

**LACTOSE DIGESTION AND TOLERANCE AFTER YOGURT  
TABLET CONSUMPTION IN ADOLESCENTS**

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Thesis

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TABLET CONSUMPTION IN ADOLESCENTS**

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Milk is the major source of calcium, high quality protein and other nutrients. It contains the unique disaccharide lactose—consisting of galactose and glucose which require hydrosis by a brush border enzyme  $\beta$ -galactosidase or lactase. High incidence of lactose maldigestion in both Thai children and adults were reported. Lactase deficient person may avoid milk and other dairy products consumption because intolerance symptoms such as abdominal pain, flatulence and diarrhea may occur. Previous studies in Thailand, explored several means to reduce the undesirable symptoms when milk was consumed. For example, reducing milk consumption volume per time, having milk after meals, drinking milk with solid food and having fermented milk in form of yogurt. It is well established that lactase deficient subjects digest and tolerate lactose in yogurt better than that in milk. Yogurt tablet is a new product which has been developed from fresh yogurt with a longer shelf life in room temperature and still contains certain viable lactic acid bacteria.

This study aims to evaluate the effect of fresh yogurt consumption compared with yogurt tablet consumption on lactose digestion and tolerance based on breath hydrogen production in adolescents. Lactose maldigestion and intolerance were studied in 107 Thai adolescent students from Mahidolwithayanusorn Secondary School, aged 14-16 years, using 250 ml of milk as the test diet. Among these subjects, 32% were lactose maldigesters with 29% were lactose intolerant maldigesters. However, among lactose digesters, 10% were lactose intolerants. Thirty volunteers out of 34 lactose maldigesters were subsequently tested for breath hydrogen and gastrointestinal symptoms after consumption of 2 cups (300 g) of fresh yogurt and 28 yogurt tablets. At least 5 days were required for washout period between each experiment. Subjects who consumed fresh yogurt produced significantly lower levels of breath hydrogen than those who had had 250 ml of milk and yogurt tablet ( $p < 0.05$ ). Breath hydrogen level after yogurt tablet consumption was not significantly lower than that produced after drinking 250 ml of milk. Mild gastrointestinal symptoms experienced by the subjects did not differ after consumption of fresh yogurt and yogurt tablet. It is conclude that consumption of yogurt tablets was not as effective as fresh yogurt in reduction of breath hydrogen production, but it had the same effect in reduction of gastrointestinal symptoms.

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ประไพรี ศรีจักรวาล, Ph.D. ประภาศรี ภูวเสถียร, Ph.D. วิสิษฐ จະวะสิต, Ph.D. 99 หน้า

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นมเป็นแหล่งอาหารที่สำคัญของธาตุแคลเซียม โปรตีนคุณภาพดี และสารอาหารอื่นๆ แต่ในนมมีน้ำตาลแลคโตสซึ่งเป็นน้ำตาลโมเลกุลคู่ ต้องอาศัยเอนไซม์แลคเตสในลำไส้ย่อยให้เป็นน้ำตาลโมเลกุลเดี่ยวจึงจะดูดซึมได้ คนไทยมีภาวะการย่อยน้ำตาลแลคโตสบกพร่องอยู่เป็นจำนวนมาก ทำให้เกิดอาการในระบบทางเดินอาหารเช่น ปวดท้อง ท้องเสียเป็นต้น ที่ผ่านมามีการหาวิธีที่จะช่วยภาวะดังกล่าวที่เกิดขึ้น เช่นลดปริมาณนมที่ดื่มในแต่ละครั้ง ดื่มนมพร้อมอาหารอื่น ดื่มนมหลังมื้ออาหาร และการบริโภคโยเกิร์ตแทนนม เนื่องจากน้ำตาลแลคโตสในโยเกิร์ตสามารถย่อยได้ดีกว่าน้ำตาลแลคโตสในนม จากการศึกษาในประเทศไทยได้ทำผลิตภัณฑ์โยเกิร์ตรูปแบบใหม่คือ โยเกิร์ตอัดเม็ด นอกจากนี้จะมีคุณค่าทางโภชนาการแล้วยังมีระยะเวลาเก็บได้ยาวนานขึ้น ดังนั้นการศึกษานี้จึงมุ่งหวังที่จะศึกษาภาวะการย่อยน้ำตาลแลคโตสและอาการที่เกิดขึ้นภายหลังการรับประทานโยเกิร์ตอัดเม็ด โดยเปรียบเทียบกับกลุ่มที่รับประทานโยเกิร์ตครีม แล้วบันทึกผลการเกิดก๊าซไฮโดรเจน และอาการไม่สบายท้องโดยศึกษาในกลุ่มอาสาสมัครที่เป็นเด็กนักเรียนโรงเรียนมหิดลวิทยานุสรณ์จำนวน 107 คน อายุระหว่าง14-16 ปี โดยให้อาสาสมัครดื่มนม 250 มิลลิลิตร แล้ววัดปริมาณก๊าซไฮโดรเจนในลมหายใจพร้อมกับบันทึกอาการไม่สบายท้องที่เกิดขึ้น พบว่า 32% ของอาสาสมัครทั้งหมดมีภาวะบกพร่องในการย่อยน้ำตาลแลคโตส ซึ่งในจำนวนนี้มี 29% มีอาการไม่สบายท้อง ในกลุ่มที่สามารถย่อยน้ำตาลแลคโตสได้พบว่า 10% มีอาการไม่สบายท้อง อาสาสมัคร 30 คน ที่มีภาวะบกพร่องในการย่อยน้ำตาลแลคโตสทำการศึกษาต่อโดยรับประทานโยเกิร์ตครีม 2 ถ้วย (300 กรัม) หลังจากนั้นอีก 5 วัน จึงรับประทานโยเกิร์ตอัดเม็ดจำนวน 28 เม็ด พบว่าก๊าซไฮโดรเจนในลมหายใจหลังรับประทานโยเกิร์ตครีมมีค่าต่ำกว่าการรับประทานนม และโยเกิร์ตอัดเม็ดอย่างมีนัยสำคัญทางสถิติ ( $P < 0.05$ ) และก๊าซไฮโดรเจนในลมหายใจหลังรับประทานโยเกิร์ตอัดเม็ดมีค่าต่ำกว่าหลังการดื่มนมอย่างไม่มีนัยสำคัญทางสถิติ และอาการไม่สบายท้องหลังรับประทานโยเกิร์ตครีม และ โยเกิร์ตอัดเม็ด ไม่มีความแตกต่างกัน

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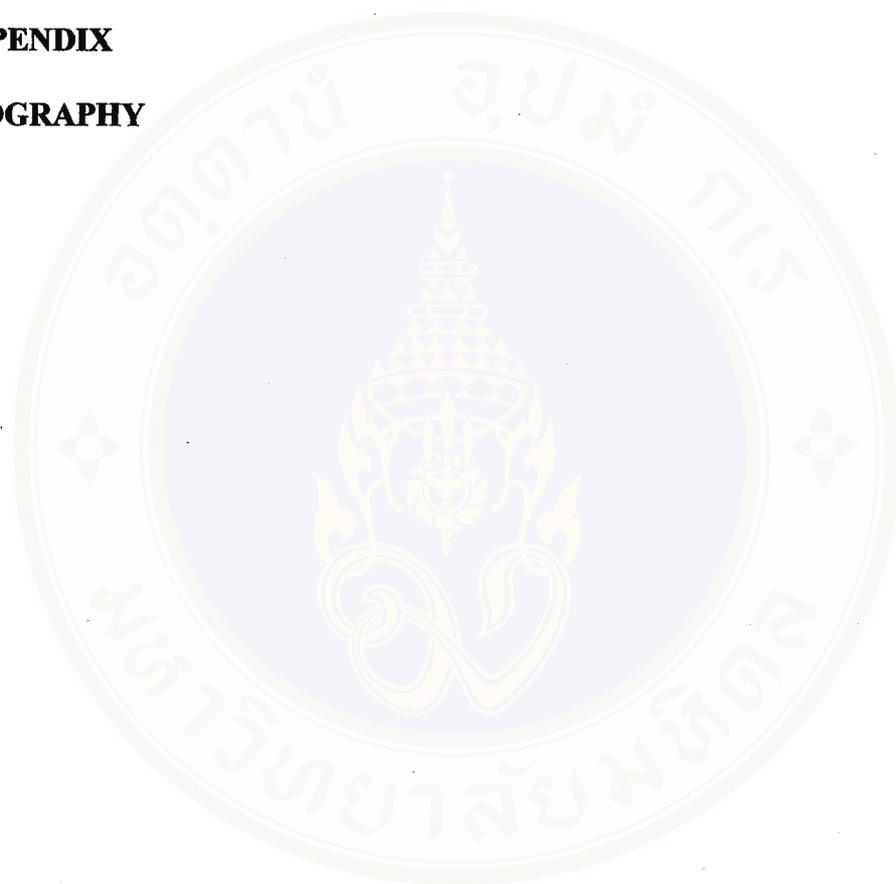
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## LIST OF ABBREVIATIONS

h	hour
min	minute
kg	kilogram
g	gram
mg	milligram
µg	microgram
ml	millitre
ppm	part per million
H <sub>2</sub>	Hydrogen
CH <sub>4</sub>	Methane
HPLC	High performance liquid chromatography
<i>L. acidophilus</i>	<i>Lactobacillus acidophilus</i>
<i>L. bulgaricus</i>	<i>Lactobacillus bulgaricus</i>
<i>E. coli</i>	<i>Escherichia coli</i>
<i>S. thermophilus</i>	<i>Streptococcus thermophilus</i>
LDL	Low density lipoprotein
HDL	High density lipoprotein

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## CHAPTER I

### INTRODUCTION

Milk and dairy products are important sources of many nutrients including high quality protein, calcium, potassium, phosphorus and riboflavin. It contains the unique disaccharide lactose - consisting of galactose and glucose linked by a  $\beta$ -1, 4 bond-absorption of which requires hydrolysis by a brush border enzyme  $\beta$ -galactosidase, commonly called lactase (1). All young mammal and human infants (except those with a congenital defect) are born with high levels of the enzyme which enables them to digest lactose. Lactase activity declines after weaning in most racial/ethnic groups except most white North Americans and Northern Europeans. By approximately three to five years of age, when the child is consuming a variety of foods, lactase level becomes low (2). Population with low lactase activity is now termed lactase deficiency, lactose maldigesters. The undigested lactose transit into the large intestine where it is fermented by the colonic bacteria, forming lactic acid, hydrogen, carbon dioxide or methane. The possible symptoms resulting from lactose maldigestion include abdominal cramping, bloating, flatulence, and diarrhea. The undesirable gastrointestinal symptoms occur in lactose maldigesters and intolerance may cause the sufferers to reduce milk and milk product consumption to a minimum or to exclude them from their diets. An inadequate calcium intake, particularly throughout childhood and early adulthood, is thought to increase the risk of postmenopausal osteoporosis (14). It becomes more critical in lactose intolerance person

to develop osteoporosis. The relationship between lactase deficiency and osteoporosis were reported (15, 16).

High prevalence of lactose maldigestion, from about 60 to 100% of the studied in African and Asian races population are reported (4, 5, 6, 7, 8). Studies on the lactose digestion status in Thai children and adults were performed in the last three decades (9, 10, 11), high incidence of lactose maldigestion were reported (9, 10, 11, 12, 13). Though, the study of lactose maldigesters and intolerance in Thai adolescent has yet been performed.

Previous studies explored several means to reduce the undesirable symptoms when milk is consumed, for example, reducing of milk volume at a time (12), having milk during and after meal (12, 17), drinking milk with solid foods (18), and having fermented milk in the form of yogurt (13, 19).

It is well established that lactase deficient subjects digest and tolerate lactose better from yogurt than from milk (20-25). This most likely results from two mechanisms: (i) the action of the bacterial lactase activity contained and vehiculated by yogurt bacteria in the human intestine and (ii) a slow gastric emptying and/or intestinal transit of lactose, thus, allowing the residual intestinal lactase and the colon microflora to more effectively metabolize the lactose (23, 24, 26, 27).

Yogurt tablet is the new concept of nutritious snack which developed from fresh yogurt with a longer shelf life in a room temperature. It still contained viable lactic acid bacteria. Then it can be used as source of probiotic, refer beneficial effects in the human intestine. The hypothesis of this study is that yogurt tablet can reduce the problems of lactose intolerance and maldigestion in Thai adolescents who require high amount of calcium.

**Specific objectives of the study were to study :**

- 1. to screen lactose maldigesters and intolerants among Thai adolescents, using breath hydrogen test, and a 250 ml- cow's milk as a test diet.**
- 2. to study the effect of fresh yogurt consumption on lactose digestion and tolerance, based on breath hydrogen production in Thai adolescents**
- 3. to compare the effect of fresh yogurt and yogurt tablet consumption on lactose digestion and tolerance, based on breath hydrogen production in Thai adolescents.**

## CHAPTER II

### LITERATURE REVIEW

Milk has been called “the almost perfect food”, rich in proteins, vitamins, minerals and other nutrients. Therefore, it is surprising that those who cannot digest milk sugar make up the majority of the world’s population.

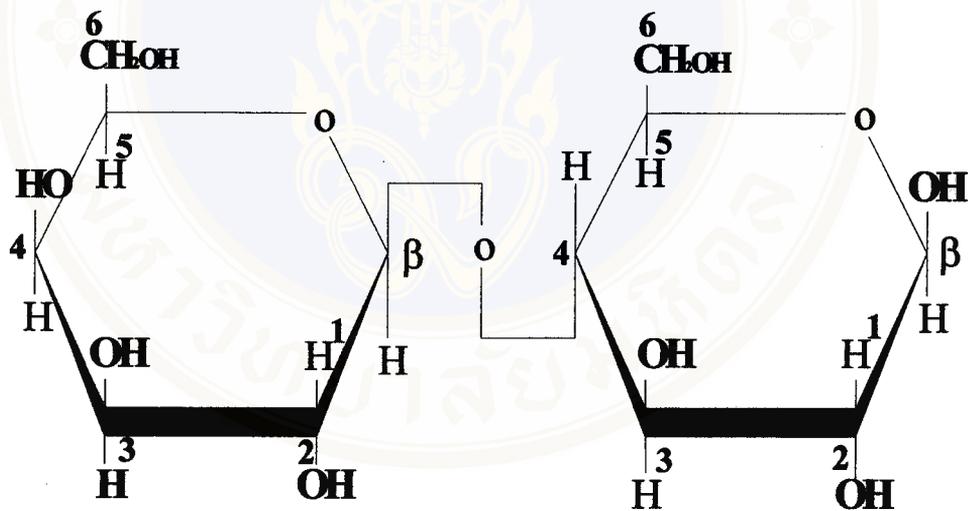
Lactose, or milk sugar, is the main carbohydrate found in milk of nearly all mammals and is not found in other natural foodstuff. Lactose is not utilized by the body until it is first hydrolyzed by enzyme lactase. The enzyme presents in the digestive tract of many mammals, including human, calves, goats, sheep and dogs. Lactase activity is greatest at birth but after weaning about 90% is lost (37).

#### **Physiology of lactose digestion**

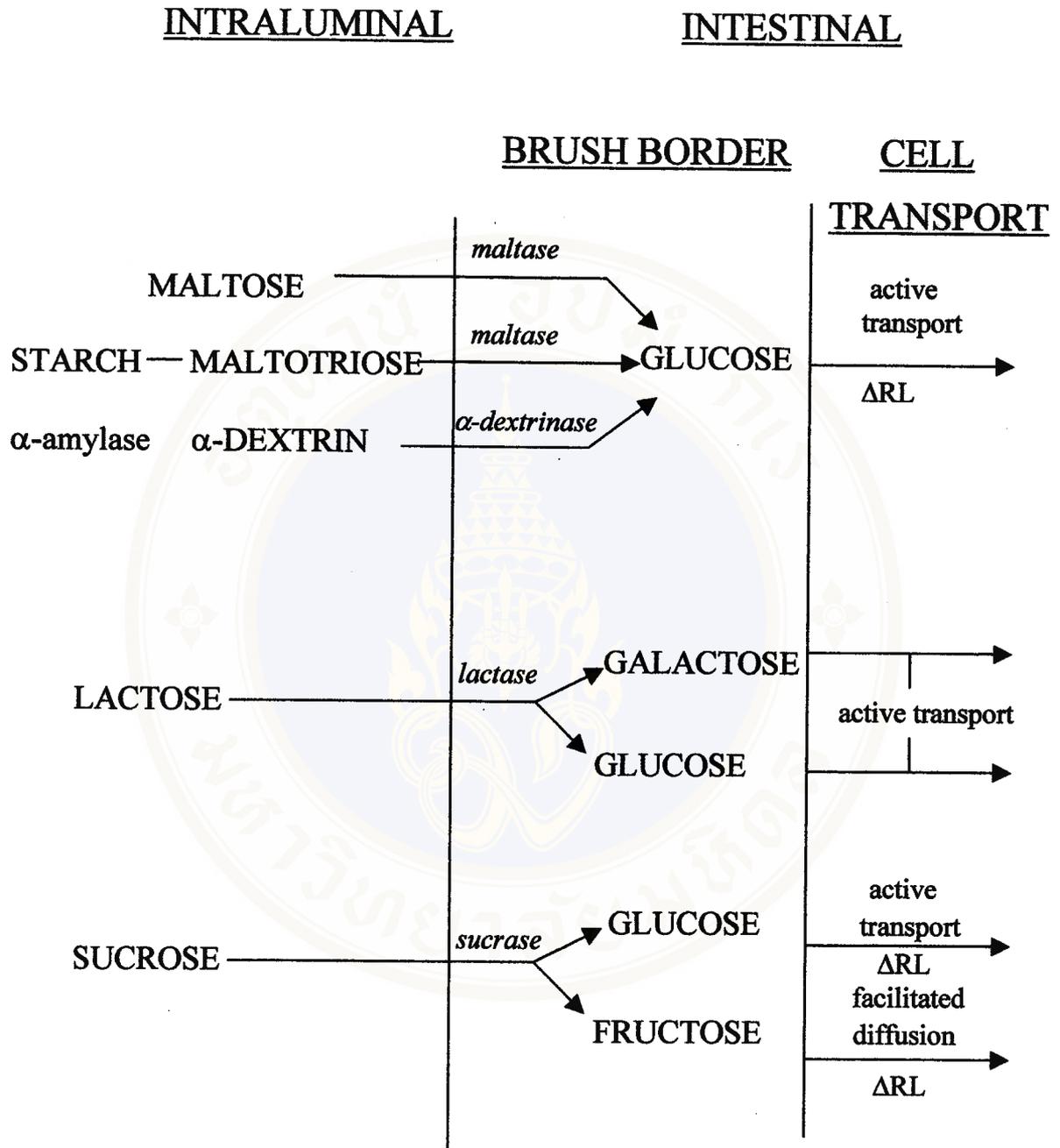
Human milk consists of 7 percent lactose, while whole cow’s milk contains 4.8 percent. Lactose is a disaccharide made up of equal portions of two monosaccharides, glucose and galactose (Figure 1). A unique intestinal enzyme, lactase, a  $\beta$  - galactosidase, breaks the chemical bond between glucose and galactose, freeing them for absorption (38). Lactase is one of five disaccharide - hydrolyzing enzymes located on the brush border of the intestinal epithelium. Activity of the lactase enzyme is highest in the duodenal and in the terminal ileum (39).

