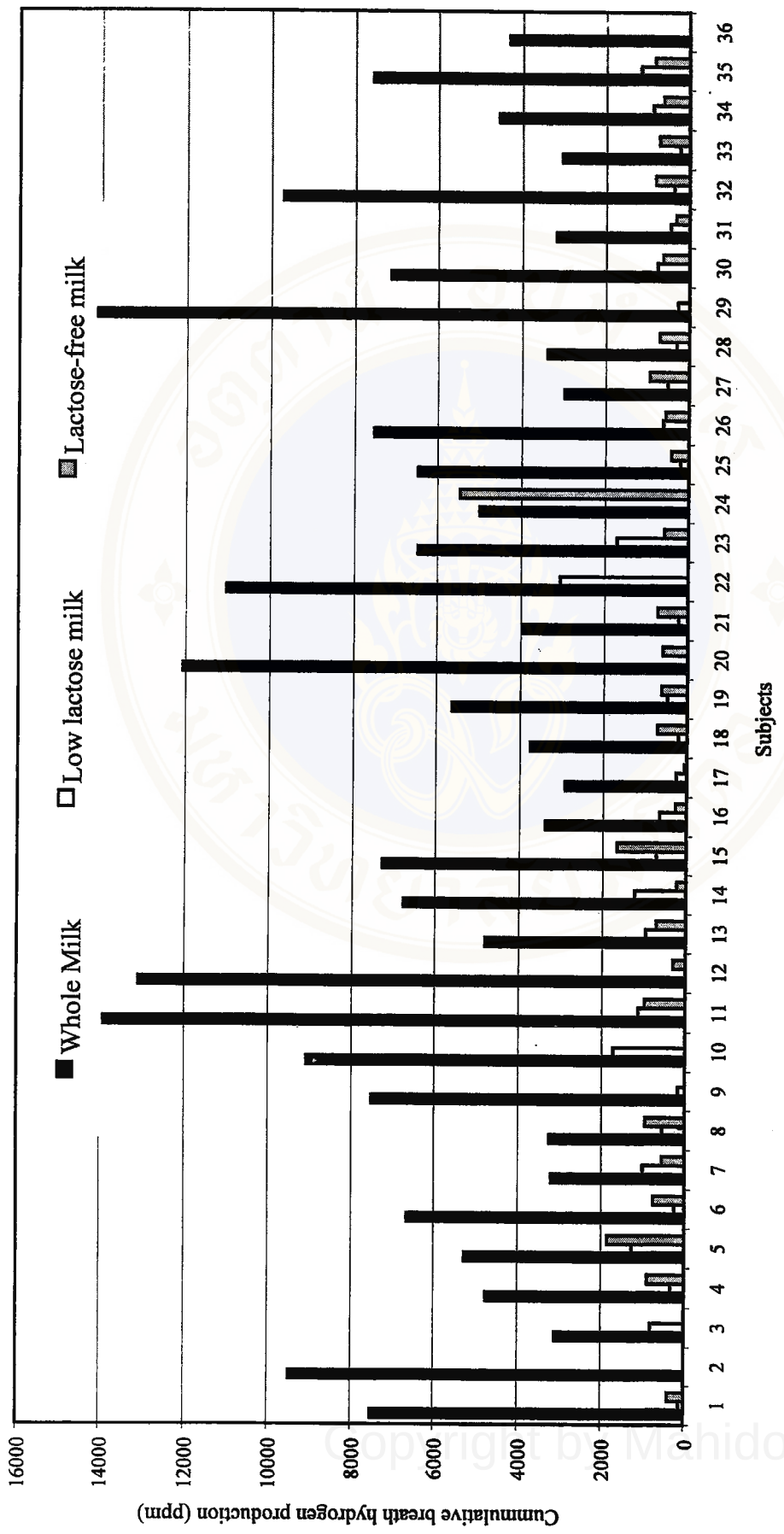

Figure 12. Individual cumulative breath hydrogen production (ppm.min) over the 7 h period in lactose maldigesters (n=36) after ingestion of 250-ml whole milk, low lactose milk and lactose-free milk

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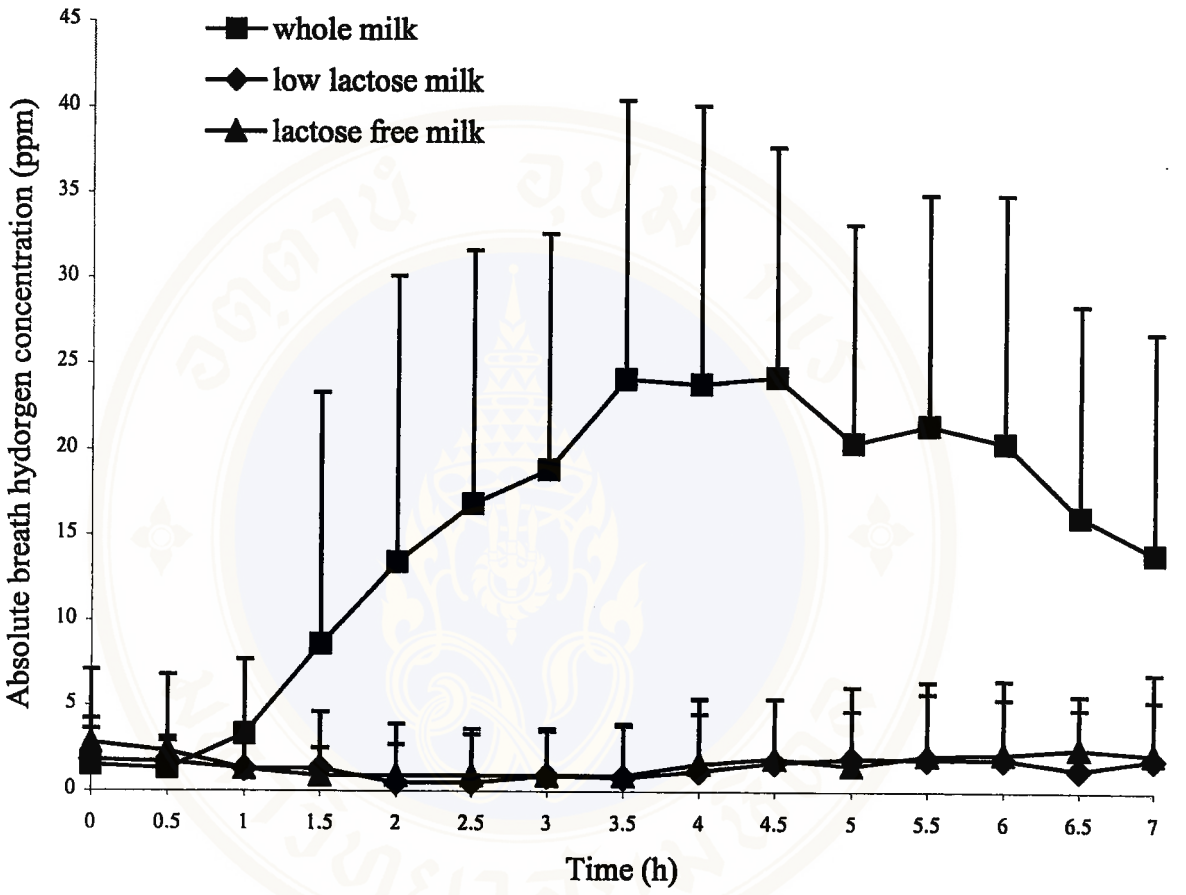

