

**The Nutritional Use of Thai Medicinal Plants and the Etiology of Breast Cancer in  
Thai Women**

by

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## **List of Abbreviations**

NCCD	Non-communicable chronic disease
SHBG	Sex hormone binding globulin
IGFBP	Insulin-like growth factor binding protein
IGF	Insulin-like growth factor
BMI	Body mass index
WHR	Waist-to-hip ratio
GST	Glutathione S-transferase
DMBA	7, 12-dimethylbenz( $\alpha$ )anthracene
AMH	Anatomically modern humans
USO	Underground storage organs
P:S	Polyunsaturated: saturated fat ratio
LC-PUFA	Long-chain polyunsaturated fatty acids
PUFA	Polyunsaturated fatty acid
FFQ	Food frequency questionnaire
USD	U.S. Dollar
TEB	Terminal end bud
TDLU	Terminal ductal lobuloalveolar units
CHO	Carbohydrate
PRO	Protein
ROS	Reactive oxygen species
LDL	Low-density lipoproteins

## **Abstract**

### **The Nutritional Use of Thai Medicinal Plants and the Etiology of Breast Cancer in Thai Women**

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#### **Chair: A. Roberto Frisancho**

This study represents a novel and unique approach to the study of breast cancer in developing countries by examining breast cancer risk through an evaluation of the nutritional use of traditional medicinal plants within the context of the Paleolithic diet. To achieve this goal, anthropological and epidemiological data were collected in order to ascertain the risk factors associated with the increasing incidence of breast cancer in Thailand.

The major findings of this dissertation are as follows: (1) a later age at menarche among Thai women, rather than protecting against breast cancer (the pattern typically found in Western populations), produces an increased risk due to the prolongation of the adolescent growth period and a greater exposure to the action of growth-promoting hormones; (2) central fat distribution is associated with an increased risk of breast cancer among Thai women, even in the absence of anthropometric markers associated with the threshold for obesity; (3) adherence to the nutritional use of traditional medicinal plants among Thai women contributes to a protective effect against breast cancer, in that

consuming higher rates of medicinal plants results in a reduction in breast cancer risk; and (4) dietary quality, rather than the quantity of dietary components, determines breast cancer risk among Thai women.

## **Chapter 1**

### **Introduction and Goals of the Study**

#### Introduction

Over the past decade, research on traditional medicine has increased significantly due to a revived interest in alternative treatments for chronic diseases such as diabetes and cancer. Although improvements in nutrient and energy intake and the rapid development of pharmaceuticals have reduced the prevalence of infectious disease burden throughout the world, these same advances have simultaneously contributed to the rise in non-communicable chronic diseases (NCCDs) like breast cancer. In response to the rise of NCCDs worldwide, researchers have begun to examine the use of traditional medicine for the potential treatment of diseases such as breast cancer. This recent interest in traditional pharmacology is especially relevant since epidemiological studies examining the lifestyle behaviors and genetic variables contributing to breast cancer risk have yielded inconsistent results.

Rather than utilizing standard epidemiological approaches such as anthropometry and dietary intake, this study represents a novel and unique approach to the study of breast cancer in developing countries in that it examines breast cancer risk via an evolutionary perspective. Through an evaluation of the nutritional use of traditional medicinal plants within the context of the Paleolithic diet, widely believed to represent the dietary pattern to which anatomically modern humans have adapted, this study builds upon previous epidemiological techniques by addressing cancer risk in terms of a

nutritional ecological model. In this study, it is postulated that the intake of medicinal plants protects against breast cancer by representing a cultural adaptation in developing populations which best reflects the Paleolithic dietary model, in that health is optimized via a complex relationship between the Thai population, the food products made available by the environment, and the intake of essential micronutrients.

### Overview of Breast Cancer Epidemiology

Although non-Western countries have lower cancer rates than Western nations, developing populations still account for more than half of the world's cancer burden, since they represent almost seventy-five percent of the world's population, (Magrath and Litvak 1993). Higher rates of cancer mortality in developing countries can be attributed to both the lack of access to medical resources and to the ineffectiveness of local medical infrastructures to provide basic palliative treatment. This is especially true of breast cancer, which represents the most commonly diagnosed cancer and the leading cause of cancer deaths in women worldwide (Althuis et al 2005).

While Western countries have experienced a moderate decline in breast cancer incidence and mortality over the past two decades due to improvements in screening and preventative measures, developing countries in Asia, Latin America and Africa have seen a drastic increase in the number of breast cancer cases, with studies showing close to a 60-80% increase in breast cancer incidence over the past ten years (Lacey et al 2002). Many researchers have attributed this recent increase in breast cancer incidence to the spread of Western lifestyle behaviors, such as high energy diets, decreases in physical activity and changes in reproductive patterns, into Asia, Latin America and Africa (Althuis et al 2005). However, epidemiological studies into the traditional risk factors

(i.e. diet and body composition) associated with breast cancer in developing countries have highlighted two important differences when comparing the pattern of breast cancer incidence in Western and developing nations- the role of age and weight gain upon breast cancer risk.