Of all the dietary sugars, lactose is hydrolyzed the most slowly (38). For example, the hydrolysis of lactose occurs at only half the rate of sucrose hydrolysis. The rate at which lactose is assimilated is dependent on the rate of the hydrolysis. The

relative slowness of lactose in broken down, accompanied by a lack of reserved enzyme, helps explain why many people are vulnerable to lactose maldigestion (Figure 2). It has been suggested that a faster-than-normal rate of gastric emptying in persons with low lactase activity may also contribute to symptoms of intolerance after milk ingestion (40). It is thought that when lactose is not hydrolyzed, the osmoreceptors, which lie deeper in the intestinal mucous than the lactase enzyme, are not stimulated to inhibit gastric emptying.



**Figure 1** Chemical structure of lactose ( $\beta$ -form) O- $\beta$ -D-galactopyranosyl-(1-4)- $\beta$ -D-glucopyranose



**Figure 2** Schematic diagram of absorption of dietary carbohydrate at the intestinal brush border

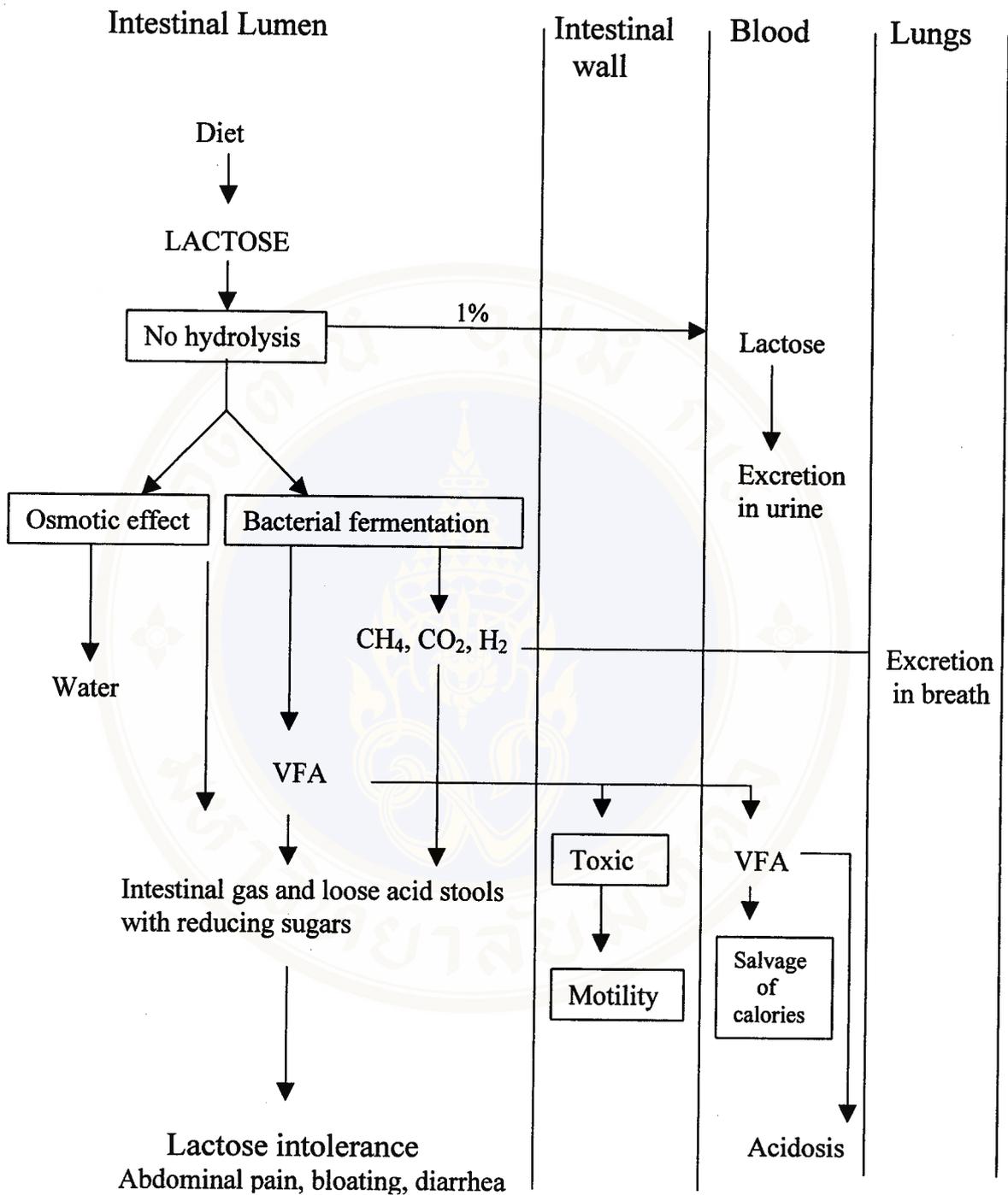
RL, the rate-limiting step in the overall digestion and absorption of the sugars

### **Lactose maldigestion**

It is estimated that approximately 1% of unhydrolyzed lactose in the small intestine is absorbed by passive diffusion into the bloodstream, which is then excreted into the urine unmetabolized. As the remainder of unabsorbed lactose reaches the jejunum, it exerts an osmotic effect, causing water and sodium to be secreted into the intestinal lumen. Transit of the contents of the small bowel is accelerated. Large amounts of lactose may then enter the colon, where it is fermented by colonic bacteria. Approximately 65 – 100% of the undigested lactose reaching the colon is metabolized to short chain organic acids, hydrogen, methane, and carbon dioxide gases. Some of the organic acids are absorbed into the bloodstream, while some are excreted in feces, resulting in acidic stools (**Figure 3**) (38). In some individuals sufficient sulphate exists, sulphate reducing bacteria (SRB) are primarily responsible and H<sub>2</sub>S is produced. During conditions of low sulphate availability, methanogenic bacteria and acetogenic bacteria are able to combine H<sub>2</sub> with CO<sub>2</sub> to form methane and acetate respectively (**Figure 4**) (41). Methane is then exhaled in addition to CO<sub>2</sub> and H<sub>2</sub>. Methane formation is most active in the distal colon (42).

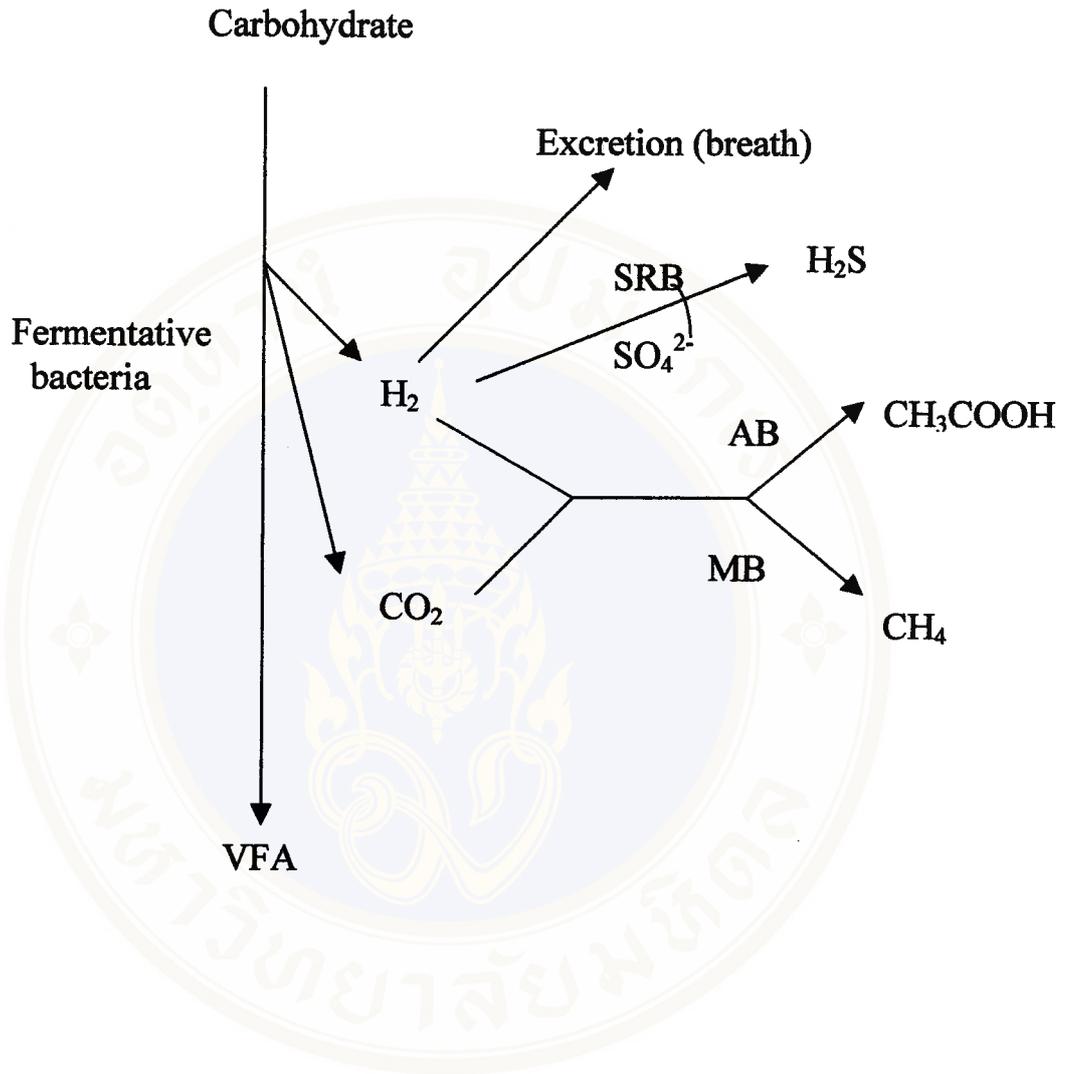
### **Prevalence of lactose maldigestion**

Prevalence of lactose maldigestion in various groups of population has been extensively reviewed (2, 43). There are geographical, ethnic and age-related difference in the ability to digest lactase (13). A high frequency to lactose maldigestion has also been documented in South African Black adults and children (2). Rao *et al* (1994) indicated that the prevalence of lactose maldigestion significantly increase with age in blacks compared to whites and that the magnitude of



**Figure 3** Pathophysiologic events following lactose maldigestion.

VFA indicates volatile fatty acids.



**Figure 4** Postulated mechanisms for hydrogen disposal human colon

SRB = sulphate reducing bacteria

MB = methanogenic bacteria

AB = acetogenic bacteria

VFA = volatile fatty acids

the problem may be greater in black maldigesters than in white maldigesters (44). There were different frequencies of lactose maldigestion found in populations of similar racial composition in different environments (45,46). In consideration of the differences in types and doses of test diets and the methods of investigation of lactose maldigestion as well as age group of populations, these make interpretation and comparison on the prevalence of lactose maldigestion difficult.

**High prevalence of lactose maldigesters (60 – 100%) were found among the following :**

1. Mediterranean and middle East region: Jews, Southern Italians, Arabs,  
Greek Cypriots.
2. Asia : Thais, Indonesians, Crannies, Koreans
3. African : South Nigerian peoples, Hausa, Bantu
4. North and South America: Esteems, Canadian and U.S. Indians, Chami  
Indians.
5. Pacific region : Neu Guineans, Figians

**Low prevalence of lactose maldigesters (0 – 30%) were found among the following :**

1. Europe : Danes, Finns, Germans, French, Dutch, Poles, Czechs,  
Northern Italians, British.
2. African : Hinna, Tussi, Fulani.
3. India : Punjab and New Delhi areas.
4. Australia : Australian white

### **Studies of lactose maldigestion in Thailand**

Studies on the lactose digestion status in Thai children and adults were performed in the last three decades. Many studies in humans focused only on adults or reported results over a wide age range without providing an age breakdown. 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 (8). Keusch *et al* (1969) found that by the age of 2 years, about 80% of normal Thai children manifested lactose maldigestion (47). In view of the Japanese children, starts at about the age of 3 years and reached a stable level of around 85% at about 6 years (48). Flatz *et al* (1969) conducted lactose tolerance test using 1g lactose/kg body weight as the test diet, in 114 healthy well nourished individuals in Northern Thailand (10). They found that all of 75 adults and 24 children aged 4-12 years were lactose maldigestion. Subsequent study sought out similar cases in adults from Ayuthaya Province, using lactose tolerance test with change oral lactose dose to 0.8 g/kg body weight, the degree of lactose maldigesters was 96 % of these subjects (11).

Further studies applied hydrogen breath test and used a 250 ml-milk as the physiological dose of lactose (12,13,19). The decline percentage of lactose maldigesters was observed. Banjapong (1987), using the criterion of breath hydrogen production  $\geq 20$  ppm, found that 28 out 32 healthy adult subjects (88%) were lactose maldigesters (12). Using a higher cut-off point of breath hydrogen concentration  $\geq 25$  ppm, Yankirati (1993) showed that 48% of Thai adults were lactose maldigesters (13). Besides milk, lactose solution (12g equal to the amount in 250ml milk) was tested in 39 healthy Thai adults in 1991 by Soontornchai (49) and 77% of lactose maldigester

was found. Kittawee (1997) reported 15% lactose maldigester, when milk was given at mid morning break to 101 children, aged 5-6 years, with breakfast (17). Chancharupong (1997) used 250 ml milk as the test diet, found that 41% out of 110 Thai adolescents, aged 14-16 years were lactose maldigesters with 30% being lactose intolerant maldigester (19).

The difference in the former and the later studies in degree of lactose maldigestion in Thailand were due mainly to the differences in the type and doses of test diet and the methods of measurements.

### **Lactose maldigestion and lactose intolerance**

Not all maldigesters are intolerant to milk; only around 20% experience some or all of the gastrointestinal symptoms with as a little as one cup of milk (50). In principle, apparent lactose intolerances should not be found in lactose digesters but many researchers found lactose intolerance among lactose absorbers (51, 52). Lactose intolerance is a subjective condition which depends not only the parameters, but also on social and cultural habits and on psychological factor (53). Webster *et al* (1995) indicated that the prevalence of abdominal pain, bloating, flatulence, diarrhea and a constipation was similar in children with or without lactose maldigestion (54). In the double blind study of Kwon *et al* (52), using 50g lactose as test diet, of 42 lactose absorbers, 13 reported gastrointestinal symptoms after ingestion of lactose free only or both lactose free and lactose containing diet. Among these subjects, only one reported gastrointestinal symptoms after receiving lactose -containing food.