Figure 13. Average changes in absolute breath hydrogen concentration (ppm) after ingestion of 250-ml whole milk (■), low-lactose milk (◆), and lactose-free milk (▲).

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CHAPTER V

DISCUSSION

5.1 Milk consumption habit in middle age Thai people

Studied middle age adults were teachers and instructors, it was not surprising that most of them drank milk which showed their concern about bone health. However, only 33% of them drink milk regularly. The main reasons were having experience of undesirable gastrointestinal symptoms and do not like milk flavor.

5.2 Lactose maldigestion and intolerance in Thai middle age adults

In this study, a 250-ml (1 serving, containing 12 g lactose) whole milk was used as the physiological test diet to identify lactose maldigesters and intolerants which was agreed with the test volume of several experiments (15, 16, 17, 133). A high dose of lactose (50g), which equals to drinking of about 1 liter of milk, was used as a test diet in previous studies (12, 13). This is obviously an unusual feeding habit, which resulted in the findings of high prevalence of lactose maldigester (96-100%) in Thai adults. Sategna-Guidettic *et al* (182) suggested that milk rather than pure lactose must be used in clinical evaluation of lactose malabsorption and intolerance because milk lactose is better digested and induce intolerance in fewer subjects.

Hydrogen breath test is a high sensitivity and specificity compared with other method, in addition, it is non-invasive rapid and sensitive enough to detect the maldigestion of as small as 2 g of lactose (92, 119, 180). Thus, the hydrogen breath test was selected to determined lactose maldigestion in this study. A rise in breath

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hydrogen concentration of ≥ 20 ppm above base line was the criterion for classification as a lactose maldigester (102, 132, 133, 134). Based on breath hydrogen production, 57% of the studied Thai middle age adults were lactose maldigesters.

Apart of breath hydrogen production, methane in expired air was also measured in the subjects. This is because previous studies have shown that breath methane excretors have lower breath hydrogen production following ingestion to test does of the non-absorbable sugar than non-methane producers (141, 142). False negative results may occur in the subjects unable to produce hydrogen if methane is not measured. Based on the methane production of greater than 2 ppm above ambient air to defined as methane producer, 40% of the studied middle age adults were methane producers. Previous studies (37, 38) found that 14% and 21% Thai adolescents were methane producers. Since methane is produced from hydrogen, therefore, the sum of absolute hydrogen and methane production in breath of at least 15 ppm is considered to indicate for lactose maldigestion (183). According to this criteria, additional 14% of the subjects who were previously identified as lactose digesters, were thereafter identified as lactose maldigesters. It is therefore strongly recommended that breath methane should be concurrently measured in the study of lactose digestion. Based on the breath hydrogen and methane production, 71% of the middle age Thai adults were identified as lactose maldigesters. Kvammana (39) applied this criteria and found 40% of the studied Thai adolescents were lactose maldigesters.

About 36% of the studied middle age adults had no experience of gastro intestinal symptoms after drinking of a 250-ml whole milk (lactose tolerants). This suggested that, the majority of these group (64%) still had gastrointestinal discomfort

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after milk consumption, but in this case, only with mild degree of symptoms (flatulence, abdominal cramp, diarrhea). Previous study reported 60-75% of lactose intolerants in Thai adults (15, 16). Mild diarrhea was occurred in 1/3 of the lactose intolerants (15 in 45 lactose intolerants). Eight of them were found among subjects who were previously identified as lactose digesters due to their hydrogen production was lower than 20 ppm. They were finally classified as additional lactose maldigesters. This is on the basis that diaarhea occurs only when the rate of delivery of undigested lactose to the colon exceeds the rate at which the bacteria ferment the undigested lactose, this in turn can reduce the sensitivity of the diagnosis of lactose maldigestion by reducing the magnitude of hydrogen production (49, 113). Based on the criteria of hydrogen plus methane production and diarrheal symptom, 18 adults were re-classified as additional lactose maldigesters. In total, 83% (58 out of 70) of the studied middle age adults were lactose maldigesters.

No clear correlation has been established between lactose maldigestion, lactose intolerance and milk intolerance in previous studies (69, 184). In this study, when subjects with diarrhea were excluded, there was a significant correlation between symptom intensity of individuals and their cumulative breath hydrogen production in lactose maldigesters. However, mild gastrointestinal symptoms (without diarrhea) were also found in some of the lactose digesters (4 lactose digester-intolerants out of 12). This finding was similar to the previous studies (38, 37, 39, 61, 62, 66, 185). It was suggested that the subjects may misattribute a variety of abdominal symptoms to the lactose intolerance (63). These results suggested that symptomatic response cannot be used as a single parameter to indicate the lactose maldigestion. Unlike the hydrogen breath test which has been recognized, with high confidence, as the most

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reliable indirect method to confirm that the gastrointestinal symptoms after milk consumption is due to the unabsorbed lactose (102, 103, 186). Nevertheless, gastrointestinal symptoms, especially the presence of diarrhea, must be included in the study of carbohydrate digestion to indicate severe maldigestion.