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

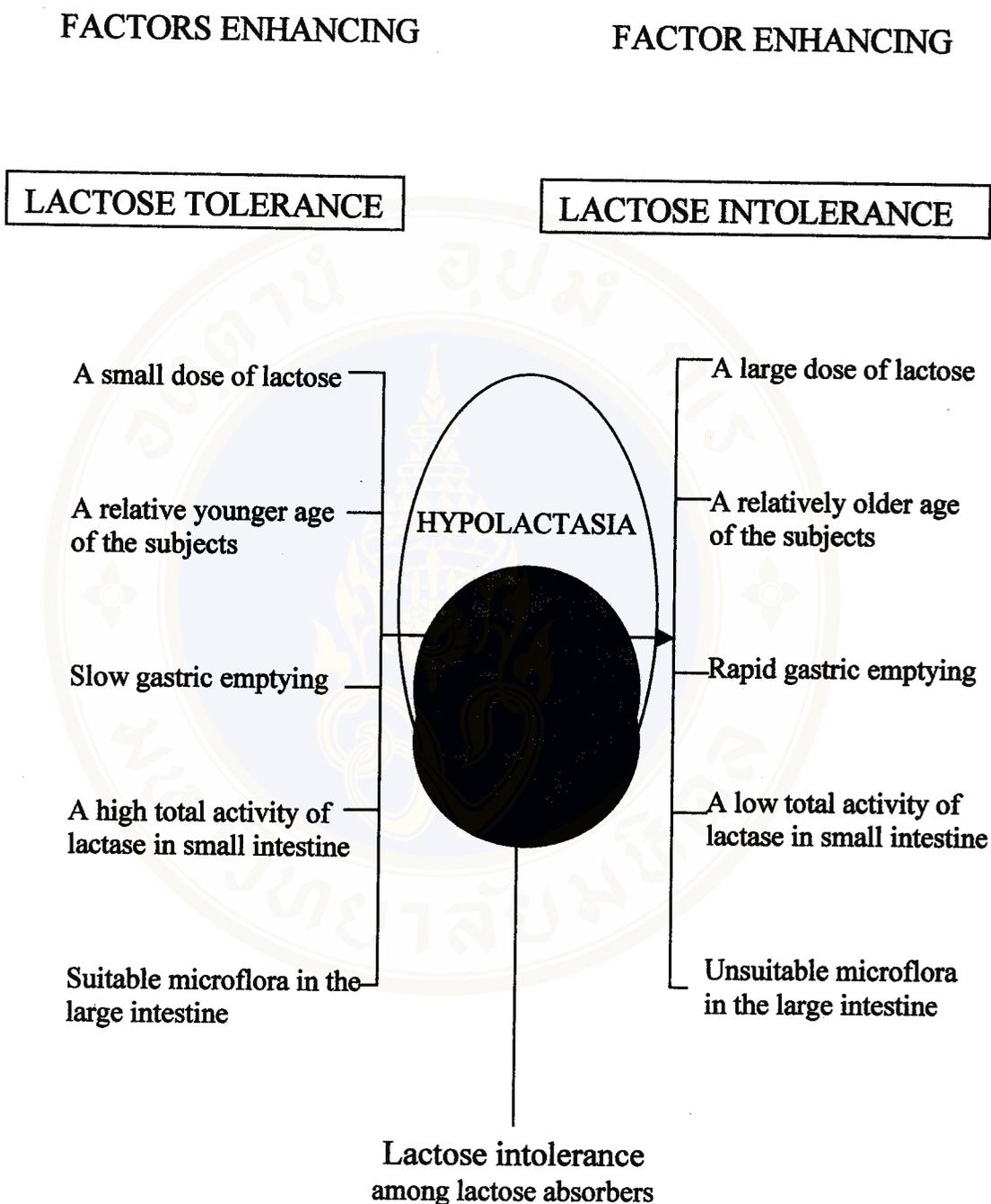
transit), the conditions of ingested (e.g., with or without food), the condition and activity of the large intestine microflora and the residual lactase activity throughout the small intestine (**Figure 5**). These variables form the basis by which many individuals with lactose malabsorption attributable to “adult - onset” lactase deficiency tolerate significant amounts of milk without developing symptoms.

Recent research using a double-blind method, has shown that individuals who claim intolerance—even severe intolerance are not always maldigesters as demonstrated by a breath hydrogen test (53, 54, 55). Documented maldigesters may incorrectly be associated symptoms to lactose (53).

### **Hydrogen breath test**

Hydrogen breath test is the most popular method used for diagnosis of lactose malabsorption, due to its non-invasive, simplicity, specificity and sensitivity. The principle of the breath hydrogen analysis was established by Levitt and colloway (57). Hydrogen breath test have been used for gastrointestinal diagnosis of the past dozen years. Sensitivity and specificity in the diagnosis of lactose malabsorption are 100% (58). Beside, Rosado and Solomons (38) reported that sensitivity and specificity of the hydrogen breath - analysis Test are 80% and 100% respectively.

Breath samples can be collected by expiration into a bag of syringe (**Figure 6**). A special valve mouthpiece has been divided in which the first 500 ml exhaled during an expiration cycle is collected in one compartment, and the remainder of the tidal (alveolar) column in a second compartment. By this way, residual air from the



**Figure 5** Factors influencing lactose intolerance symptoms in subjects with hypolactasia

anatomic dead space and true alveolar gas can be conveniently separated during interval collection (59). If the breath sample cannot be analyzed immediately, it can be stored for varying length of time. Special gas tight glass syringe and oiled glass, or even disposable plastic syringe can maintain a constant hydrogen concentration for up to 12 hours. Foil paper enveloped impregnated with Mylar has been constructed by some investigators and can maintain a gas sample for almost 50 days (60,61). These are not commercially available, however, a common, rubber-stoppered, evacuated glass tube can preserve the original hydrogen concentration of a gas sample for a 3 weeks (62). Unreliable recover of gas from the tube necessitates extreme caution in using this storage system (62). The collected breath samples were analyzed for hydrogen concentration using gas chromatography namely a Quintron Microanalyzed model DP (Figure7) (38).

#### **Advantages of hydrogen breath test (65, 66)**

The breath hydrogen test is the best and simplest test to establish the presence of lactose malabsorption, although it is not a measure of lactose hydrolysis and subsequent monosaccharide uptakes. The test is a useful, non-invasive method for detecting carbohydrate malabsorption.

Recently, it has become clear that many marginally intolerant patients are not suspected of having carbohydrate intolerance until it is identified by the breath hydrogen test. As reported, at the meetings of the AGA in San Francisco in May, 1986, and in Chicago in 1987, many patients were unsuspected lactose malabsorbers. The breath hydrogen test can be used to identify and follow up the improvement of the patients with lactose intolerance after lactose restriction. Without the test, the

relationship may be difficult to establish, and it may be difficult to convince the doubting patient to conscientiously avoid milk products during the test.

The breath hydrogen test response provides a general guide as to the severity of the malabsorption. It is useful to have at least semi-quantitative data which suggest how severe the lactose malabsorption might be. Therefore, the test can be guide for recommending the level at which milk or milk products can be included in the diet. Total avoidance may be unnecessary burden for some patients and a threat to health unless supplemental calcium is added to their diets.

### **Factors influence hydrogen breath test**

Various idiopathic conditions can alter the validity of hydrogen breath test or influence the interpretation of results. A fundamental understanding of these cautions is essential to the successful application of the hydrogen breath analysis test.

1) **Sleeping:** allowing the patient to sleep during the test will cause an increase in breath hydrogen and probably in methane. There are 2 reasons for such increase. Hypoventilation, which is an inadequate rate of air turn - over in the lung, slows down the rate of hydrogen removal from the blood. Sleep also decrease intestinal motility, which slows down the movement of carbohydrates through the colon and allows a longer time for hydrogen accumulation. Thus, intermittent sleeping during the test will interfere and should not be allow (68).

2) **Colonic pH:** hydrogen production is affected by colonic pH. A decrease in stool pH from 7.0 to 5.5 will cause a drop in hydrogen generation to 1/4 the former rate (98). The rate is returned promptly by increasing the pH. Thus, the hyperacidic colon contents may inhibit the generation of hydrogen, or cause the generation of

methane instead of hydrogen by colonic bacteria (69). Colonic pH is frequently acidic in individuals on certain modified diets (70) and in normal newborns (71).

**3) Smoking:** tobacco smoke contains high level of hydrogen, so smoking (by the patient or by anyone in the experimented area) will produce high hydrogen levels and will cause extreme baseline instability of the instrument (29).

**4) Laxatives and enema:** laxatives and enema can decrease the hydrogen (and methane) response in malabsorbers (72). The decrease may results from reducing the resident time for carbohydrate in the colon, leading to reduce time for fermentation. The reduced production may also results from a change in the concentration of the bacteria and from a change in the environment such as increasing the acidity, with could inhibit bacterial action (73). Rapid transit of the carbohydrate through the colon due to hypermotility will also reduce the exposure time and decrease hydrogen production in the colon.

**5) Exercise:** the effect of exercise reduces breath hydrogen. Payne *et al* (1983) noticed that at least when sequential comparisons of breath hydrogen response were carried out on individual subjects, exercise immediately prior to the tests should be eliminated (74).

**6) Menstrual cycle:** in luteal phase (days 18-20 when progesterone increases), will delay gastrointestinal transit since transit time was lengthened 25% from approximately 100 minutes in the follicular phase (days 8-10 when progesterone levels are low) to 125 minutes during the luteal phase (75).

**7) Antibiotics:** administering a course of antibiotics may sterilize the colon, so the colony count of bacteria is low and non-existent (76,77). However, not all antibiotics have this effect on the breath test. It was reported that penicillin and

metronidazole may even increase hydrogen excretion (78). This presumably due to their drug fermentative-depressant effect on the gut flora, however, not reproduced in the fecal cultures, where hydrogen remained unchanged.

**8) Dinner meals:** The accuracy of a breath hydrogen testing could be influenced by the dinner meal ingested on the day before the test. A dinner containing unabsorbed polysaccharides results in elevated fasting hydrogen excretion on the following day (80,81). If the subjects consumed an unrestricted dinner, mean fasting breath hydrogen was about 3 times higher than with the carbohydrate-restricted dinner (79).

**9) Other diseases:** diseases such as pneumatosis cystoid intestinalis (82), cystic fibrosis (83), small bowel bacterial growth (84), celiac sprue (85) or sickle cell disease (86) may elevate fasting breath hydrogen excretion.

### **Criterion for interpretation**

Criterion for interpretation of breath hydrogen test in the detection of carbohydrate malabsorption may vary, but all are based on comparisons of hydrogen concentrations in interval samples with a pretest value obtained after an overnight fast. Hydrogen concentration risen from basal level  $\geq 20$  ppm is generally regarded as consistent with lactose malabsorption. Rasado and Solomons (1983) determined the sensitivity and specificity of various cut-off values for the maximum increase in breath hydrogen concentration (59). They found that, at least for a dose of intact cow's milk, with a lactose content of 18 g (360 ml milk), the conventional criterion of  $\geq 20$  ppm is as efficient for detecting the same individuals as being lactose maldigesters as the more restrictive concentration of  $\geq 25$  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 gives 80-100% sensitivity and 100% specificity (59,63). 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 (64).

The area under the discontinuous curve of the change breath hydrogen concentration is often used to quantify response to oral carbohydrate. Rumessen *et al* (1990) suggested that both areas under the curve and peak hydrogen concentration seem valid for comparison of various dose of lactulose ingested. Furthermore the magnitude of area under the curve and hydrogen peak were closely correlated.

### **Breath methane**

In general when bacterial metabolize (or ferment) carbohydrates, they produce acids, water and gases, mainly carbon dioxide and hydrogen (89). Nearly half of hydrogen producers also produce methane. Furthermore, methane production has been identified in those who fail to produce hydrogen following ingestion of non-digestible sugars (90, 91). The pattern of gas production is related to the type of bacteria resident in the colon and it can not be predicted in advanced.

The pattern of methane excretion is independent of that of hydrogen. About 35 – 50% of normal adults are methane excretors. Studies of the development of methane producers shows that children the age of 3 years are usually methane – free, the incidence increases slowly to the age of 12 - 13, after which there is a sharp increase to the adults level by age 17 – 18, a gradual increase to about age 50, the an unchanged

level until the 7<sup>th</sup> or 8<sup>th</sup> decade of life. Studies of the determinants of methane have not been conclusive. It is suggested that methane production is less “substrate – dependent” than is hydrogen, though dietary differences may change the incidence of methane – producers (for instance, there is more in Mexico and less in India than in the U. S.). It has been reported that methane is partially derived from endogenously produce glycoprotein (from mucus and the wall of the intestine). It is probably related to the type of bacteria resident in the colon, but no explanations are adequate by themselves (87, 88).

There is a relationship between hydrogen and methane production in which methanogenic bacteria are able to convert the hydrogen to methane in the colon. Therefore, when disaccharides are metabolized by bacteria, sometimes only hydrogen is produced, sometimes both hydrogen and methane will appear in the alveolar air, and sometimes only methane will be increased. Thus, although knowledge is still incomplete about this interaction, it is important to look at both components if an understanding of the breath test gases is to be complete (88).

One dissenting paper exists in the literature. Montes and co-workers (67) reported a high correlation between the hydrogen response and the methane response in malabsorbers, and found the percentage of malabsorbers to be the same (51%). They concluded that methane producing status was not necessary for the interpretation of the lactose breath - test. However, most reports suggest that a significant percentage of malabsorbers will be missed if breath methane is not measured during the lactose malabsorption test.

So, in the study of lactose maldigestion and intolerance, it should be considered to measure breath methane inconjunction with breath hydrogen to prevent the false negative test, especially in lactose digesters.

## **Yogurt**

The definition of “yogurt” according to the Codex Alimentarius (1975) is “**a coagulated milk product obtained by specific lactic acid fermentation, through the action of *Lactobacillus delbrueckii* var. *bulgaricus* and *streptococcus salivarius* var. *thermophilus*, from cow’s milk with or without addition of powdered milk, powdered skim milk etc.**” The microorganisms in the final products must be viable and abundant. The use of lactic acid bacteria other than the 2 species cited in the definition is not permitted in most European countries. In this case, a yogurt containing *Bifidobacterium* spp. or *Lactobacillus acidophilus* would be classified as a “fermented milk”. In other countries, such as the UK, Canada and the USA, the addition of lactic acid bacteria to the 2 species used to make yogurt is permitted.

## **Fermented milk and lactose maldigestion**

Population with a high rate of lactose deficiency have been consuming fermented milk as their milk source for hundreds of years with no apparent intolerance. It was suggested in 1970 (92) 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 (93,20). Several studies have confirmed that hydrogen production with yogurt is significantly less than that with

milk (13, 20, 54, 93, 97) and that symptoms are improved in both adults and children (13, 95).

### **Mechanisms for improve digestion with fermented milk**

Yogurt differs from milk in that fermentation of milk with the lactic acid bacteria *Streptococcus thermophilus* and *Lactobacillus bulgaricus* leads to the production of lactic acid and the subsequent coagulation of milk proteins, with the resultant characteristic consistency. Other molecules are produced during metabolism as well. The final product contains  $10^7$ - $10^8$  live bacteria per ml, and has a pH of 3.9-4.3.

The level of lactose in traditional yogurt can alleviate maldigestion since 20-30 g lactose/l of yogurt are hydrolyzed by the lactic acid bacteria. However, today milk solids are usually added to milk prior to fermentation, thus increasing the initial amount of lactose. The level in the final product is therefore not necessarily less than that found in milk (94). The beneficial effect of yogurt work regardless of the quantity of lactose in the products, and therefore is not due to a lower level lactose. The mechanisms can be described as follows.

#### **a) Indirect effects of fermentation**

Fermentation leads to a thick texture and viscosity which delays gastric emptying and slows gastrointestinal transit time (98-100). This allows more time for residual intestinal lactase and for yogurt's bacteria to work.

#### **b) Direct effects of fermentation**

The lactic acid bacteria in yogurt contain enzymes such as lactase ( $\beta$ -galactosidase) which increase the level of lactase in the small intestine. The buffering

capacity of yogurt enables the bacteria to survive the acidity of the stomach (101). Bile in the small intestine may then enhance the permeability of the bacterial cells, allowing lactose to enter and to be digested (102-103). The enzymes must be active in order to exert such an effect. Previous studies on miniature pigs (104,105) suggested that intact bacterial cells in the food products are necessary for this effect, possibly because the cell wall protects the enzymes during their transit through the upper gastrointestinal tract.

#### **c) Long term adaptation**

Lerebours *et al.* (106) did not note an adaptation of human small intestinal lactase activity as a result of yogurt ingestion for 1 week. An increase in lactase activity was noted, however, in the brush border of both conventional and axenic (germ-free) mice after 14, 28 and 42 days of yogurt consumption (107). As has been suggested for milk (96), prolonged consumption of fermented milks may lead to an adaptation of the colonic microflora and a long-term improved digestion of lactose. This is not likely, however, because of the overall stability of the colonic microflora (109). Chancharupong (1997) found that prolonged consumption of yoghurt for 14 days had an effect on lactose digestion, changing few lactose maldigesters to lactose digesters. The effect was found not sustainable and did not appear on the 3<sup>rd</sup> day after cessation of yoghurt period (19). Additional research will provide information for possible long term adaptation of the small intestine and the colon.

#### **d) Lactose maldigestion in children**

Yogurt may provide a different mechanism other than increased absorption for children with lactase deficiency. Yogurt leads to a breath hydrogen test with a delayed time to rise and a lower rate of rise. As a result, the children experienced decreased

symptoms with yogurt, even though the level of maldigestion is not altered. Heat-treated yogurt shows an intermediate effect (110).

### **Health benefit of yogurt**

#### **1) Improvement of lactase deficiency**

It is well accepted that lactose digestion in yogurt made with live *Lactobacillus bulgaricus* and *Streptococcus thermophilus* is significantly improved in lactase deficient individuals when compared with milk or heated yogurt (13, 19, 23). Reduced intestinal discomfort is related to microbial activity (99, 100, 111). Yogurt ingestion leads both to less hydrogen production in the breath hydrogen test (lactose maldigestion) and to reduce symptom (lactose intolerance) (20, 21, 100). Kolars and co-workers (20) fed lactose maldigesters with 18 g of lactose in yogurt, water or milk. They found that only one-third the level of breath hydrogen was recorded when consuming lactose in yogurt as compared to the consumption of lactose in water or milk.

Milk fermented with individual species of bacteria possess varying ability to improve lactose digestion. *L. bulgaricus* fermented milks demonstrated higher capability to reduce symptoms than milk fermented with *Bifidobacterium bifidum*. Strain selection may be important as well (111). With current knowledge, it is difficult to conclude that there are differences in lactose digestion between species of bacterial fermented.

#### **2) Lower blood cholesterol levels**

Since Mann and Spoerry's observation in 1974 that East African Maasai warriors who consume 4 liters per day of fermented milk had low blood levels of

serum cholesterol, and that, when intake increase to 8 liters per day, they had additional decrease in the levels (112), many studies have been conducted to find a hypocholesterolemic factor in fermented milks. Results are difficult to interpret because of confounding variables, such as exercise and varying levels of initial serum cholesterol. The exact mechanism is unknown.

There is a concern that consuming yogurt on a regular basis may increase serum cholesterol because of the milk fat. Halpern *et al* (114) and Mcnamara *et al* (113) both showed that in young normal lipidemic adults consuming 450g (around 16 ounces) per day of low fat yogurt (7g fat/d) for up to 4 months, there were no deleterious effects on serum cholesterol, triglycerides, HDL or LDL levels. Yogurt low fat, can be consumed regularly without concern for raising cholesterol levels.