From self-record (questionnaire survey), subjects with gastrointestinal symptoms was about 18% which was much lower than the lactose intolerants found after 250-ml milk (64%) was given during the studied period. The same finding was reported in the study of Chancharupong (38). More awareness of symptoms during the experiment period of the subjects than drinking milk in daily life could result in the higher record of symptoms.

5.3 Effect of imitated milk products consumption on lactose digestion and tolerance in middle age adult lactose maldigesters

Hydrogen production, as indicated by peak hydrogen concentration after low-lactose milk (2 g lactose) and lactose-free milk consumption were significantly lower than after whole milk consumption and none indicated lactose maldigestion based on hydrogen production. Cumulative breath hydrogen production showed significantly differences in the same direction with that of peak hydrogen concentration. However, at present no criteria for cut-off point in using of cumulative breath hydrogen to identify lactose maldigesters. However, 10% of these subjects were methane producers, they retained their lactose maldigestion status.

Based on the gastrointestinal symptoms, 36% reported gastrointestinal symptoms and 6 % of the subjects had diarrhea after low-lactose consumption. Several previous studies reported the small lactose dose given did not cause significant

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difference in the severity of gastrointestinal symptoms (64, 66, 165, 187). There are many factors such as social, culture habits, attitudes toward milk and psychological are significant factors in the prevalence of such symptoms (66). Moreover, several studies have shown that lactose maldigesters may report symptoms from consuming milk that are not because of its lactose content (66, 69, 70).

Benjapong's study (16) showed that half a cup of milk consumption (approximate 6 g lactose) in subjects could reduce the degree of lactose maldigestion and intensity of gastrointestinal symptoms. Miller *et al* (166) suggested that milk with as little as 50% lactose reduction can produced a significant reduction in the signs and symptoms of intolerance in lactose maldigesters. However, Paige *et al* (44) found that 90% lactose-hydrolyzed milk had more effective than other level. They showed that 50% lactose hydrolyzed milk (6 g% lactose) consumption in lactose maldigesters had lower blood sugar rise than 90% lactose-hydrolyzed milk (1.2 g% lactose) and symptoms could not be considered a factor in detected as lactose maldigesters.

From this study it can be concluded that the majority of the studied middle age adults could tolerate low-lactose and lactose-free milk based on the production of hydrogen, methane and gastrointestinal symptom (diarrhea) after imitated milk consumption. Since lactose-free milk showed significant lower intensity than that produced after low-lactose milk (2g lactose) consumption, therefore, for the sensitive middle age adults, the lactose-free milk is recommended.

Dairy products provide the majority of daily calcium intakes for most people as well as making substantial contributions to other nutrient intake. Lactase deficiency and consequent milk avoidance has been likely to cause some forms of osteoporosis (32, 188). Average calcium intake of the studied middle age adults was 63% (505±226

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mg/day) of the Thai RDA (88) and in total 83% of them were lactose maldigesters and 64% were lactose intolerants. Thus, imitated low-lactose or lactose-free milk in this study was a choice for lactose intolerants.

In previous study, showed that lactose can enhance calcium absorption (97) and other nutrients in milk especially protein and minerals were recovered in greater quantities in lactase deficiency (30). Nielsen *et al* (189) found that calcium absorption to be significantly higher with the diet containing hydrolyzed milk than with the lactose-free diet supplemented with extra calcium. The finding in this study showed that both type of milk can markedly reduce the lactose intolerance gastrointestinal symptoms compared to the whole milk. However, the cost of raw materials used in the preparation of lactose-free milk was higher than low-lactose milk (46). It is suggested that low-lactose milk can be used for general lactose intolerants and lactose-free milk (lactose=0g) is recommended only for the sensitive lactose intolerants.

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CHAPTER VI

CONCLUSION

Lactose digestion and tolerance were studied in 70 Thai middle age adults, using hydrogen breath test with a 250-ml milk as the test diet. The effect of low- lactose imitated milk and lactose-free milk consumption on lactose digestion and tolerance was investigated in 36 lactose maldigesters. It was found that 57% of the studied subjects were lactose maldigesters when hydrogen in breath was used as the criteria for classification of lactose maldigestion. When both breath hydrogen and methane production were considered, 71% of subjects were identified as lactose maldigesters. An additional of 12% of previously classified lactose digesters with diarrhea were included. In total, 83% of the studied middle age adults were identified as lactose maldigesters and 64% were lactose intolerants.

In addition of reducing of the amount of milk consumed or drinking milk with meal or having fermented milk, consumption of imitated low-lactose milk (with 2 g lactose) or lactose-free milk be another choice for lactose intolerants. This is because imitated low-lactose milk (with 2 g lactose) and lactose-free milk can markedly reduced the degree of lactose maldigestion as well as the intensity of lactose intolerance. Due to the lower cost of the product, imitated low-lactose milk can be a good choice for non-sensitive lactose intolerants to promote nutritional status especially, calcium. However, the imitated low-lactose milk, 2 g lactose, may not be the optimal level, further study of the higher level may be needed in order to minimise the production cost. Lactose-free milk is, however, still necessary for the highly sensitive lactose intolerants.

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RECOMMENDATION AND SUGGESTION

FOR FURTHER STUDY

1. In the study on lactose digestion, false negative can be detected when hydrogen production is the only parameter used. This can be due to the ability of methane producers who can convert hydrogen to methane by bacteria in colon. Thus, breath methane should be measured in conjunction with hydrogen breath test for reliable results in low hydrogen producer subjects.

2. Although gastrointestinal symptoms are subjective and variable among the subjects, they (diarrhea in particular) must be included in the study of lactose digestion, to indicate severe maldigestion.

3. In this study, the imitated low-lactose at 80% level of the whole milk was used. Further research should be considered using several levels of lactose in milk in order to get the more suitable level of lactose, which could be used to reduce lactose intolerance.

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APPENDIX A

Questionnaire form for milk and milk product consumption

Code No.....

แบบสอบถามบริโภคนิสัยการดื่มนมและผลิตภัณฑ์นม

ประวัติทั่วไป

1. ชื่อ-สกุล .....

2. อายุ ..... ปี

3. น้ำหนัก .....กก. ส่วนสูง .....ซม.

4. เพศ ☐ ชาย ☐ หญิง

5. การศึกษา ☐ ระดับประถมศึกษา ☐ ระดับอนุปริญญา
☐ ระดับมัธยมศึกษา ☐ ระดับปริญญาตรี
☐ ระดับอาชีวศึกษา ☐ สูงกว่าปริญญาตรี

ประวัติการเจ็บป่วย

6. ท่านมีโรคประจำตัวหรือไม่

☐ ไม่มี ☐ มี โปรดระบุ .....

7. ท่านเคยเป็นโรคเกี่ยวกับระบบทางเดินอาหารหรือไม่

☐ ไม่เคย ☐ เคย โปรดระบุ .....

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8. ท่านมียาที่รับประทานอยู่เป็นประจำหรือไม่

☐ ไม่มี ☐ มี โปรดระบุ .....

ประวัติการดื่มนมและผลิตภัณฑ์นม

9. ปกติท่านดื่มนมหรือไม่

☐ ไม่ดื่ม (หากตอบว่าดื่มให้ข้ามไปตอบข้อ 15)

☐ ดื่มทุกวัน

☐ ดื่ม 2-3 ครั้ง/สัปดาห์

☐ นานๆ ครั้ง โปรดระบุ.....

10. <u>ส่วนใหญ่</u> ท่านมักจะดื่มนมเวลาใดและมีอาการไม่สบายต่างๆ หรือไม่

☐ ก่อนอาหาร → ☐ ไม่มีอาการ
→ ☐ มีอาการ (ระบุ) .....

☐ หลังอาหาร → ☐ ไม่มีอาการ
→ ☐ มีอาการ (ระบุ) .....

☐ ขณะท้องว่าง → ☐ ไม่มีอาการ
→ ☐ มีอาการ (ระบุ) .....

11. หลังดื่มนมไปแล้ว

11.1 แสดงอาการไม่สบายต่างๆ เช่น .....

☐ ทุกครั้งที่ดื่ม ☐ เป็นบางครั้ง ☐ ไม่เป็น(ข้ามไปตอบข้อ 12)

11.2 เวลาที่แสดงอาการ

☐ หลังดื่มนมทันที ☐ ทิ้งระยะเวลาพอสมควรประมาณ.....ช.ม.

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12. ท่านชอบดื่มนมรสอะไร

☐ จืด ☐ หวาน

☐ ช็อคโกแลต ☐ นมสตรอเบอรี่ยี่ห้อ .....

☐ รสอื่นๆ โปรดระบุ .....

13. ปริมาณของการดื่มนมเฉลี่ยต่อ 1 ครั้ง

☐ 1/2 แก้ว - กล่อง - ถุง / ครั้ง

☐ 1 แก้ว - กล่อง - ถุง / ครั้ง

☐ 2 แก้ว - กล่อง - ถุง / ครั้ง

☐ อื่นๆ โปรดระบุ .....

14. ปริมาณของการดื่มนมเฉลี่ยใน 1 วัน

☐ 1/2 แก้ว - กล่อง - ถุง / วัน

☐ 1 แก้ว - กล่อง - ถุง / วัน

☐ 2 แก้ว - กล่อง - ถุง / วัน

☐ มากกว่า 2 แก้ว-กล่อง-ถุง/วัน (ระบุ) .....แก้ว-กล่อง - ถุง/วัน

15. ท่านไม่ดื่มเพราะเหตุใด

☐ ดื่มแล้วมีอาการไม่สบายต่างๆ เช่น .....

☐ ไม่ชอบรสชาตินม

☐ เหตุผลอื่นๆ เช่น.....

16. นอกจากดื่มนมแล้ว ท่านรับประทานผลิตภัณฑ์อื่นๆที่ทำจากนมบ้างหรือไม่ เช่น

โยเกิร์ต นมเปรี้ยวพร้อมดื่ม ไอศกรีม เนยแข็ง

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☐ ไม่เคยรับประทาน

☐ รับประทาน โปรดระบุชนิด .....จำนวน..... ถ้วย-กล่อง/ครั้ง

และใน 1 วันท่านรับประทานจำนวน..... ถ้วย-กล่อง/วัน

17. หากรับประทานผลิตภัณฑ์อื่นๆ ที่ทำจากนมดังเช่นข้อ 16 หลังจากรับประทานแล้วมีอาการไม่สบายท้องหรือไม่

☐ ไม่มีอาการ

☐ มีอาการ (ระบุ) .....

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APPENDIX B

Record form for 24 hour dietary intake

แบบสอบถามอาหารที่รับประทานภายใน 24 ชั่วโมง

ชื่อ-สกุล ..... อายุ ..... ปี เพศ ( ) ชาย ( ) หญิง

วันที่บันทึกรายการ..... ( ) วันราชการ ( ) วันหยุด

อาหารที่รับประทานในวันนี้ ( ) ปกติ ( ) พิเศษ

มื้ออาหาร	เมนู	ส่วนประกอบอาหาร	น.น./กรัม หรือ ปริมาณ	เจ้าหน้าที่กรอก	
				Food code	Raw Wt (gm)

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APPENDIX C

Record form for gastrointestinal symptoms

แบบบันทึกอาการต่างๆ ที่เกิดขึ้นภายหลังการดื่มนม

ชื่อ-สกุล ..... ตื่นนอนเวลา ....น. เวลาที่เริ่มดื่มนม ..... น.