### **3) Reduction of the risk of cancer**

A epidemiological study from France showed that people consuming yogurt had less risk of developing large colorectal adenomas (115). In addition, the consumption of yogurt in elderly subjects with atrophic gastritis led to a decrease in the procarcinogenic fecal enzymes nitroreductase and azoreductase (116). Most studies reported a decreased in these enzymes during the study period when live lactic acid bacteria were consumed with a return to baseline level during follow-up when no lactic acid bacteria were consumed. The mechanisms and long-term effect of these changes are not clear.

The epidemiological studies (117,118) have found that colon cancer risk was inversely related to the consumption of diets which includes fermented milk. Other results (119) show no relation to colorectal cancer risk. Conclusions for the reduction of risk of colon cancer can not yet be extrapolated from these preliminary results.

Other dietary factors have been considered in the prevention of colon cancer, including fiber and calcium.

#### **4) Treatment of urogenital infections**

The predominant microorganism in the normal microflora of the female urinary tract is lactobacilli, in particular, *L. acidophilus*. Hormonal changes, age, and menopause can alter the flora. During urinary tract infections, pathogens such as *Candida albicans* (a yeast responsible for candidiasis) and *E. coli*, travel from the colon to the urethra and the bladder.

A recent study by Hilton *et al.* (120) suggested that daily oral ingestion of 227 g (8 ounces) of yogurt containing *L. acidophilus* may be useful in reducing the recurrent of candidal vaginal infections. In the small prospective cross-over study, women consuming yogurt experienced fewer recurrences of vaginal candidiasis than during control period. Further research on this aspect is necessary.

#### **5) Immunodulating effects**

Yogurt has been shown to enhance various parameters of the immune system in vitro models (121) and in mice (122-124). In humans, one study found an improvement in clinical symptoms of nasal allergy, but no changes in any parameters tested (125). A recent report with atrophic subjects found no significant modification of immune system caused by yogurt (126). Very high concentration of yogurt bacteria have led to increase in IFN $\gamma$ ,  $\beta$  lymphocytes, and natural killer cells (99), and yogurt consumption increase 2',5' - $\alpha$  synthetase activity (a reflection of production of IFN $\gamma$ ) (127).

## 6) Prevention and the treatment of intestinal disturbance

Diarrhea is a significant world-wide health problem. Many intestinal disturbances are caused by food or water contaminated by bacteria or viruses, or by poor hygiene. Certain lactic acid bacteria have been found to be helpful in preventing or treating some types of bacteria-induced diarrhea because of their ability to alter the activity of the intestinal microflora and compete with potential pathogens (114,128), as well as their ability to produce bacteriocins such as acidophilin and bulgarican (129).

Infant and young children are particularly susceptible to the ill effect of diarrhea. Gastroenteritis can be caused by a variety of pathogens including rotavirus. Boudra *et al.* (130) found that children aged 3-36 months with persistent diarrhea had decreased symptoms and increased weight gain when given a yogurt-based diet compared with those given a milk-based diet.

Other forms of diarrhea may be treated in part with lactic acid bacteria. For example, consumption of yogurt fermented with *L. bulgaricus* and *S. thermophilus* leads to a slower gastrointestinal transit time than milk and help decrease diarrhea associated with lactase deficiency (100,131).

## CHAPTER III

### MATERIALS AND METHODS

#### 3.1 Subjects

Subjects were the students in mathayomsuksa 4 at Mahidolvithayanusorn school, aged ranged 14-16 years old. All were healthy, without history or symptoms of gastrointestinal diseases or diarrhea, non had recently used antibiotics or laxatives during 4 weeks prior to study. All subjects were asked to fill in a questionnaire on a habit of milk and milk products consumption and on the gastrointestinal symptoms usually experienced. The questionnaire is given in **Appendix B**. They agreed to participate in this study after the risks and benefit had been fully explained.

One-hundred and seven individuals, 53 males and 54 female students participated in screening for lactose maldigester. Thirty lactose maldigesters, 9 males and 21 females, out of 34 who were identified as lactose maldigester, participated in the study of effect of yogurt and yogurt tablet consumption on lactose digestion and tolerance.

Subjects were requested not to consume milk and milk products for a day and fast from 10 p.m. of the night preceding each test day. In addition, due to the significant amount of hydrogen produced after eating some kinds of fruits and vegetables, the subjects were requested not to consume some vegetables and fruits especially beans, dried legumes, banana, unripped mangoes, cabbage, collard and other high fiber foods one day

An experiment diet was given after overnight fast for 8-10 h. Normal daily activity was allowed during sample collection. Smoking, sleeping and doing exercise work or exercise (enough to cause hyperventilation) were prohibited.

### 3.2 Tests diets

**Milk:** Ultra-heat treated (UHT) cow's milk, FOREMOST™ (Foremost dairies company (Bangkok) Co., Ltd) was used as test diet for studying lactose maldigestion and intolerance. Lactose content in a 250 ml-milk was 12.33 g.

**Yogurt:** Plain yogurt and strawberry flavored yogurt, DUTCH MILL™ (Dutch Mill (Thailand) Co., Ltd), containing living bacteria as *Streptococcus thermophilus* and *Lactobacillus bulgaricus*, were chosen as test diet. The total estimated bacteria count was  $2.0 \times 10^9$  cfu/g. Two cups of yogurt, 2x150 g, contained 11.34 g lactose, were as test diet to study the effect of yogurt consumption on lactose digestibility and tolerance.

**Yogurt tablet:** Yogurt tablet prepared from plain yogurt at Department of Biotechnology, Mahidol University, containing living bacteria as *Streptococcus thermophilus* and *Lactobacillus bulgaricus*, were chosen as test diet. The total estimated bacteria count was  $1.4 \times 10^5$  cfu/g. Twenty-eight tablets, (1.24 g each) contained 11.35 g lactose, were used as test diet to study the effect of yogurt tablet consumption on lactose digestibility and tolerance. Twenty-eight tablets were given to each subject in order to provide approximately 11.34 g of lactose as fresh yogurt.

### 3.3 Breath sample collection

Breath sample was collected by means of end-expiratory breath sampling technique using the Quintron Gas sampler system (Figure 6). The system consists of a 750-ml alveolar air collection bag with a one-way flap-valve, a 100-ml discard bag, a T-piece with a one-way flap-valve and a mouth piece. The discard bag was assembled in line with the mouthpiece whereas the collection bag was connected to a one-way flap valve at the right direction of the T-port. 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 doing this, the discard bag was first filled with the first portion of the expired air. Due to the pressure in the discard bag, the valves on the T-piece and in the collection bag were then open. The collection was then filled with the remaining expired air of the alveolar air. The collection bag was then removed from the T-port. A subsample of gas (40-50 ml) in the collection bag was aspirated into a plastic syringe, fitted with a three way stopcock and used for hydrogen 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 a test diet. Normal daily activities were allowed during breath collection. The subjects restrained from smoking, sleeping and doing excessive work or exercise (enough to cause hyperventilation). They were allowed to drink water only during the period of breath collection.

### 3.4 Quantitative determination of hydrogen in expired air

Breath hydrogen in the expired air was analyzed by Microlyzer, Quintron Instrument Co., Inc. Milwaukee, Wisconsin, Model DP Microlyzer<sup>TM</sup>. It is a special purpose gas analyzer, which has been designed to measure small quantities of hydrogen gas in expired and alveolar air samples. The measurement of breath hydrogen is useful for the detection of intestinal malabsorption of sugars, for the measurement of intestinal transit time and for the detection of bacterial overgrowth (into the intestinal from the colon). The principle of the Model DP Microlyzer<sup>TM</sup> is based on the technique of gas chromatography. Hydrogen is separated from other gases present in the breath by using an external chromatographic column, called a sivrite-10 cartridge. It allows the hydrogen to pass through freely, but traps all other interfering gases (those which can burn or react with oxygen). When operated the Model DP Microlyzer<sup>TM</sup>, insert the male end of the sivrite-10 cartridge into the female luer fitting of the Flush port below the Sample valve (Figure 7). The gas sample (20 ml) was injected through the Sivrite-10 cartridge and pushed the sample stem in to its stop. Thus only hydrogen gas was carried passed the sensor by an inert carrier gas which flowed continuously through the system. The carrier gas for the Model DP Microlyzer<sup>TM</sup> was room air that had been conditioned with a large for-column which removed moisture and stabilized the baseline of the instrument. When the bolus of the hydrogen was carried into the sensor block, its presence produced an electrical signal by interacting with the solid state sensor. The peak value of the processed signal was directly related to the hydrogen concentration in the sample. The circuit converted the signal to a decimal form which displayed as parts per million (ppm)

hydrogen on a digital panel meter. The breath sample in the 50-ml syringe was analyzed, in duplicated, within 24 h. after collection.

A compressed reference gas standard of known hydrogen concentration (Quingas-1) was used to calibrate the digital concentration (ppm) reading. The standard gas was 2 fold diluted with the room air and injected to the instrument. The concentration of the hydrogen was directed read. The calibration curve was plotted prior to the sample reading every day and standard checking was performed regularly.

### 3.5 Calculation of hydrogen concentration

Hydrogen concentration, above the baseline, after consumption of test diets was calculated by 2 methods:

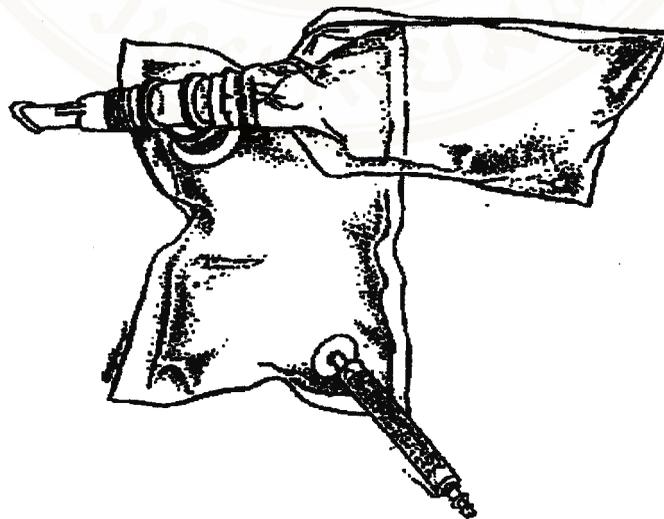
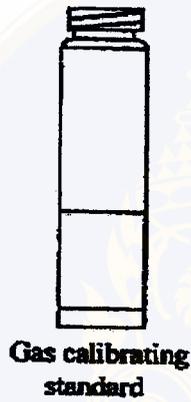
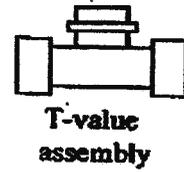
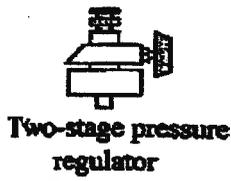
- 1) the absolute concentration of hydrogen as peak height of magnitude hydrogen at 30-min intervals expressed as ppm
- 2) cumulative breath hydrogen excretion was computed as the area under the hydrogen production curve over the baseline which the following equation for the sum of the areas of consecutive trapezoids (132) and were expressed as ppm.min.

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

Where A = the area under the hydrogen production peak

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

T = 30 min.



Collection system

Figure 6 Accessories for the Microlyzer

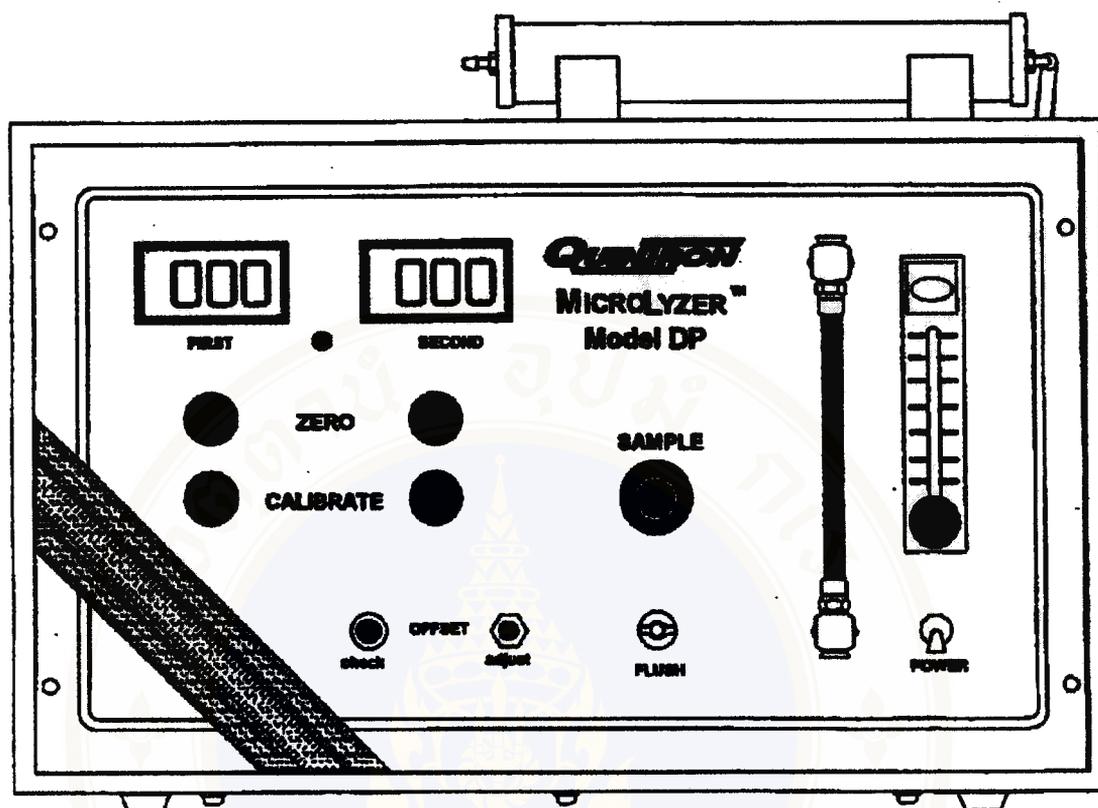


Figure 7 Quintron Microlyzer Model DP

### 3.6 Statistical analysis

The results were expressed as mean  $\pm$  SD and were considered statistically significant if two-side P values were  $< 0.05$ . The difference between 3 treatments were compared using One-way analysis of variance (ANOVA).

### 3.7 Gastrointestinal symptoms

Subjects were asked to record gastrointestinal symptoms, which occurred after test diet consumption for 24 h. The record form for the symptom is given in Appendix C. The following set of symptoms, 1) diarrhea, 2) abdominal pain, 3) abdominal bloating and

flatulence were recorded as present or absent. Among these symptoms, abdominal pain, abdominal bloating and flatulence, and diarrhea were graded from 0-3 (0: no symptom, 1: mild, 2: moderate, 3: severe symptom) (133). The average intensities of each gastrointestinal symptom were calculated.

### **3.8 Experimental design**

Three experiments were carried out and the hydrogen breath analysis was used as an index of incomplete of lactose absorption. The experiments were conducted as follows:

#### **Experiment 1: Identification of lactose maldigestion and intolerance**

**Objective:** To identify lactose maldigestion and intolerance among 107 adolescents using a 250 ml-cow's milk as a test diet

**Method:** After an overnight fast, a sample of expired air was collected and a 250-ml cow's milk was given. The breath samples were collected at 30-min intervals for 7 h. after drinking milk. 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  $\geq 20$  ppm over baseline value during 7-h collection period was the criteria for classification as a lactose maldigester. Since the subjects were allowed to consume lunch, the peak hydrogen rised after 5 h. period which may due to the carry-over effect of lunch were not counted for lactose maldigester. The cumulative hydrogen production as an area under the consecutive hydrogen production curves, was

also evaluated. The symptom after consumption of milk were recorded and used to indicate lactose intolerance.

**Experiment 2:** Effect of yogurt consumption on breath hydrogen production and tolerance

**Objective:** To study the effect of fresh yogurt consumption in lactose maldigesters

**Method:** 2 cups of 150 g fresh yogurt (a cup of plain yogurt and a cup of strawberry flavored yogurt) were given to lactose maldigesters after an overnight fast. Similar to Experiment 1, breath samples were collected at 30-min intervals for 7 h.

**Experiment 3:** Effect of yogurt tablet consumption on breath hydrogen production and tolerance

**Objectives:** To study the effect of yogurt tablet consumption in lactose maldigesters

**Method:** Twenty-eight yogurt tablets were given to lactose maldigesters after an overnight fast. Breath samples were collected at time intervals similar to Experiment 1 and 2.

At least 5 days for washout period was required between each experiment. Milk consumption pattern was recorded by all subjects participated in this study.

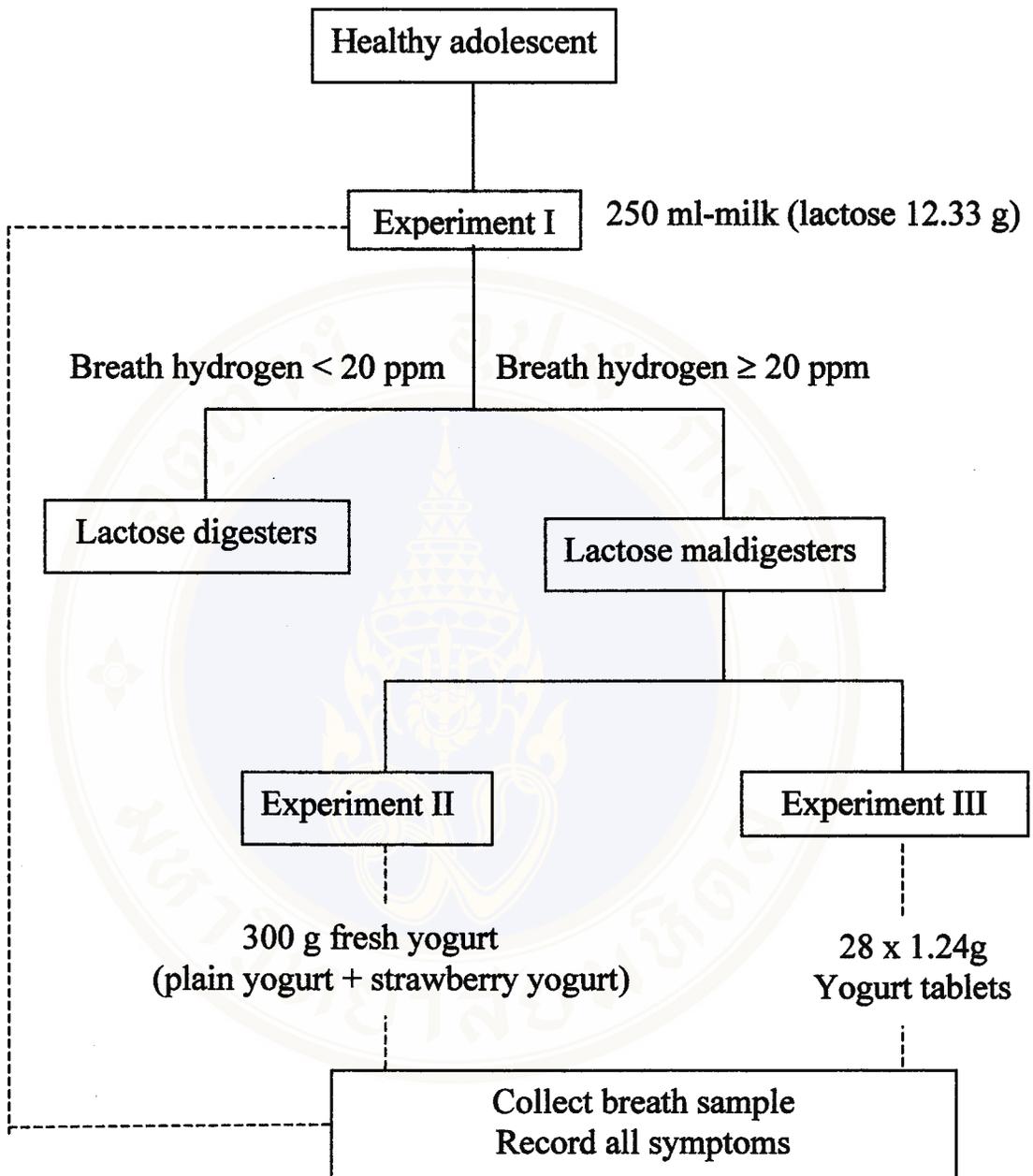


Diagram 1 Study plan

### 3.9 Analysis of lactose content (134)

Fresh yogurt sample was analyzed for lactose content. It was prepared by weighing 10 g sample into a 50-ml volumetric flask. 10 ml of 10% standard lactose solution was added and diluted with 5 ml distilled water. Protein in the yogurt sample was precipitated by slowly adding of 5 ml of 10%  $ZnSO_4$  while mixing 5 ml of 0.5N NaOH was added. 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. The solution was filtered through Whatman No.1 filter paper. Lactose in the filtrate was analyzed by high performance liquid chromatography (HPLC) against lactose standard. Standard solutions (1,2 and 3 g%) yogurt tablet and milk samples were prepared in the same way as yogurt samples. The method of analysis is shown in **Table 1**.

**Table 1** Summary of standard and sample preparation for lactose determination by HPLC

Reagent	Milk sample	Yogurt sample	Yogurt tablet sample	Standard lactose
Sample	20 ml	10 g	2-5 g	-
Standard lactose	-	10 ml	-	5,10,15 ml
Distilled H <sub>2</sub> O	10 ml	10 ml	30 ml	25, 20,15 ml
10% ZnSO <sub>4</sub>	5 ml	5 ml	5 ml	5 ml
Mixed				
0.5N NaOH	5 ml	5 ml	5 ml	5 ml
Mixed vigorously and left for 15 min at room temperature				
Adjusted to final volume 50 ml and mix				
Filter through Whatman No.1	↓	↓	↓	↓
Filtrate	Filtrate	Filtrate	Filtrate	Lactose (1, 2, and 3 g%)
HPLC analysis of lactose				

**Equipment:** The chromatographic system consisted of a pump (Varian, Associates, Sienny vale,USA; model 5000) completed with a stainless steel cartridge LichroCART® column (Lichrospher® 100 NH<sub>2</sub>, particle size = 5 µm), dimension length =250 mm, i.d.=4 mm (Merck 516121) and a refractive index detector (Varian, Associated, Sienny Vale, USA; model series RI-3) connected to a 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. The amount of lactose was quantitatively measured by comparing the peak area of the unknown with the standard.

## CHAPTER IV

### RESULTS

#### 4.1 Lactose content in milk

Lactose content in a 250 ml-milk, 2 cups of fresh yogurt and 28 yogurt tablets were 12.33, 11.34 and 11.35 g, respectively (Table 2).

**Table 2** Lactose content in milk, fresh yogurt and yogurt tablets sample

Sample	Assigned Serving size	Lactose content (g)	
		Per 100 g sample	Per assigned serving
Milk	250 ml	4.93	12.33
Fresh yogurt (average)*	300 g (2 cups)	3.78	11.34
Yogurt tablet	35 g (28 tablets)	32.68	11.35

\*Averaged content in plain and strawberry flavored yogurt

#### 4.2 Characteristics of subjects

Subjects who answered the questionnaire were 114 adolescents, 58 males and 56 females with the aged range from 14-16 years old. From questionnaire survey, milk and yogurt consumption habits of the subjects are shown in Table 3. Most of them (81%) consumed at least one box of milk (250 ml) daily. More than 50% had no gastrointestinal symptoms and 19% of them had only mild symptoms. About 84% of them consumed 1-5 cups of yogurt per week and had no gastrointestinal symptoms.

**Table 3** Milk and milk product consumption habit in 114 adolescents

Milk and milk product consumption habit	Number ( % )	
	Female (56)	Male (58)
<b>1. Drink milk</b>		
- no	3 (5%)	1 (2%)
- yes	53 (95%)	57 (98%)
<b>2. Experience of gastrointestinal symptoms after milk consumption</b>		
- no	37 (70%)	34 (60%)
- yes	16 (30%)	23 (40%)
<b>3. Amount of milk consumed</b>		
- < 250 ml per day	9 (17%)	12 (21%)
- 250 ml per day	25 (47%)	17 (30%)
- 500 ml per day	16 (30%)	20 (35%)
- >500 ml per day	3 (6%)	8 (14%)
<b>4. Consume yogurt</b>		
- no	7 (12.5%)	4 (7%)
- yes	49 (87.5%)	54 (93%)
<b>5. Experience of gastrointestinal symptoms after yogurt consumption</b>		
- no	47 (96%)	50 (93%)
- yes	2 (4%)	4 (7%)
<b>6. Amount of yogurt consumed</b>		
- < 1 cup per day	38 (78%)	48 (89%)
- 1 cup per day	7 (14%)	4 (7%)
- 2 cups per day	3 (6%)	1 (2%)
- >2 cups per day	1 (2%)	1 (2%)

### 4.3 Identification of subjects with lactose maldigestion and intolerance in Thai adolescents

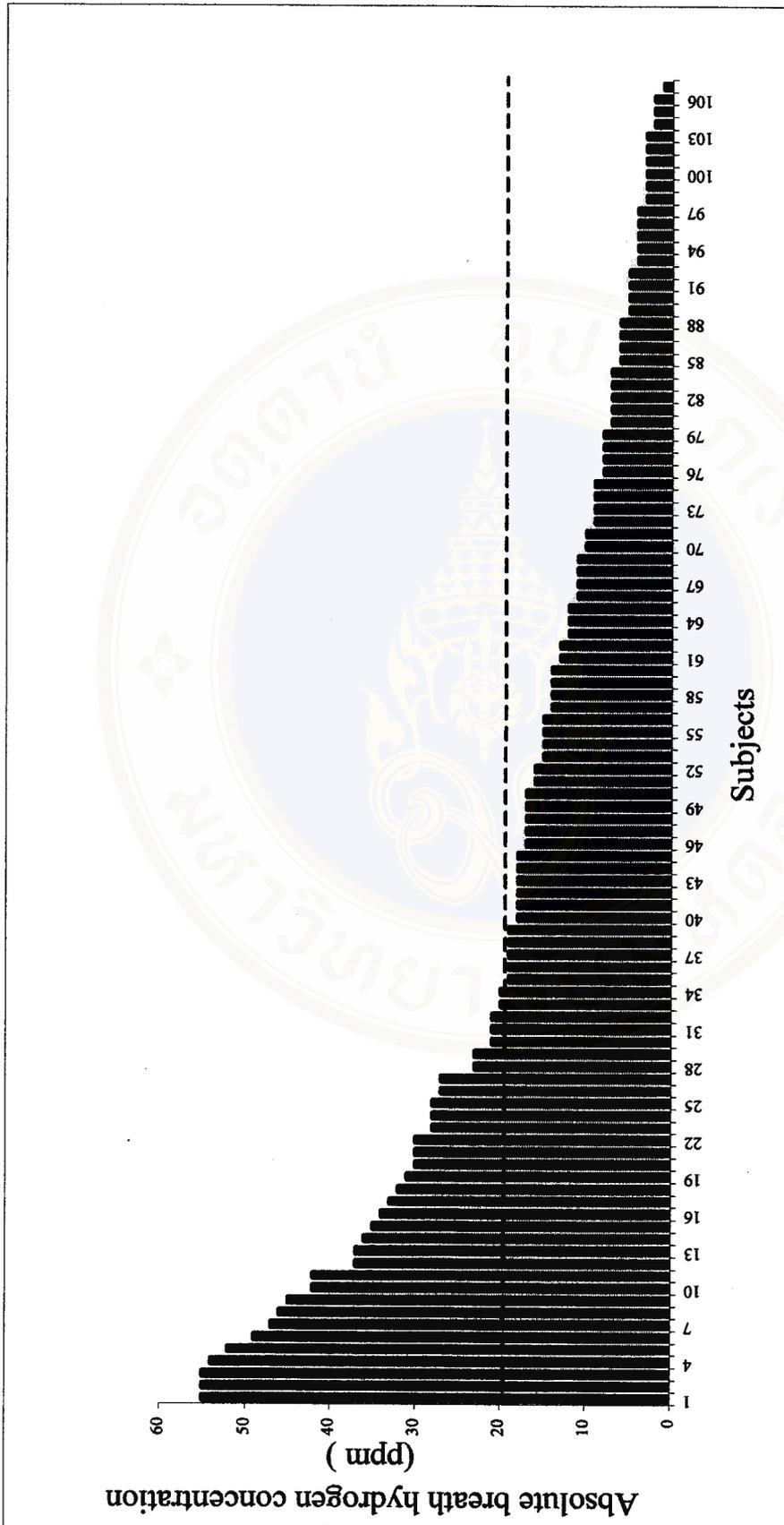
#### 4.3.1 Breath hydrogen production

Hydrogen in expired air was measured over a 7 h period in 107 Thai adolescents, after drinking of 250 ml-milk. **Figure 8** shows individuals' absolute magnitude peak of breath hydrogen (maximum increment of breath hydrogen production over baseline) with 34 out of 107 adolescents (32%) whose absolute hydrogen production was  $\geq 20$ ppm. They (21 females and 13 males) were classified as lactose maldigesters (**Table 4**).

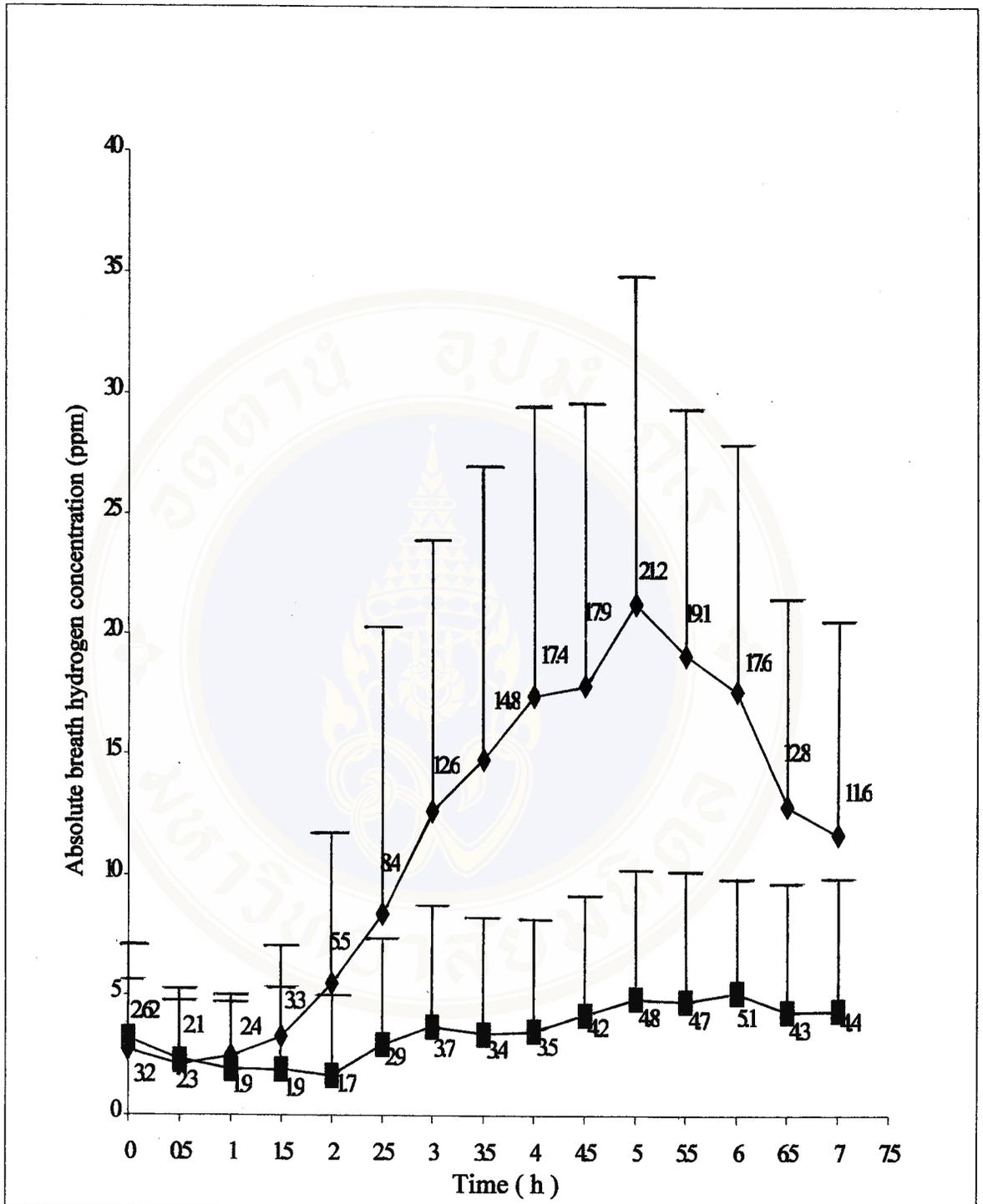
**Table 4** Status on lactose digestion in the subjects

Subjects	Number (%)		Total (%)
	Female (%)	Male (%)	
Lactose digesters (n=73)	33 (30.8%)	40 (37.4%)	68
Lactose maldigesters (n=34)	21 (19.6%)	13 (12.2%)	32
Total subjects (n=107)	54 (50.4%)	53 (49.6%)	100

The average absolute peak hydrogen concentration of the lactose maldigesters (n=34) was  $35.1 \pm 11.3$  ppm, whereas that of the lactose digesters (n=73) was  $10.3 \pm 5.7$  ppm. Average pattern of changes in absolute breath hydrogen concentration produced by lactose maldigesters and lactose digesters, throughout the 7 h period, is summarized in **Figure 9** and **Appendix E**. Absolute peak height of each subject appeared at different period of time. The absolute peak hydrogen concentration in the group of lactose maldigesters appeared between 4.5 to 5.5 h. after drinking milk.



**Figure 8** Absolute peak hydrogen concentration (ppm) in expired air of individuals after ingestion of 250 ml-milk, n=107. 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 maldigesters .



**Figure 9** Average changes in absolute breath hydrogen concentration (ppm) after ingestion of 250 ml-milk in lactose maldigesters (n=34) (◆) and lactose digesters (n=73) (■)

### 4.3.2 Breath methane

Thirty out of 107 adolescents (28%) produced breath methane  $> 3$  ppm above the ambient level and the maximum value was 42 ppm. Twenty-three lactose digesters (21%) and 7 lactose maldigesters (7%) were classified as breath methane producers (Table 5). In this study, based on the criteria of breath  $H_2 \geq 20$  ppm, the degree of lactose maldigestion in Thai adolescents was 32%. When the criteria of breath  $H_2+CH_4 \geq 15$  ppm was used, 9 out of the 23 previous identified lactose digesters were found as additional lactose maldigesters,. Thus, using both criteria the number of lactose maldigesters was 43, equals to 40% of the adolescents (Table 6).

**Table 5** Number of breath methane producing subjects after milk consumption

Status of subjects	Number of subjects	Number of subjects (%)	
		$CH_4 \leq 3\text{ppm}$	$CH_4 > 3\text{ppm}$
Lactose digesters	73	50 (47%)	23 (21%)
Lactose maldigesters	34	27 (25%)	7 (7%)
Total subjects	107	77 (72%)	30 (28%)

**Table 6** Number of lactose maldigesters based on different criteria

Criteria	Number of lactose maldigesters (%)
Breath $H_2 \geq 20$ ppm	34 (32%)
Breath $H_2 + CH_4 \geq 15$ ppm	9 (8%)
Total subjects	43 (40%)

### 4.3.3 Gastrointestinal symptoms

Abdominal pain, diarrhea, bloating and flatulence, recorded by the subjects, were considered as gastrointestinal (GI) symptoms. Ten out of 34 lactose maldigesters (29.4%) experienced with these symptoms, were classified as lactose intolerant. The rest who had no experience were classified as lactose tolerants (70.6%). Seven out of 73 of lactose digesters had gastrointestinal symptoms. The data was shown in **Table 7**.