เวลาหลังการดื่มนม	ไม่มีอาการ	ปวดท้อง			ผายลม /เรอ			คลื่นไส้			อาเจียน			ท้องเสีย			อาการอื่นๆ ระบุ		
		1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3

1 = อาการเล็กน้อย : 1-3 ครั้ง/ชั่วโมง 2 = อาการปานกลาง : 4-6 ครั้ง/ชั่วโมง 3 = อาการหนัก : 7-9 ครั้ง/ชั่วโมง

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APPENDIX D

Absolute breath hydrogen concentration at 30 min. interval, peak hydrogen concentration (ppm), area under curve of breath hydrogen production (ppm-min.) and methane concentration in lactose maldigesters (n=40)

Subject number	Test period	Time after taking test product (hr)															Peak H_2 concentration	Area under curve	Methane concentration
		0	0.5	1	1.5	2	2.5	3	3.5	4	4.5	5	5.5	6	6.5	7			
1 (1)	M	1	0	0	0	-1	5	15	45	7	46	52	33	23	19	12	52	7515	0
	L	2	0	0	0	0	0	0	1	0	0	0	0	2	0	0	2	120	4
	H	1	0	1	0	0	0	1	0	1	1	2	1	3	2	1	3	390	2
2 (2)	M	0	2	4	49	20	43	11	19	28	33	16	14	23	25	58	49	9480	0
	L	1	4	0	14	-3	-7	-7	-7	-6	-6	-6	-8	-6	-8	-8	14	-1485 *	0
	H	6	0	2	6	-2	-3	-4	-3	-3	-1	-1	0	-5	1	5	6	-225 *	2
3 (3)	M	2	0	0	2	3	34	13	9	9	11	11	6	5	0	-1	34	3105	0
	L	2	1	0	2	0	1	2	1	2	2	3	2	6	3	2	6	810	3
	H	0	0	0	3	-1	0	-1	0	-1	-1	-1	-1	-1	-1	0	3	-150 *	5
4 (4)	M	0	2	2	3	0	9	22	25	25	17	20	25	14	-3	-5	25	4755	2
	L	1	1	0	0	-1	0	-1	1	2	0	2	0	1	1	9	2	330	2
	H	3	1	1	0	0	0	0	2	2	2	1	2	3	13	2	3	885	3
5 (5)	M	0	4	2	1	1	7	-	48	30	15	14	16	15	16	13	48	5265	10
	L	0	2	2	1	2	1	1	1	2	1	5	11	7	4	4	11	1260	6
	H	18	12	0	4	6	5	5	3	8	4	3	-3	2	2	3	8	1845	3
6 (6)	M	3	0	14	19	15	14	17	23	23	25	20	16	17	13	9	25	6660	2
	L	4	0	1	0	0	0	-1	0	1	0	0	1	1	2	2	1	240	0
	H	0	5	1	2	1	1	1	1	0	1	2	2	4	3	2	4	750	2

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APPENDIX D (cont.)

Absolute breath hydrogen concentration at 30 min. interval, peak hydrogen concentration (ppm), area under curve of breath hydrogen production (ppm-min.) and methane concentration in lactose maldigesters (n=40)

Subject number	Test period	Time after taking test product (hr)															Peak H_2 concentration	Area under curve	Methane concentration
		0	0.5	1	1.5	2	2.5	3	3.5	4	4.5	5	5.5	6	6.5	7			
7 (7)	M	2	0	2	1	1	1	4	7	21	18	12	14	-	17	15	21	3195	17
	L	0	2	1	4	4	4	3	2	3	2	2	2	2	2	1	4	1005	2
	H	1	0	1	0	-1	0	0	1	-1	3	2	4	4	3	3	4	540	11
8 (8)	M	0	0	5	6	16	14	17	23	-1	7	4	10	-4	9	4	23	3240	3
	L	2	1	0	0	1	0	1	1	1	3	3	1	2	1	4	3	540	3
	H	10	8	4	0	1	1	3	1	1	1	1	1	2	2	1	3	945	3
9 (9)	M	0	3	0	4	8	30	39	31	40	42	11	12	12	12	13	42	7515	0
	L	3	4	4	0	1	-1	0	0	0	-2	-2	-2	1	1	0	4	165	4
	H	1	7	0	0	-2	-2	-2	-2	-2	1	0	0	0	0	-1	1	-60*	3
10 (10)	M	0	1	0	3	3	19	27	-	41	38	33	57	48	27	17	57	9075	0
	L	1	0	3	2	3	4	5	5	4	4	5	9	6	5	4	9	1725	0
	H	0	0	1	0	0	-1	-2	-1	0	0	-1	-	2	1	-2	2	-60*	3
11 (11)	M	0	2	2	71	76	53	14	54	47	24	-	31	42	32	31	76	13905	3
	L	1	2	1	0	7	4	4	7	6	2	2	2	0	0	0	7	1125	3
	H	0	0	0	0	0	1	1	1	13	6	3	5	3	-2	3	13	975	2
12**	M	0	0	8	4	3	0	4	24	20	25	31	36	26	11	39	36	6345	2
	L																		
	H																		

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APPENDIX D (cont.)

Absolute breath hydrogen concentration at 30 min. interval, peak hydrogen concentration (ppm), area under curve of breath hydrogen production (ppm-min.) and methane concentration in lactose maldigesters (n=40)

Subject number	Test period	Time after taking test product (h)															Peak H_2 concentration	Area under curve	Methane concentration
		0	0.5	1	1.5	2	2.5	3	3.5	4	4.5	5	5.5	6	6.5	7			
13 (12)	M	6	0	3	25	25	28	20	57	79	58	43	28	27	25	29	79	13065	0
	L	3	1	0	0	-2	-2	-2	-2	-4	-2	-2	-1	1	-1	-2	1	-465 *	3
	H	1	0	1	0	0	3	3	0	1	-	1	-	-	0	1	3	300	3
14 (13)	M	0	1	19	16	22	15	20	14	11	16	13	-1	5	6	6	22	4800	0
	L	1	2	1	0	0	1	1	1	4	4	4	8	3	2	1	8	960	3
	H	0	3	1	1	1	0	2	0	-1	2	2	2	4	5	3	5	705	0
15 (14)	M	3	0	6	12	32	24	20	20	26	22	25	-	27	4	12	32	6765	4
	L	0	3	0	0	0	-1	1	2	2	0	0	0	20	5	18	20	1230	30
	H	1	1	1	0	0	0	0	0	0	0	0	1	1	1	4	1	225	14
16 (15)	M	3	2	0	0	-1	0	7	1	18	31	40	48	39	36	40	48	7275	2
	L	0	3	0	0	0	0	1	2	2	4	4	5	2	0	2	5	720	3
	H	14	23	0	6	0	2	3	1	2	2	2	2	2	3	0	6	1650	2
17 (16)	M	1	0	1	1	6	9	9	11	10	7	11	22	8	14	6	22	3375	3
	L	2	0	0	0	-1	0	0	7	5	4	2	3	1	0	-1	7	645	4
	H	0	0	0	0	0	0	-1	-1	0	0	1	3	1	3	6	3	270	3
18 (17)	M	0	0	1	5	12	7	10	24	10	2	4	6	3	9	8	24	2910	0
	L	6	3	0	0	0	0	-1	-1	-1	0	0	2	1	2	1	2	255	3
	H	0	0	0	0	-1	-1	-1	-1	-1	-1	-1	9	0	0	0	9	60	3