Therefore, in total of 107 adolescents, 32% were lactose maldigesters with 29% of them were lactose-intolerant whereas 68% were lactose digesters with 10% of them were lactose-intolerant (**Table 7**). The conclusion of the results is shown in the following table.

**Table 7** Lactose digestion and intolerance after drinking 250 ml-milk in 107 adolescents

	Number of subjects (%)		Number of subjects (%)	
Criteria	Lactose maldigesters		Lactose digesters	
Breath H <sub>2</sub> ≥ 20 ppm	34 (32%)		73 (68%)	
GI symptoms	Intolerant	Tolerant	Intolerant	Tolerant
	10 (9.4%)	24 (22.4%)	7 (6.5%)	66 (61.7%)

#### **4.4 Effect of yogurt and yogurt tablet consumption on lactose digestion and tolerance in lactose maldigesters**

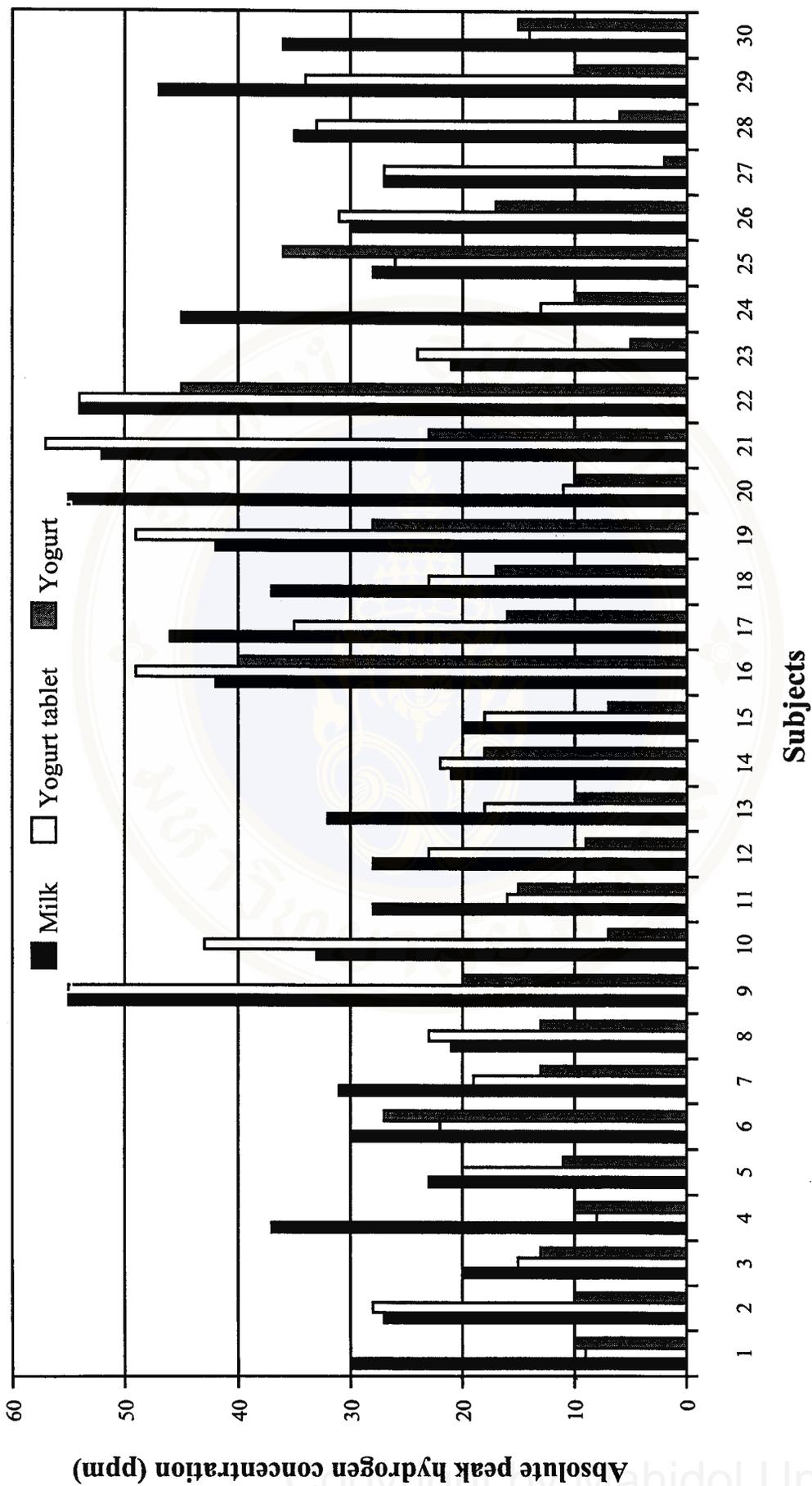
The aim of this study was to investigate the effect of yogurt and yogurt tablet consumption in lactose maldigesters by breath hydrogen test. Thirty out of 34 lactose maldigesters agreed to participate in this study.

##### **4.4.1 Breath hydrogen**

The maximum absolute peak height of breath hydrogen produced in individuals after consumption of milk, yogurt and yogurt tablets ranged from 20-55, 2-45 and 8-57 ppm, respectively.

The average pattern of change in absolute breath hydrogen concentration produced by lactose maldigesters after milk, yogurt and yogurt tablet consumption throughout 7 h period was shown in **Figure 12** and **Appendix F**. After consumption of yogurt and yogurt tablets, 97% and 60% of the lactose maldigesters, respectively, showed lower absolute peak height and cumulative breath hydrogen concentration than after drinking milk (**Figure 10** and **Figure 11**). The mean $\pm$ SD of absolute peak height production in individuals after milk, yogurt and yogurt tablet consumption were  $34.4 \pm 10.9$ ,  $15.8 \pm 10.3$ ,  $27.3 \pm 14.1$  ppm, respectively (**Table 8**). The mean $\pm$ SD of area under curves after milk, yogurt and yogurt tablet consumption were  $4822 \pm 1494$ ,  $2020 \pm 1457$  and  $3940 \pm 2390$  ppm.min, respectively (**Table 8**).

The level of cumulative breath hydrogen production and absolute peak hydrogen concentration produced by the subjects after yogurt consumption were statistically different ( $p < 0.05$ ) from those produced after yogurt tablet consumption. No differences in these parameters was found in the subjects after milk and yogurt tablet consumption.



**Figure10** Individual absolute peak hydrogen concentration (ppm) over the 7 h period in lactose maldigesters (n=1-30) after ingestion of milk, yogurt tablet and yogurt

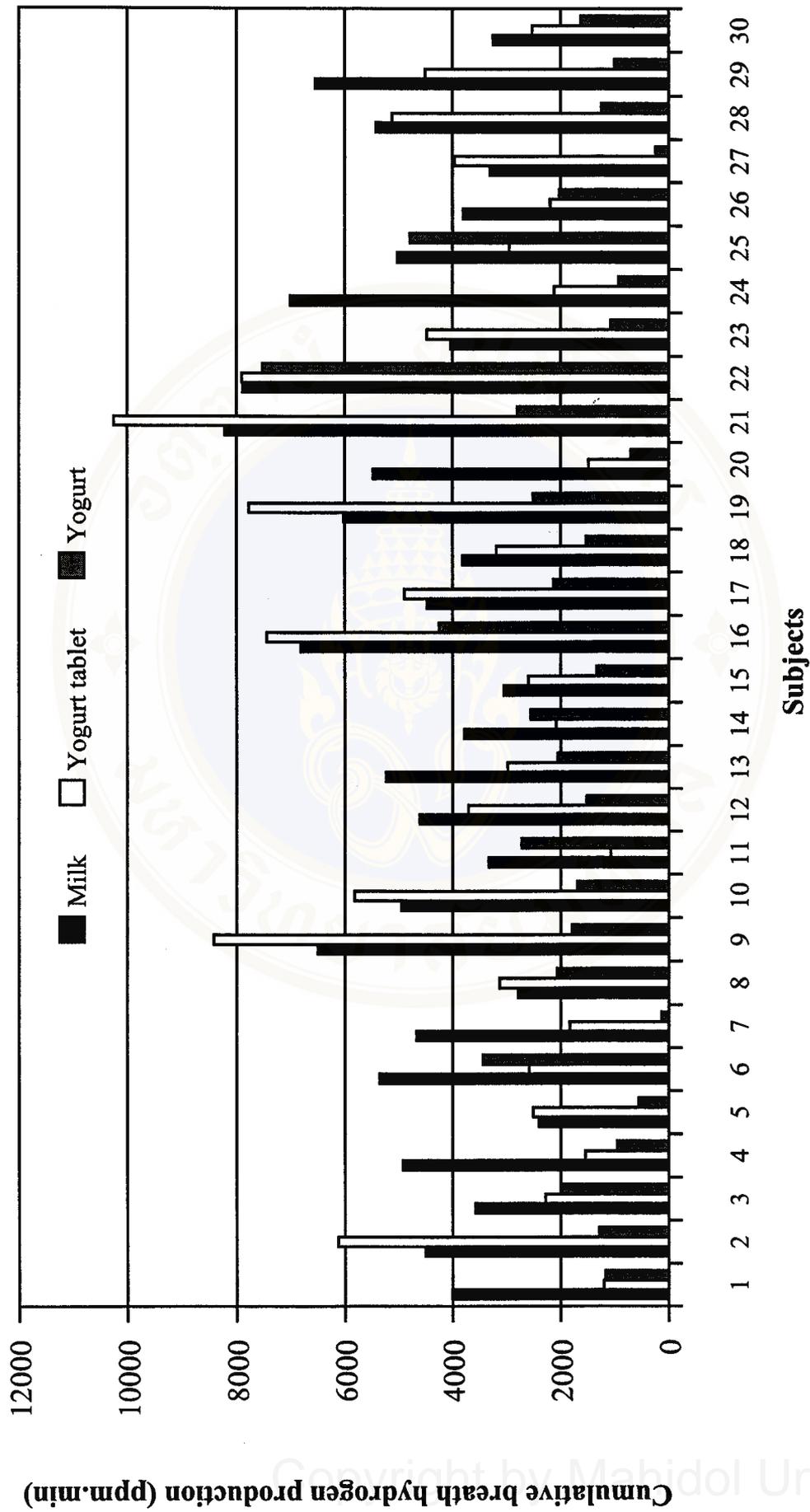
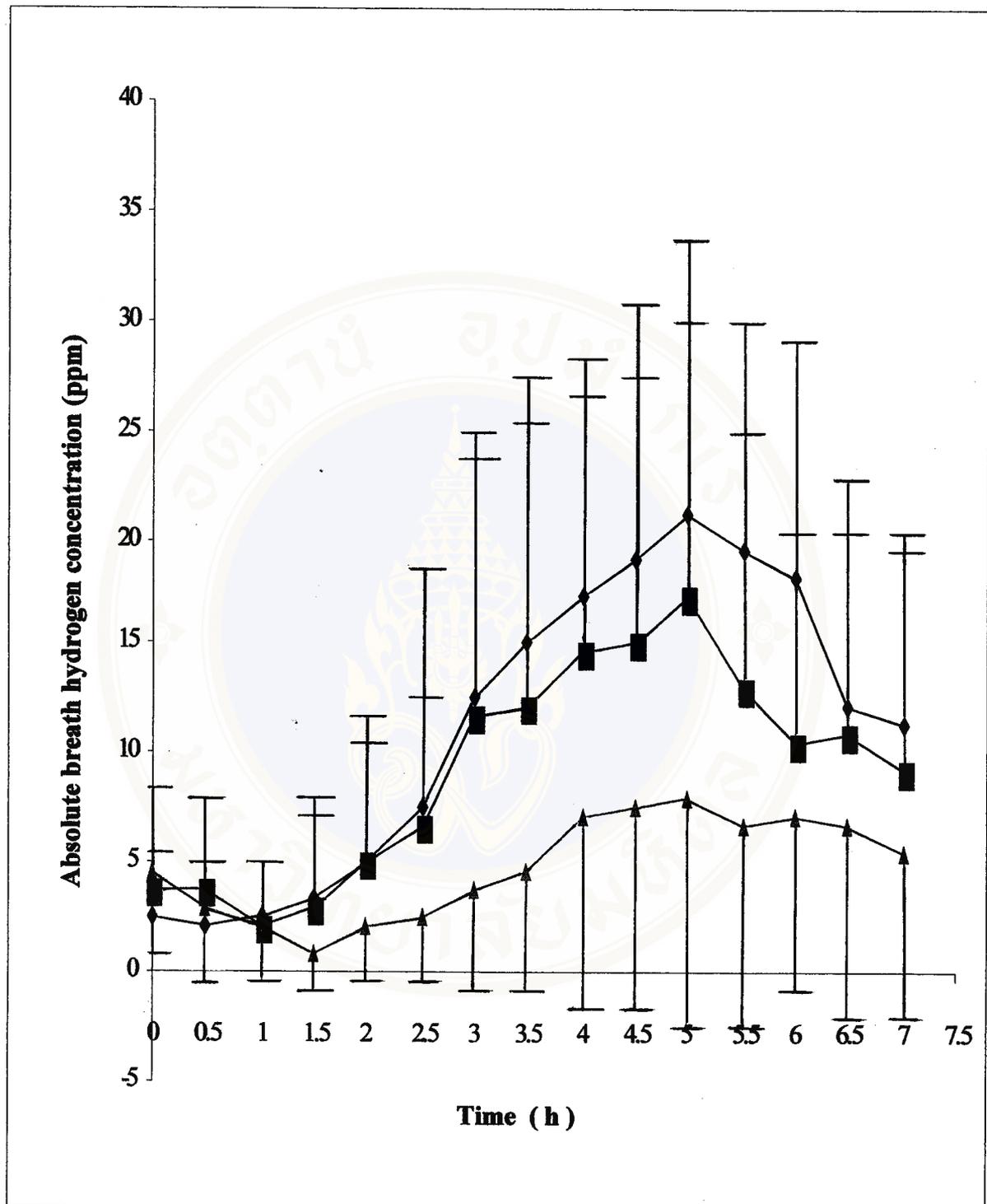


Figure 11 Individual cumulative breath hydrogen production (ppm.min) over the 7 h period in lactose maldigesters (n=30) after ingestion of milk, yogurt tablet and yogurt



**Figure 12** Average changes in absolute breath hydrogen concentration (ppm) after ingestion of 250 ml-milk (◆), yogurt tablet (■) and fresh yogurt (▲) in lactose maldigesters (n=30)

**Table 8** Absolute peak hydrogen concentration and cumulative breath hydrogen production (area under hydrogen production curve) , as mean $\pm$ SD, after consumption of milk, yogurt and yogurt tablets

Experiment diets	Number of subjects	Absolute peak height (ppm)	Area under curve (ppm.min)
Milk	30	34.4 $\pm$ 10.9 <sup>a</sup>	4822 $\pm$ 1494 <sup>a</sup>
Yogurt	30	15.8 $\pm$ 10.3 <sup>b</sup>	2020 $\pm$ 1457 <sup>b</sup>
Yogurt tablet	30	27.3 $\pm$ 14.1 <sup>a</sup>	3940 $\pm$ 2390 <sup>a</sup>

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

## CHAPTER V

### DISCUSSION

#### 5.1 Identification of lactose maldigester and intolerance in Thai adolescent

##### 5.1.1 Measurement of breath hydrogen and methane production

In this study the breath hydrogen test was employed to identify lactose maldigesters because the test is non-invasive and high sensitivity, in comparison to other methodologies. It has capacity to detect the incomplete absorption of carbohydrate in 240 ml milk or as small as 2 g of lactose (135). Many investigators (5, 8, 12, 13, 17-19, 63, 136) applied this technique to identify lactose maldigesters in different population groups. The increment of a peak breath hydrogen of  $\geq 20$  ppm above baseline of individual subject was used in various studies as a cut-off criterion for classification of lactose maldigesters (8, 12, 63, 136). However, the area under the hydrogen production curve could have been promising criteria to identify the lactose maldigesters in the future. The cut-off point of this criteria must be identified.

Breath methane is derived exclusively from fermentation reactions of strictly anaerobic bacteria (methanogens) within the intestinal lumen. On the basis of several large series of studies (146, 147), it has been estimated that 33-50% of healthy adults excrete breath methane in appreciable quantities,  $>3$  ppm above ambient levels. However, the factors - genetic makeup, ethnic origin, diet, environment or an interaction of all these factors - that determine an individual's methane excretor status are not well understood.

Previous studies found that both in fasting state and after ingestion of nonabsorbable sugars, subjects with low breath hydrogen excretion had a significantly greater both methane concentrations than did their counterparts with high hydrogen excretion (148). The hydrogen breath test may have the false negative results. Therefore, in the study of lactose maldigestion and intolerance, breath methane should be measured in conjunction with hydrogen breath test. The level of methane of at least 12 ppm is an indicative of lactose maldigesters without reference of hydrogen production (88). 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 (88).

### 5.1.2 Test diet

In this study that, a 250 ml-milk was used as the test diet, the degree of lactose maldigestion in Thai adolescents, 14–16 years old, was 32%. Many previous studies reported higher degree of lactose maldigesters in adolescent, but they used high concentration of lactose solution as the test diet. For example, Thing *et al* (1988) studied lactose maldigestion in Chinese adolescents, aged 15–16 years old, using 0.5 g lactose/kg body weight as the test diet (about 22.5 g /45 kg bodyweight), they found that 69% of the Chinese adolescents were lactose maldigesters (8). Using 50 g lactose in 250 ml water as the test diet, 80% of Asian–American adolescents were found to be lactose maldigesters (137). Studies on the prevalence of lactose maldigestion in the last three decades in Thai children and adults, high concentration of lactose solution (1g / kg body weight for children and 50 g lactose in adults) was used as the test diet and 100% or almost 100% of lactose maldigesters were reported (9, 10, 11, 47). Administration of 50 g lactose in the previous studies on lactose maldigester is equal

to drinking of about 1 liter of milk. This is obviously an unusual feeding habit (6). Sategna–Guldetti, *et al* (138) also suggested that milk, rather than pure lactose, must be used as a physiological test diet in clinical evaluation of lactose malabsorption and intolerance. Besides, the amount of  $\geq 12\text{g}$  lactose clearly demonstrated maldigestion using hydrogen breath test (135). In this experiment, therefore, a 250 ml-milk which contains 12.33 g lactose was used as the test diet. This is in accordance with the test volume of milk in several current experiments (8, 12, 63, 136).