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APPENDIX D (cont.)

Absolute breath hydrogen concentration at 30 min. interval, peak hydrogen concentration (ppm), area under curve of breath hydrogen production (ppm-min.) and methane concentration in lactose maldigesters (n=40)

Subject number	Test period	Time after taking test product (h)															Peak H_2 concentration	Area under curve	Methane concentration
		0	0.5	1	1.5	2	2.5	3	3.5	4	4.5	5	5.5	6	6.5	7			
19 (18)	M	5	0	4	22	36	17	5	-2	2	9	14	5	6	3	3	36	3750	4
	L	1	1	0	0	0	0	0	0	1	0	1	3	0	0	1	3	210	4
	H	5	4	0	1	1	0	1	1	1	1	1	2	6	2	0	6	705	5
20 (19)	M	0	1	0	1	2	1	8	9	26	29	21	27	27	25	21	29	5625	3
	L	0	1	3	0	0	0	0	1	0	2	3	1	2	2	2	3	480	3
	H	0	0	0	1	1	0	1	1	2	1	0	0	6	2	11	6	615	3
21 (20)	M	1	6	0	3	6	13	60	42	34	37	38	45	53	53	23	60	12060	3
	L	0	1	13	3	2	-7	7	6	0	-3	0	-5	-10	-10	-12	7	-270*	0
	H	3	1	2	0	1	2	1	0	0	1	0	2	3	3	4	3	585	0
22 (21)	M	1	0	5	4	2	9	17	3	25	18	12	16	7	2	3	25	3960	0
	L	1	0	0	1	0	1	-1	0	1	0	1	2	1	1	0	2	225	3
	H	1	0	1	0	-1	0	3	7	7	2	1	1	2	0	1	7	720	0
23 (22)	M	5	0	9	11	29	53	50	54	26	36	15	17	22	32	23	54	11040	2
	L	9	4	0	14	6	9	6	-7	-6	11	19	19	9	9	9	19	3060	2
	H	4	0	1	2	3	4	-3	-6	-2	-1	-2	1	-2	1	-1	4	-75*	2
24 (23)	M	4	3	1	0	8	19	25	44	25	22	22	25	18	1	2	44	6480	2
	L	3	2	0	0	1	1	1	4	10	14	12	4	3	3	1	14	1710	3
	H	9	2	1	0	-1	-1	-1	0	2	-1	-1	1	7	7	-1	7	570	4

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APPENDIX D (cont.)

Absolute breath hydrogen concentration at 30 min. interval, peak hydrogen concentration (ppm), area under curve of breath hydrogen production (ppm-min.) and methane concentration in lactose maldigesters (n=40)

Subject number	Test period	Time after taking test product (h)															Peak H_2 concentration	Area under curve	Methane concentration
		0	0.5	1	1.5	2	2.5	3	3.5	4	4.5	5	5.5	6	6.5	7			
25 (24)	M	0	2	5	0	1	2	2	15	26	26	20	23	13	20	23	26	4995	2
	L	1	1	0	0	-3	-1	2	0	0	0	-1	-1	-3	0	3	2	-120*	2
	H	8	7	15	0	15	13	12	13	15	19	17	19	15	12	12	19	5460	1
26 (25)	M	2	1	1	0	0	3	15	16	22	28	25	36	37	26	10	37	6480	0
	L	0	1	2	1	0	0	0	-1	0	1	1	1	1	0	-1	2	195	3
	H	2	2	0	0	0	1	0	0	1	1	1	1	2	3	2	3	420	3
27**	M	10	4	0	0	5	6	19	-3	25	27	3	34	14	14	10	34	4740	2
	L																		
	H																		
28**	M	1	1	1	0	0	3	13	16	30	24	16	20	16	8	7	30	4560	3
	L																		
	H																		
29 (26)	M	9	6	0	4	37	21	16	18	21	14	20	26	31	22	21	37	7530	0
	L	0	1	0	0	0	0	2	3	6	8	1	0	0	0	-1	8	615	0
	H	2	2	0	1	2	1	2	2	1	1	1	1	1	2	1	2	555	2
30 (27)	M	0	1	0	1	0	1	3	21	19	19	7	7	13	4	7	21	2985	8
	L	0	0	0	0	0	3	2	1	-	0	3	3	1	3	2	3	510	5
	H	0	0	0	1	1	1	2	3	3	4	4	3	3	5	3	5	945	12

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APPENDIX D (cont.)