### **5.1.3 Degree of lactose maldigestion in Thai adolescents**

In this study, using breath hydrogen measurement and a 250 ml-milk as tested diet, 32% of adolescents was found being lactose maldigesters. The degree was slightly lower than the findings in previous reports, 39-41% in adolescents (18, 19), which applied the same test diet, same criteria and method for identification of lactose maldigesters. However, when lactose maldigesters (9 subjects) based on breath methane and hydrogen production was added up, similar degree (40%) of lactose maldigesters in adolescents were found. Thus, it seems possible that methanogenesis could reduce available hydrogen to the extent that breath hydrogen excretion does not rise by 20 ppm. In conclusions, 43 out of 107 adolescents (40%) were lactose maldigesters after 250 ml-milk consumption.

### **5.1.4 Gastrointestinal symptoms**

In lactose maldigesters, the residual lactose is transported to the colon where lactose fermentation is taken place by colonic bacteria, resulted in the production of short chain fatty acids and gas (hydrogen, carbon dioxide and methane). This in turn induced different degree of gastrointestinal symptoms of flatulence, diarrhea and abdominal pain—the common symptoms among lactose maldigesters after consumption

of dairy products. Inclusion of 6–7 g lactose in a diet was found to induce the increment of intolerant symptoms (135, 140). Direct relationship was reported between the dose of lactose consumed and the severity of intolerant (139, 141). In the present study, 29% of the lactose maldigesters were lactose intolerant with mild gastrointestinal symptoms.

Theoretically, symptoms attributable to lactose maldigestion should occur when receiving lactose-containing diet. Nevertheless, 71% of lactose maldigesters in the present study had no experience of any symptoms. The same phenomenon (64% of lactose maldigesters) was shown by Kwon *et al* (139) although 50 g lactose was given to the subjects. It was suggested by Saunders and Wiggins (138) that the development of diarrhea or gaseous symptoms depend partly on the balance between the production and the removal of fermentation products. The gases produced during fermentation may be rapidly absorbed into the blood stream, thus the malabsorption does not necessarily cause the detectable gastrointestinal symptoms.

In this study, it was unexpected that 10% of the lactose digesters were lactose intolerants. In principle, apparent lactose intolerance should not be found in lactose digesters. In the double blind study of Kwon *et al* (139), using 50 g lactose as the test diet, of 42 lactose absorbers, 13 reported gastrointestinal symptoms after ingestion of lactose free only or both lactose free and lactose containing diet. Among these subjects, only one remaining reported gastrointestinal symptoms after receiving lactose-containing foods only. These results suggested that subjects may misattribute a variety of abdominal symptoms to lactose intolerance (142). Saurez and Levitt (140) suggested that subjects who believe themselves to be lactose intolerant, small doses of lactose may unrealistically have an underlying symptomatic state, which they misattribute

to lactose intolerance. Therefore, the reporting gastrointestinal symptoms in the lactose maldigesters after drinking of 250 ml- milk in this study may not be attributed to lactose intolerance.

In conclusion, only 29% of lactose maldigesters adolescents were lactose intolerant due to drinking 250 ml-milk although they drink milk regularly. This might be caused by the adaptation of gastrointestinal microflora in the subjects (139, 140).

## **5.2 Effect of fresh yogurt consumption on lactose digestion and tolerance in lactose maldigester adolescents**

Consuming 2 cups of fresh yogurt effectively decreased the degree of lactose maldigestion in adolescents (identified by consuming 250 ml-milk). The finding was supported by: 1) decreasing numbers of lactose maldigesters 2) 97% of lactose maldigesters showed reduction in absolute peak height of breath hydrogen production and 100% of them showed reduction in average cumulative breath hydrogen excretion, 3) reducing the severity of the gastrointestinal symptoms in lactose maldigesters. The finding was in accordance with the previous human studies (13, 19, 20-25).

Improved lactose digestion with yogurt appears partly due to the slight reduction of lactose content during fermentation, but is primarily due to autodigestion within the intestine by the microbial  $\beta$ -galactosidase enzyme (20). Three related factors appear to be important to the survival and expression of microbial enzyme activity from yogurt were: 1) the buffering of stomach acid by yogurt, 2) protection by the intact microbial cell to degradation by stomach acid or enzymes, 3) action of digestive enzymes and bile acids on the microbial cell which releases  $\beta$ -galactosidase activity (54), and 4) the effect of semi-solid of yogurt which delays gastric emptying and

slows gastrointestinal transit times (98-100). This allows more time for residual intestinal lactase and for yogurt 's bacteria to work.

Not only reduction in breath hydrogen production but also the decreasing in the numbers of gastrointestinal symptoms were observed when yogurt was consumed compared to milk. Similar findings were reported by several researchers (20, 21, 25, 110). Even active-cultures yogurt brands do not contain the same level of lactase activity, they can, compared with milk, reduce the occurrence and severity of symptoms associated with lactose maldigestion (144). However, if yogurt is pasteurized after the addition of active cultures (which is sometimes done to extend shelf life of the product), they will lose their ability to hydrolyze lactose in the gut (24, 28).

Some researchers found that flavored yogurts were more acceptable than plain (28). The same finding was experienced among the adolescent subjects in this study. Varela-Moreairas, *et al*, (28) found that consumption of flavored yogurt produced a breath hydrogen level slightly higher than that of plain yogurt, but none of the subjects experienced any symptoms after eating variety of yogurt (145). This probably due to the replacement of added fruits which reduced the amount of yogurt containing living microorganisms, thus reduced the  $\beta$ -galactosidase activity in flavored yogurts.

In this study, two cups of yogurt, one of plain and another of strawberry flavored yogurt, were given to the lactose maldigesters. This was to provide more acceptable and to reduce the effect of flavored yogurt, if any. Nevertheless, the consumption of the yogurt obviously exhibited reduction in breath hydrogen among lactose maldigesters.

In conclusion, consumption of yogurt can reduce numbers of lactose maldigesters and intolerants.

### **5.3 Effect of yogurt tablet consumption on lactose digestion and tolerance in lactose maldigester adolescents**

Consumption of yogurt tablets, with lactose content equal to that of fresh yogurt, reduced absolute peak breath hydrogen concentration and cumulative breath hydrogen production but much lesser degree than the fresh yogurt (reduced number of lactose maldigestors of about 60% compared to 97-100%). This might be due to the difference in the number of active bacteria in the products. The estimated active bacteria in 2 cups (300 g) of fresh yogurt was  $6.0 \times 10^{11}$  cfu which was clearly higher than those presented in 28 yogurt tablets ( $4.9 \times 10^6$  in 34.7 g). The effects of yogurt tablets consumption in producing breath hydrogen (by the average change of absolute peak hydrogen and cumulative breath production) in the subjects with lactose intolerance did not differ significantly from that produced after milk consumption. Although consumption of yogurt tablets was not as effective as fresh yogurt in reduction of breath hydrogen, but it exhibited similar effect as fresh yogurt on decreasing the gastrointestinal symptoms among lactose maldigesters.

## CHAPTER VI

### CONCLUSION

Lactose maldigestion and tolerance were studied in 107 Thai adolescents, using a 250 ml-milk as the test diet. The effect of fresh yogurt and yogurt tablet consumption on lactose digestion and tolerance was investigated. It was found that 32% of the studied adolescents were lactose maldigesters when hydrogen in breath was used as the criteria for classification of lactose maldigestion. When both hydrogen and methane production in breath were measured, 40% of adolescents were identified as lactose maldigesters.

Consuming 2 cups of yogurt decreased the degree of lactose maldigestion as indicated by lower breath hydrogen production in lactose maldigesters. It had no significant effect on lactose digestion when 28 yogurt tablets were consumed but they have the same effect as fresh yogurt in reducing the gastrointestinal symptoms.

In general, yogurt tablet is consumed about 5-6 tablets at a time. Due to the low concentration of lactose in a serving (2-3 g lactose), lactose maldigestion or lactose intolerance should not occur. Therefore, yogurt tablets as a milk product containing some calcium and protein, can be served as nutritious snack for children or other age groups. Besides, it can be used as a source of probiotics. However, it must be aware of causing tooth decay because the high sugars content in yogurt tablets.

## **RECOMMENDATION AND SUGGESTION FOR FURTHER STUDY**

1. Yogurt tablet is a new form of yogurt. It can be used as a potential source of probiotic, calcium and protein for children and adults especially those with lactose intolerants. It has advantages for use in rural area whereas refrigeration may not be available.
2. Future research should be emphasis on the possibility of using yogurt tablet as the starter for yogurt production. However, yogurt tablet should be developed to contain more viable lactic acid bacteria.
3. At present, the criteria of using breath hydrogen excretion  $\geq 20$  ppm is commonly considered to indicate the lactose maldigesters. In most studies, both the cumulative breath hydrogen concentration and the absolute peak height were measured. However, the cumulative breath hydrogen concentration has not yet been considered as the criteria to identify lactose maldigesters. It should be considered as another parameter to indicate lactose maldigestion. The criteria based on this parameter must be set up.

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## APPENDIX A

### GLOSSARY

**Lactase**  $\beta$ -galactosidase, an enzyme of the hydrolase class that catalyzes the hydrolysis (digestion) of lactose, a disaccharide, into its monosaccharide components of glucose and galactose. Lactase is present on the brush border of the intestinal mucosa where such digestion takes place.

**Lactose** A disaccharide which yields upon hydrolysis the monosaccharides, glucose and galactose. Since milk is the sole natural source of lactose, it is commonly referred to as milk sugar.

**Lactose intolerance** The clinical sign and symptom following lactose maldigestion which include bloating, flatulence, abdominal pain, and diarrhea. "Tolerance" and "intolerance" are not synonymous with "digestion" and "maldigestion" and should be used only in reference to a defined dose of lactose delivered in a specific vehicle (i.e. the subject was intolerance to 50g of lactose in aqueous solution).

**Lactose maldigestion** Reduced digestion of lactose due to low lactase activity as determined by a lactose tolerance test.

**Lactose malabsorption** This word has been used uncorrected in previous literature. Lactose is not absorbed as such but must first be split into glucose and galactose.

**Breath hydrogen test** A test that measures expired air for hydrogen content. If lactose is poorly digested it passes into the large intestine where it is fermented by bacteria, with hydrogen as a by product, the hydrogen diffuses into the bloodstream and is expired through the lungs. This increase in breath hydrogen after lactose consumption is thus a measure of the degree of lactose maldigestion.

## APPENDIX B

## แบบสอบถามบริโภคนิสัยการดื่มนมและผลิตภัณฑ์นม

## ประวัติทั่วไป

1. ชื่อสกุล \_\_\_\_\_ วันเดือนปีเกิด \_\_\_\_\_

2. ที่อยู่ บ้านเลขที่ \_\_\_\_\_ ซอย \_\_\_\_\_ ถนน \_\_\_\_\_

ตำบล/แขวง \_\_\_\_\_ อำเภอ/เขต \_\_\_\_\_ จังหวัด \_\_\_\_\_

โทร \_\_\_\_\_

3. ชั้นมัธยมศึกษาปีที่ \_\_\_\_\_ ห้อง \_\_\_\_\_ แผนการเรียน \_\_\_\_\_

4. เพศ 1. ( ) หญิง 2. ( ) ชาย

5. น้ำหนัก \_\_\_\_\_ กิโลกรัม

6. ส่วนสูง \_\_\_\_\_ เซนติเมตร

7. จำนวนพี่น้องในครอบครัว \_\_\_\_\_ คน เป็นบุตรคนที่ \_\_\_\_\_

8. ปัจจุบันพักในหอพักของโรงเรียนหรือไม่

( ) 1. พัก ห้องหมายเลข \_\_\_\_\_

( ) 2. ไม่พัก เพราะ \_\_\_\_\_

### ประวัติการเจ็บป่วย

9. มีโรคประจำตัวหรือไม่

- ( ) 1. ไม่มี ( ) 2. มี โปรดระบุ \_\_\_\_\_

10. มีอาการเจ็บป่วยในระยะเวลาที่ผ่านมาหรือไม่

- ( ) 1. ไม่มี ( ) 2. มี โปรดระบุ \_\_\_\_\_

11. เคยป่วยเป็นโรคเกี่ยวกับทางเดินอาหารหรือไม่

- ( ) 1. ไม่เคย ( ) 2. เคย โปรดระบุ \_\_\_\_\_

12. ยาที่รับประทานเป็นประจำ

- ( ) 1. ไม่มี ( ) 2. มี โปรดระบุ \_\_\_\_\_

13. ปัจจุบันรับประทานยาอะไรอยู่หรือไม่

- ( ) 1. ไม่รับประทาน ( ) 2. รับประทาน โปรดระบุ \_\_\_\_\_

### ประวัติการดื่มหมและผลิตภัณฑ์นม

14. ปกติดื่มหมหรือไม่ (ถ้าดื่มข้ามไปตอบข้อ 16)

- ( ) 1. ไม่ดื่ม ( ) 2. ดื่ม

## 15. ถ้าไม่ดื่ม โปรระบุเหตุผล

- ( ) 1. ดื่มแล้วมีอาการ เช่น \_\_\_\_\_
- ( ) 2. ไม่ชอบรสชาติหรือกลิ่นของนม
- ( ) 3. อื่นๆระบุ \_\_\_\_\_

## 16. ถ้าดื่มนม โปรระบุระยะเวลา

- ( ) 1. ดื่มตั้งแต่เด็กจนถึงปัจจุบัน
- ( ) 2. เพิ่งเริ่มต้นดื่มมาประมาณ \_\_\_\_\_ ปี

## 17. หลังดื่มนมไปแล้วแสดงอาการท้องเสีย ระยะเวลาที่แสดงอาการ

- ( ) 1. ทุกครั้ง ( ) 2. เป็นบางครั้ง ( ) 3. ไม่มีอาการ

## 18. ถ้าดื่มนมแล้วแสดงอาการท้องเสีย ระยะเวลาที่แสดงอาการ

- ( ) 1. หลังดื่มนมทันที ( ) 2. ทั้งระยะเวลาพอควร

## 19. ชอบดื่มนมรสอะไร

- ( ) 1. นมพร้อมไขมัน ( ) 2. นมจืด
- ( ) 3. นมหวาน ( ) 4. อื่นๆ โปรระบุ \_\_\_\_\_

## 20. ปริมาณของการดื่มนมต่อครั้ง โปรระบุว่าเป็นแก้วหรือกล่องหรือถุง

- ( ) 1. 1/2 แก้ว-กล่อง-ถุง/ครั้ง
- ( ) 2. 1 แก้ว-กล่อง-ถุง/ครั้ง
- ( ) 3. 2 แก้ว-กล่อง-ถุง/ครั้ง
- ( ) 4. >2 แก้ว-กล่อง-ถุง/ครั้ง โปรดระบุ \_\_\_\_\_ แก้ว-กล่อง-ถุง/ครั้ง

21. ความถี่ของการดื่มนมโดยเฉลี่ย โปรดระบุว่าเป็นแก้วหรือกล่องหรือถุง

- ( ) 1. 1 แก้ว-กล่อง-ถุง/วัน
- ( ) 2. 2 แก้ว-กล่อง-ถุง/วัน
- ( ) 3. >2 แก้ว-กล่อง-ถุง/วัน โปรดระบุ \_\_\_\_\_ แก้ว-กล่อง-ถุง/ครั้ง
- ( ) 4. ดื่มเป็นครั้งคราว โปรดระบุ \_\_\_\_\_ แก้ว-กล่อง-ถุง/สัปดาห์

22. มักดื่มนมเวลาใด

- ( ) 1. ก่อนอาหาร
- ( ) 2. หลังอาหาร
- ( ) 3. ขณะท้องว่าง

23. ปกติดื่มนมร่วมกับอาหารหรือไม่

- ( ) 1. ดื่มนมอย่างเดียว
- ( ) 2. ดื่มนมร่วมกับอาหาร โปรดระบุชนิดของอาหาร \_\_\_\_\_

24. ปกติรับประทานโยเกิร์ตหรือไม่ (ถ้ารับประทานข้ามไปตอบข้อ 26)

- ( ) 1. ไม่รับประทาน ( ) 2. รับประทาน

25. ถ้าไม่รับประทาน โปรดระบุเหตุผล

( ) 1. รับประทานแล้วมีอาการเช่น \_\_\_\_\_

( ) 2. ไม่ชอบรสชาติหรือกลิ่นของ โยเกิร์ต

( ) 3. อื่นๆ โปรดระบุ \_\_\_\_\_

26. ถ้ารับประทานชอบ โยเกิร์ตรสชาติใด

( ) 1. รสธรรมชาติ ( ) 2. รสส้ม ( ) 3. รสสับปะรด

( ) 4. รสสตอเบอร์รี่ ( ) 5. อื่นๆ โปรดระบุ \_\_\_\_\_

27. เมื่อรับประทาน โยเกิร์ตมีอาการเช่นเดียวกับเมื่อดื่มนมหรือไม่

( ) 1. มีอาการเหมือนดื่มนม

( ) 2. ไม่มีอาการ

( ) 3. อื่นๆ โปรดระบุ \_\_\_\_\_

28. ปริมาณการรับประทาน โยเกิร์ตต่อครั้ง

( ) 1. 1 ถ้วย/ครั้ง (ปริมาณ \_\_\_\_\_ กรัม/ถ้วย)

( ) 2. 2 ถ้วย/ครั้ง (ปริมาณ \_\_\_\_\_ กรัม/ถ้วย)