Absolute breath hydrogen concentration at 30 min. interval, peak hydrogen concentration (ppm), area under curve of breath hydrogen production (ppm-min.) and methane concentration in lactose maldigesters (n=40)

Subject number	Test period	Time after taking test product (h)															Peak H_2 concentration	Area under curve	Methane concentration
		0	0.5	1	1.5	2	2.5	3	3.5	4	4.5	5	5.5	6	6.5	7			
31**	M	6	9	1	0	0	0	1	9	26	30	34	29	16	20	10	34	5490	5
	L																		
	H																		
32 (28)	M	0	0	-	0	6	13	21	21	10	11	6	12	6	6	2	21	3390	37
	L	3	2	0	0	-	2	1	1	1	1	1	0	0	0	-1	2	330	24
	H	0	0	0	1	2	3	1	2	-1	5	4	1	3	2	1	5	705	32
33 (29)	M	0	2	11	8	32	38	41	55	57	55	48	45	32	31	30	57	14100	0
	L	5	4	2	0	-1	-1	-1	-2	-2	-2	-1	0	1	5	9	5	270	3
	H	4	2	0	0	-1	-2	-1	-2	-1	-1	-1	-1	-1	0	0	0	-210*	3
34 (30)	M	0	0	0	0	3	6	33	25	23	30	20	21	51	18	16	51	7140	2
	L	0	0	4	1	2	2	2	1	3	2	4	2	1	1	1	4	765	3
	H	0	0	4	0	0	0	0	1	2	0	6	3	1	1	6	6	630	6
35 (31)	M	0	0	0	0	0	-2	4	12	-1	11	28	20	22	13	-2	28	3180	2
	L	0	1	0	0	0	0	2	-1	2	2	2	2	2	2	2	2	450	0
	H	0	0	0	0	0	0	1	1	1	2	1	0	1	2	3	2	315	3
36 (33)	M	0	2	7	9	41	39	33	32	47	36	33	24	21	-	-	47	9720	0
	L	0	2	5	-	-2	-	-2	-1	2	1	-3	-1	5	2	9	5	375	2
	H	0	1	3	1	3	2	2	2	2	2	4	3	1	1	0	4	810	0

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APPENDIX D (cont.)

Absolute breath hydrogen concentration at 30 min. interval, peak hydrogen concentration (ppm), area under curve of breath hydrogen production (ppm-min.) and methane concentration in lactose maldigesters (n=40)

Subject number	Test period	Time after taking test product (h)															Peak H_2 concentration	Area under curve	Methane concentration
		0	0.5	1	1.5	2	2.5	3	3.5	4	4.5	5	5.5	6	6.5	7			
37 (33)	M	2	2	0	0	4	3	0	16	18	24	0	10	10	10	7	24	3045	2
	L	10	5	2	0	1	-1	-2	1	-3	4	3	-1	-2	-2	-1	4	225	2
	H	7	0	4	0	5	2	3	2	1	0	0	1	1	1	1	5	720	0
38 (34)	M	2	2	1	0	3	19	23	28	18	16	11	8	8	9	10	28	4560	2
	L	0	1	0	1	0	3	3	2	2	2	0	4	6	2	6	6	870	2
	H	0	1	0	2	1	0	1	1	4	3	-2	4	1	2	5	4	615	3
39 (35)	M	0	1	7	9	6	12	29	7	25	31	34	34	26	22	19	34	7575	0
	L	0	2	2	1	3	4	4	4	5	3	4	3	1	1	3	5	1155	3
	H	0	0	0	0	1	1	2	2	2	5	4	2	2	7	-1	7	825	0
40 (36)	M	1	0	4	20	28	24	8	8	8	8	13	10	6	5	3	28	4320	0
	L	1	2	0	0	-2	-1	-2	-4	-2	-2	-3	-1	0	9	0	9	-165*	0
	H	0	0	1	2	-2	-2	-2	0	0	1	1	0	1	0	0	2	0	3

Note

M = milk

L = low lactose milk

H = hydrolyzed milk

* = area under curve less than 0 = 0

** = subjects who left the experiment before the end of the study

( ) = next running number for lactose and lactose-free milk experiment

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APPENDIX E

Absolute breath hydrogen concentration at 30 min. interval, peak hydrogen concentration (ppm) and area under curve of breath hydrogen production (ppm-min.) after whole milk consumption in lactose digesters (n=30)

Subject number	Time after taking test product (h)															Peak H_2 concentration	Area under curve	Methane concentration
	0	0.5	1	1.5	2	2.5	3	3.5	4	4.5	5	5.5	6	6.5	7			
41*	3	1	0	6	9	11	12	12	15	17	18	10	-	-2	-2	18	3285	9
42**	6	0	11	15	4	-1	-3	6	0	-8	-1	-2	-2	1	-6	15	600	0
43*	0	0	4	7	9	6	9	13	6	7	5	11	9	10	4	13	2940	3
44*	0	3	0	0	-1	11	5	-3	-1	0	-4	-1	-1	0	1	11	360	8
45*	0	1	0	4	-2	-1	1	4	6	15	11	13	11	11	7	15	2325	6
46	0	0	0	10	4	4	13	6	7	4	10	10	11	13	14	13	3000	0
47*	0	0	4	1	1	2	10	4	4	6	12	7	8	11	9	12	2235	3
48*	0	1	0	0	5	8	6	9	9	10	6	15	15	11	16	15	3090	3
49	0	7	1	0	1	-1	0	-1	0	1	-1	-2	-1	2	5	7	420	2
50	0	2	15	4	8	8	4	3	7	8	11	17	16	-3	9	17	3135	0
51	0	2	5	4	4	10	6	5	-2	3	7	11	4	0	1	11	1785	2
52*	0	3	0	1	1	-1	5	6	7	16	9	6	-4	-4	5	16	1485	4
53**	0	1	5	6	7	16	10	4	11	14	12	10	-	8	7	16	3225	0
54*	0	1	0	1	1	2	2	3	7	12	11	12	18	11	9	18	2730	3
55**	0	1	6	0	13	14	11	0	0	1	0	1	2	3	-5	14	1545	0

APPENDIX E (cont.)