( ) 3. > 2 ถ้วย/ครั้ง โปรตีน \_\_\_\_\_ ถ้วย/ครั้ง(ปริมาณ \_\_\_\_\_ กรัม/ถ้วย)

29. ความถี่ของการรับประทานโยเกิร์ต

( ) 1. 1 ถ้วย/วัน

( ) 2. 2 ถ้วย/วัน

( ) 3. > 2 ถ้วย/วัน โปรตีน \_\_\_\_\_ ถ้วย/วัน

( ) 4. เป็นครั้งคราว โปรตีน \_\_\_\_\_ ถ้วย/สัปดาห์

30. รับประทานผลิตภัณฑ์ที่ทำจากนมอื่นๆ นอกเหนือจากนมและโยเกิร์ตบ้างหรือไม่ เช่น

ไอศกรีม,นมเปรี้ยว,เนยแข็ง,เนยเหลว,นมอัดเม็ด เป็นต้น

( ) 1. ไม่รับประทาน

( ) 2. รับประทาน โปรตีน \_\_\_\_\_

31. หากรับประทานผลิตภัณฑ์ที่ทำจากนม (นอกเหนือจากนมและโยเกิร์ต) มีอาการเช่น

เดียวกับเมื่อดื่มนมหรือไม่

( ) 1. มีอาการเหมือนดื่มนม

( ) 2. ไม่มีอาการ

( ) 3. อื่นๆ โปรตีน \_\_\_\_\_

32. ปกติรับประทานอาหารประเภทใด

- ( ) 1. รสจัด                      ( ) 2. รสไม่จัด
- ( ) 3. ชอบผักมาก              ( ) 4. ไม่ชอบผัก
- ( ) 5. ชอบผลไม้                ( ) 6. ไม่ชอบผลไม้





**APPENDIX D**

Absolute breath hydrogen concentration at 30-min interval, peak hydrogen concentration (ppm) and area under curve of breath hydrogen production (ppm.min)

Subject number	Test period	Time after taking test product (h)												Peak H <sub>2</sub> concentration	Area under curve			
		0	0.5	1	1.5	2	2.5	3	3.5	4	4.5	5	5.5			6	6.5	7
1	M	1	1	0	1	1	3	7	10	10	12	21	30	18	14	10	30	4005
	Y	2	1	2	0	0	2	1	1	2	2	2	8	6	6	10	10	1170
	T	6	0	3	2	0	1	1	1	3	2	2	3	7	9	6	9	1200
2	M	4	0	2	4	3	14	10	18	27	15	22	12	9	6	12	27	4500
	Y	6	0	10	2	4	0	0	-1	8	6	-2	1	5	4	6	10	1290
	T	11	13	0	3	3	9	23	16	9	28	19	24	22	25	9	28	6120
3	M	5	1	0	10	11	13	13	11	20	10	2	8	12	3	5	20	3570
	Y	5	0	0	0	1	1	1	1	1	10	12	11	13	10	5	13	1980
	T	2	0	2	3	1	1	1	0	13	12	15	13	6	5	6	15	2280
4	M	10	8	3	0	6	6	8	8	8	8	16	12	25	37	28	37	4920
	Y	10	6	4	0	0	3	0	1	2	2	6	1	1	1	0	10	960
	T	0	6	1	5	2	6	2	0	-	2	8	4	6	8	3	8	1545
5	M	6	4	0	2	-3	-2	3	23	13	10	4	5	6	8	8	23	2400
	Y	5	2	0	1	0	0	-1	0	-1	-2	0	2	11	3	2	11	555
	T	2	0	4	5	2	10	12	10	20	6	10	1	0	2	1	20	2505
6	M	1	0	4	9	14	16	12	18	14	30	14	28	14	3	4	30	5355
	Y	8	5	3	0	2	3	4	8	8	19	27	5	15	8	6	27	3420
	T	10	3	0	3	7	12	22	14	9	3	-	1	2	5	0	22	2580

**APPENDIX D (Cont.)**

Absolute breath hydrogen concentration at 30-min interval, peak hydrogen concentration (ppm) and area under curve of breath hydrogen production (ppm.min)

Subject number	Test period	Time after taking test product (h)															Peak H <sub>2</sub> concentration	Area under curve
		0	0.5	1	1.5	2	2.5	3	3.5	4	4.5	5	5.5	6	6.5	7		
7	M	10	0	9	15	18	4	9	12	16	31	11	12	10	2	3	31	4665
	Y	0	0	0	4	4	2	13	5	2	2	1	1	2	-	4	13	1140
	T	8	7	3	0	2	5	2	10	19	7	-	-	1	1	0	19	1830
8	M	0	12	6	11	4	-11	6	5	21	4	9	2	10	9	9	21	2775
	Y	5	3	0	8	6	0	2	2	0	32	3	13	12	12	5	13	2070
	T	4	5	5	0	2	7	10	23	23	1	2	2	2	0	0	23	3120
9	M	7	5	1	0	2	-4	10	25	20	22	55	32	18	22	10	55	6495
	Y	3	0	0	1	1	1	2	2	3	3	2	5	12	16	20	20	1785
	T	0	10	8	10	15	12	42	55	31	11	10	16	18	26	7	55	8025
10	M	5	4	1	0	0	3	28	33	22	19	10	15	12	11	9	33	4950
	Y	4	0	1	5	1	5	7	7	7	3	6	2	6	3	3	7	1695
	T	4	4	5	5	7	7	0	5	8	28	38	43	17	17	16	43	5820
11	M	0	1	0	5	1	-1	-1	-1	5	17	20	28	20	13	8	28	3330
	Y	10	1	1	0	5	10	6	8	7	7	8	8	5	12	15	15	2715
	T	4	0	0	1	0	5	0	0	5	3	16	4	2	0	-4	16	1080
12	M	1	1	0	1	2	5	3	11	20	27	26	28	14	12	6	28	4605
	Y	8	3	4	0	0	2	-1	2	6	4	8	2	6	9	4	9	1522.5
	T	4	1	0	1	1	10	14	10	3	9	18	10	16	17	23	23	3705

**APPENDIX D (Cont.)**

Absolute breath hydrogen concentration at 30-min interval, peak hydrogen concentration (ppm) and area under curve of breath hydrogen production (ppm.min)

Subject number	Test period	Time after taking test product (h)															Peak H <sub>2</sub> concentration	Area under curve
		0	0.5	1	1.5	2	2.5	3	3.5	4	4.5	5	5.5	6	6.5	7		
13	M	0	4	6	2	6	2	8	15	12	14	20	28	32	15	21	32	5235
	Y	3	1	0	0	6	7	5	5	6	8	5	5	6	8	10	10	2055
	T	12	8	1	0	1	6	8	7	6	2	17	18	13	4	4	18	2970
14	M	2	0	1	3	12	15	21	10	12	15	16	5	10	5	0	21	3780
	Y	10	4	0	1	1	1	3	13	18	14	10	7	6	2	0	18	2550
	T	0	0	0	1	0	0	4	12	22	10	7	6	4	3	1	22	2085
15	M	0	4	9	1	12	16	20	13	10	5	1	4	4	2	1	20	3045
	Y	0	2	8	3	3	3	2	2	6	7	1	2	1	4	1	7	1335
	T	0	3	0	5	10	12	15	18	12	6	2	4	1	-2	1	18	2595
16	M	4	0	1	6	1	12	20	36	42	23	31	25	12	15	2	42	6810
	Y	8	9	2	0	-2	2	7	9	29	40	18	20	3	-	1	40	4245
	T	3	1	0	21	28	20	49	41	29	22	18	7	4	5	3	49	7440
17	M	0	2	2	8	0	3	4	-1	15	32	46	21	10	5	4	46	4470
	Y	8	10	5	0	7	10	16	5	9	5	1	0	2	-2	-2	16	2130
	T	0	2	4	10	11	15	10	12	24	28	35	10	6	-2	-4	35	4890
18	M	0	3	3	1	6	2	2	1	2	3	18	30	37	11	16	37	3810
	Y	0	5	4	0	3	4	3	7	1	3	1	3	17	-	-	17	1530
	T	2	1	2	0	1	0	3	1	17	20	23	8	11	14	8	23	3180

**APPENDIX D (Cont.)**

Absolute breath hydrogen concentration at 30-min interval, peak hydrogen concentration (ppm) and area under curve of breath hydrogen production (ppm.min)

Subject number	Test period	Time after taking test product (h)															Peak H <sub>2</sub> concentration	Area under curve
		0	0.5	1	1.5	2	2.5	3	3.5	4	4.5	5	5.5	6	6.5	7		
19	M	0	0	1	2	2	7	8	27	31	33	42	19	15	9	9	42	6015
	Y	3	5	0	1	2	2	2	-2	8	6	28	13	10	6	2	28	2505
	T	0	1	8	3	6	10	20	25	49	36	44	22	24	6	10	49	7770
20	M	1	2	0	0	10	12	16	20	25	55	32	10	-	-	-	55	5475
	Y	10	7	3	0	1	2	2	1	0	2	2	0	0	-2	1	10	705
	T	0	0	0	1	1	0	5	7	5	11	5	1	8	0	11	11	1485
21	M	1	0	0	1	15	48	49	52	-	19	17	28	18	20	13	52	8220
	Y	1	1	3	0	0	10	15	20	23	10	2	3	1	3	3	23	2790
	T	4	4	0	15	20	21	34	22	33	57	40	35	20	22	33	57	10245
22	M	1	6	5	0	0	1	16	18	31	37	26	29	54	25	29	54	7890
	Y	0	0	0	1	1	1	3	14	11	33	42	40	41	45	37	45	7515
	T	16	7	0	0	1	1	11	15	20	21	33	38	41	54	27	54	7905
23	M	0	0	0	0	1	9	13	15	21	19	19	14	12	8	7	21	4035
	Y	5	3	2	0	3	2	4	4	2	3	5	2	2	1	1	5	1080
	T	8	10	6	0	1	6	11	18	5	22	24	23	8	8	6	24	4470
24	M	0	1	0	1	1	4	16	33	45	29	32	23	18	16	29	45	7005
	Y	4	2	1	0	1	-2	2	1	2	0	1	0	8	10	6	10	930
	T	2	9	2	0	4	3	3	2	7	13	7	6	2	7	9	13	2115

**APPENDIX D (Cont.)**

Absolute breath hydrogen concentration at 30-min interval, peak hydrogen concentration (ppm) and area under curve of breath hydrogen production (ppm.min)

Subject number	Test period	Time after taking test product (h)																	Peak H <sub>2</sub> concentration	Area under curve
		0	0.5	1	1.5	2	2.5	3	3.5	4	4.5	5	5.5	6	6.5	7				
25	M	2	0	1	6	9	27	28	10	22	17	12	12	10	6	13	28	5025		
	Y	0	5	1	0	5	3	7	22	36	16	31	29	1	2	3	36	4785		
	T	2	0	0	0	0	2	3	8	1	23	26	1	10	16	14	26	2940		
26	M	2	0	2	2	1	1	3	6	6	12	30	26	22	12	5	30	3795		
	Y	0	0	0	0	0	1	1	3	5	8	8	17	10	12	5	17	2025		
	T	1	0	1	0	2	0	0	2	2	2	5	18	-	27	31	31	2190		
27	M	4	0	5	1	10	4	4	2	3	4	16	16	27	10	12	27	3300		
	Y	1	1	0	0	-1	2	2	0	2	1	1	1	1	0	1	2	240		
	T	14	12	9	0	4	3	6	2	3	5	11	22	27	11	20	27	3960		
28	M	6	4	6	0	4	18	35	16	10	12	24	15	19	11	7	35	5415		
	Y	5	0	6	4	4	3	1	2	3	6	3	2	2	2	2	6	1245		
	T	1	1	2	0	14	12	33	32	31	16	7	6	7	6	6	33	5115		
29	M	0	1	3	4	8	2	9	9	20	28	31	47	20	21	30	47	6540		
	Y	9	10	8	0	1	1	3	3	3	-2	0	-3	1	1	6	10	1005		
	T	0	7	0	0	5	4	4	6	10	16	34	20	13	20	22	34	4500		
30	M	0	1	4	6	1	3	0	0	1	1	6	15	36	24	20	36	3240		
	Y	2	2	0	0	0	0	-1	1	1	6	8	7	15	12	4	15	1620		
	T	1	3	2	0	2	2	3	1	4	10	14	13	13	11	11	14	2520		

**APPENDIX D (Cont.)**

Absolute breath hydrogen concentration at 30-min interval, peak hydrogen concentration (ppm) and area under curve of breath hydrogen production (ppm.min)

Subject number	Test period	Time after taking test product (h)																Peak H <sub>2</sub> concentration	Area under curve
		0	0.5	1	1.5	2	2.5	3	3.5	4	4.5	5	5.5	6	6.5	7			
*31	M	5	0	3	7	25	43	34	33	49	6	7	4	10	4	8	49	6945	
	Y	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
	T	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
*32	M	6	3	1	0	-1	6	7	8	10	14	20	23	15	10	2	23	3600	
	Y	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
	T	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
*33	M	5	1	0	1	1	4	7	5	10	17	55	17	12	20	16	55	4815	
	Y	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
	T	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
*34	M	0	2	4	2	3	0	1	2	2	7	10	26	20	34	28	34	3810	
	Y	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
	T	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	

M = milk  
 Y = fresh yogurt  
 T = yogurt tablet

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

**APPENDIX E**

Average changes in absolute breath hydrogen concentration (ppm) produced by lactose digesters and lactose maldigesters after ingestion of 250 ml-milk in each time.

Time (h)	Absolute breath hydrogen concentration (mean $\pm$ SD)	
	Lactose digesters (n=73)	Lactose maldigesters (n=34)
0	3.2 $\pm$ 4.0	2.6 $\pm$ 3.0
0.5	2.3 $\pm$ 3.0	2.1 $\pm$ 2.7
1.0	1.9 $\pm$ 2.9	2.4 $\pm$ 2.6
1.5	1.89 $\pm$ 3.4	3.3 $\pm$ 3.8
2.0	1.7 $\pm$ 3.3	5.5 $\pm$ 6.3
2.5	2.9 $\pm$ 4.5	8.4 $\pm$ 11.9
3.0	3.7 $\pm$ 5.0	12.6 $\pm$ 11.3
3.5	3.4 $\pm$ 4.9	14.8 $\pm$ 12.2
4.0	3.5 $\pm$ 4.7	17.4 $\pm$ 12.1
4.5	4.2 $\pm$ 5.0	17.9 $\pm$ 11.7
5.0	4.8 $\pm$ 5.4	21.2 $\pm$ 13.7
5.5	4.7 $\pm$ 5.4	19.1 $\pm$ 10.3
6.0	5.1 $\pm$ 4.7	17.6 $\pm$ 10.3
6.5	4.3 $\pm$ 5.4	12.8 $\pm$ 8.7
7.0	4.4 $\pm$ 5.4	11.6 $\pm$ 8.9

**APPENDIX F**

Average changes in absolute breath hydrogen concentration (ppm) in lactose maldigesters after ingestion of milk, yogurt and yogurt tablets

Time (h)	Absolute breath hydrogen concentration (mean $\pm$ SD)		
	Milk (n=30)	Yogurt (n=30)	Yogurt tablet (n=30)
0	2.4 $\pm$ 3.0	4.5 $\pm$ 3.5	4.0 $\pm$ 4.6
0.5	2.2 $\pm$ 2.8	2.9 $\pm$ 3.1	3.9 $\pm$ 4.0
1.0	2.5 $\pm$ 2.7	2.3 $\pm$ 2.8	2.3 $\pm$ 2.7
1.5	3.4 $\pm$ 3.9	1.0 $\pm$ 1.9	3.1 $\pm$ 5.0
2.0	5.3 $\pm$ 5.4	2.0 $\pm$ 2.3	5.1 $\pm$ 6.6
2.5	7.7 $\pm$ 10.8	2.6 $\pm$ 3.1	6.7 $\pm$ 5.8
3.0	12.7 $\pm$ 11.1	3.7 $\pm$ 4.4	11.7 $\pm$ 13.0
3.5	15.2 $\pm$ 12.2	3.9 $\pm$ 5.8	12.4 $\pm$ 13.0
4.0	17.4 $\pm$ 10.9	7.0 $\pm$ 8.7	14.6 $\pm$ 11.9
4.5	18.8 $\pm$ 12.1	7.5 $\pm$ 9.3	15.1 $\pm$ 12.3
5.0	21.0 $\pm$ 12.7	8.0 $\pm$ 10.7	17.5 $\pm$ 12.5
5.5	19.3 $\pm$ 10.5	6.9 $\pm$ 9.3	13.1 $\pm$ 11.6
6.0	18.1 $\pm$ 10.8	7.4 $\pm$ 8.1	10.7 $\pm$ 9.5
6.5	12.2 $\pm$ 8.0	7.0 $\pm$ 9.0	10.8 $\pm$ 11.8
7.0	11.4 $\pm$ 8.7	5.5 $\pm$ 7.6	9.3 $\pm$ 9.3

## APPENDIX G

Gastrointestinal symptoms occurred in lactose digesters during ingestion of milk and lactose maldigesters during and after ingestion of difference test diets

Test period	Status of subjects	Number of subjects experienced symptoms											
		Diarrhea				Abdominal pain				Bloating and flatulence			
		0	1	2	3	0	1	2	3	0	1	2	3
Milk	LD (73)	72	1	0	0	67	6	0	0	66	7	0	0
	LM (34)	31	3	0	0	29	5	0	0	24	10	0	0
Yogurt	LM (30)	28	2	0	0	28	2	0	0	26	4	0	0
Yogurt tablet	LM (30)	28	2	0	0	27	3	0	0	25	5	0	0

\* LD : Lactose digester

LM : Lactose maldigester

\* Grade of symptoms

0 = no symptom

1 = mild (1-3 times/h)

2 = moderate (4-6 times/h)

3 = severe (7-9 times/h)

## BIOGRAPHY



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