Absolute breath hydrogen concentration at 30 min. interval, peak hydrogen concentration (ppm) and area under curve of breath hydrogen production (ppm-min.) after whole milk consumption in lactose digesters (n=30)

Subject number	Time after taking test product (h)															Peak H_2 concentration	Area under curve	Methane concentration
	0	0.5	1	1.5	2	2.5	3	3.5	4	4.5	5	5.5	6	6.5	7			
58	2	2	0	1	2	-1	0	0	0	0	1	0	2	3	4	3	390	3
59	0	1	9	16	12	11	8	13	11	13	12	10	7	26	30	16	4920	0
60 *	0	0	0	3	10	7	9	6	13	14	10	14	13	14	9	14	3525	3
61	0	1	1	1	1	2	2	5	1	1	4	2	-1	1	2	5	660	4
62 **	1	0	0	1	-1	0	4	4	10	10	-1	7	7	16	14	10	1935	3
63	0	0	0	4	4	5	12	12	6	6	4	3	1	1	1	12	1755	2
64 **	1	0	9	10	17	-2	-2	-2	-3	-3	-3	-2	1	0	7	17	720	2
65	0	0	1	0	0	0	0	1	4	11	4	1	1	0	0	11	690	3
66	0	1	3	11	14	14	17	13	8	12	14	6	11	11	6	17	4140	0
67 **	1	1	1	0	0	7	2	3	2	3	4	1	3	0	7	7	945	2
68 *	2	0	2	2	-1	1	0	9	15	15	11	9	8	7	3	15	2415	3
69	0	4	4	2	14	4	4	1	4	3	2	4	3	3	3	14	1605	0
70	0	0	0	5	10	10	9	14	10	10	19	3	3	4	4	19	2970	2

Note: * = maldigesters subjects based on hydrogen plus methane ≥ 15 ppm

** = digester subjects who had diarrhea symptom

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APPENDIX F

Average change in absolute breath hydrogen concentration (ppm) produce by lactose maldigesters and lactose digesters after consumed 250-ml whole milk in each time. (mean ±SD)

Time (h)	Absolute breath hydrogen concentration	
	Lactose maldigesters (n=40)	Lactose digesters (n=30)
0	1.8 ± 2.5	1.6 ± 2.6
0.5	1.5 ± 2.0	1.1 ± 1.5
1.0	3.3 ± 4.3	2.9 ± 3.9
1.5	7.9 ± 14.0	4.1 ± 4.4
2.0	12.3 ± 16.2	5.0 ± 5.3
2.5	15.3 ± 14.7	5.0 ± 5.3
3.0	17.8 ± 13.6	5.4 ± 5.0
3.5	22.8 ± 16.2	5.5 ± 4.8
4.0	24.0 ± 15.5	5.8 ± 5.0
4.5	24.5 ±12.8	7.3 ± 6.3
5.0	20.4 ± 12.8	6.9 ± 6.1
5.5	22.2 ± 13.2	6.3 ± 5.7
6.0	20.1 ± 13.8	5.8 ± 6.2
6.5	15.8 ± 11.7	5.8 ± 6.8
7.0	14.2 ± 13.0	5.7 ± 6.8

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APPENDIX G

Average change in absolute breath hydrogen concentration (ppm) in 36 lactose maldigesters after consumed whole milk low lactose milk and lactose-free milk (mean ± SD)

Time (h)	Absolute breath hydrogen concentration		
	Whole milk	Low lactose milk	Lactose-free milk
0	1.5± 2.1	1.8 ± 2.4	2.8 ± 4.3
0.5	1.3 ± 1.6	1.7 ± 1.4	2.3 ± 4.5
1.0	3.3 ± 4.4	1.3 ± 2.4	1.3± 2.6
1.5	8.6 ± 14.7	1.3 ± 3.3	0.9 ± 1.6
2.0	13.4 ± 16.7	0.5 ± 2.2	0.9 ± 3.0
2.5	16.8 ± 14.8	0.5 ± 2.8	0.9 ± 2.7
3.0	18.8 ± 13.8	0.9± 2.6	0.9 ± 2.7
3.5	24.1 ± 16.3	0.8 ± 3.1	0.9 ± 2.9
4.0	23.8 ± 16.3	1.2. ± 3.3	1.6 ± 3.8
4.5	24.2 ± 13.5	1.7 ± 3.7	1.9 ± 3.5
5.0	20.4 ± 12.8	1.9 ± 4.2	1.5 ± 3.2
5.5	21.4 ± 13.5	1.9 ± 4.5	2.1 ± 3.6
6.0	20.4 ± 14.5	1.9 ± 4.6	2.2 ± 3.2
6.5	16.1 ± 12.3	1.3 ± 3.5	2.5 ± 3.1
7.0	13.9 ± 12.9	1.9 ± 5.0	2.2 ± 3.1

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APPENDIX H

Gastrointestinal symptoms occurred in subjects during and after consumption of different test diets

Test period	Subjects	Number of subjects experienced symptoms												Total subjects who had symptoms
		Abdominal pain			Bloating and flatulence			Dirrhea			Nausea*			
		1	2	3	1	2	3	2	4	6	1	2	3	
Whole milk	LD (30)	1	-	-	9	3	2	8	-	-	4	1	-	17
	LM (40)	5	-	-	17	3	1	7	-	-	3	-	-	28
	LM (36)	5	-	-	15	2	1	7	-	-	2	-	-	25
Low lactose milk	LM (36)	1	1	-	9	-	1	2	-	-	-	-	-	13
Lactose free milk	LM (36)	-	-	-	2	-	-	-	-	-	-	-	-	2

Note: LD = Lactose digester, LM = Lactose maldigesters

Example of calculation of symptom intensity score: LM (36), whole milk consumption

Symptoms: Abdominal pain score: 5 (number of subjects) x 1 (intensity) = 5

Bloating and flatulence score: (15 x 1) + (2 x 2) + (1 x 3) = 22

Diarrheal score: 7 x 2 = 14

Total score = 5 + 22 + 14 = 41

Full score = 12 (sum of severe intensity*) x 36 (N) = 432

Symptom intensity score = 41/432 = 0.09

* nausea was excluded for intensity score calculation due to its unspecific symptom of lactose intolerance

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APPENDIX I

Calcium intake (mg/d) in 70 subjects was calculated by INMUCAL V.3.0 (INMU D.7.0) from 3 day in 24 h dietary records.

Calcium intake (mg/d)	Number of subjects
≤200	4 (6%)
201-400	21 (30%)
401-600	25 (36%)
601-800	10 (14%)
> 800	10 (14%)
Total	70 (100%)

Mean calcium intake in 70 subjects was 505 ± 226 mg/day

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BIOGRAPHY

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