**The Pattern of Breast Cancer Incidence in Western Nations.** In the developed nations of North America and Europe, the risk of breast cancer increases with both age and anthropometric markers of obesity, with the highest prevalence of breast cancer cases occurring in overweight/obese postmenopausal women over the age of fifty.

Postmenopausal weight gain is believed to elevate breast cancer risk by increasing the effects of the metabolic syndrome (insulin resistance) and a suite of hormonal changes which predispose the epithelial cells of mammary tissue to carcinogenesis.

Insulin resistance in obese, postmenopausal women is associated with the downregulation of sex hormone binding globulin (SHBG) and insulin-like growth factor binding proteins 1 and 2 (IGFBP), which are responsible for removing endogenous estrogen and insulin-like growth factors (IGF) from blood plasma (Harvie et al 2003). Because postmenopausal women produce estrogen in their fat tissue through the aromatization of ovarian and adrenal androgens, obese postmenopausal women have higher levels of endogenous estrogen in their blood plasma than non-obese women (McTiernan 2000). When coupled with the effects of insulin resistance, the downregulation of SHGB and IGFBP 1 and 2, coupled with high endogenous levels of sex hormones, lead to a greater bioavailability of estrogen, progesterone and IGF (Harvie et al 2003).

Increases in endogenous estrogen levels play a significant role in the etiology of breast cancer because estrogen metabolism produces metabolites which promote tumorigenesis in mammary epithelial cells. In healthy premenopausal women, estrogen is maintained in balance with progesterone, with the menstrual cycle producing natural fluctuations in their ratio (Ursin et al 1999). After menopause, however, estrogen dominance develops as a byproduct of decreased progesterone production. Estrogen levels, which are already elevated in postmenopausal women due to estrogen dominance, are further increased by lifestyle factors such as obesity and adherence to an urbanized dietary pattern (Westerlind et al 1999).

In studies examining the role of anthropometry and the pattern of fat storage upon breast cancer risk in women from Western populations, the consensus of research demonstrates that body mass index (BMI), weight and waist-to-hip ratio (WHR) are all positively correlated with breast cancer risk among postmenopausal women (Lahmann et al 2004). Furthermore, overweight and obesity both act to increase breast cancer in postmenopausal women risk by concomitantly upregulating estrogen production and downregulating SHBG synthesis (Harvie 2003; Lahmann et al 2004).

**The Pattern of Breast Cancer Incidence in Developing Nations.** By contrast, in the developing nations of Asia, Latin America and Africa, the highest prevalence of breast cancer cases occurs among premenopausal women under the age of fifty. Furthermore, while breast cancer risk increases with anthropometric markers of obesity among Western populations, the role of weight gain in the etiology of breast cancer in developing nations is less clear-cut, with studies showing contradictory results in the association between weight status and breast cancer risk. Instead, these studies seem to

suggest that populations from developing nations may possess different risk exposures for breast cancer based on ethnicity rather than traditional factors such as anthropometry (Rose et al 2004; Adebamowo 2003; Baumgartner et al 2004; Harvie 2003).

Although no epidemiological studies have been conducted in Thailand to ascertain the association between body composition and breast cancer risk, a review of the general demographic data suggests that breast cancer rates have increased over the past 5-10 years with the concomitant rise in the frequency of overweight/obesity and the shift to Western dietary preferences (Vatanasapt et al 2002; MOPH 2005). Furthermore, the presentation of breast cancer cases in Thailand shows an age-related pattern distinct from developing nations, in which premenopausal Thai women under the age of 50 possess the highest rates of breast cancer incidence (Vatanasapt et al 2002; MOPH 2005). (By contrast, the highest rates of breast cancer in Western countries are typically associated with postmenopausal women over 50 years of age.) This younger age at initial cancer presentation, in addition to a review of data from other developing populations, suggests that other factors may play a more important role than traditional anthropometry and dietary intake in predicting breast cancer risk in Thailand. The key factor which could potentially modify breast cancer risk in Thailand may be specifically related to changes in traditional dietary preferences.

In many developing populations, traditional diets are characterized by the incorporation of diverse foods which provide healthy levels of the essential nutrients required by humans to maintain optimal health. Moreover, the traditional dietary practices utilized by developing populations often incorporate plants containing both nutritional and medicinal benefits. The relatively recent abandonment of these traditional

ethnic diets incorporating medicinal plants may play a more important role in determining breast cancer risk in developing nations than measures of body composition.

### Overview of Nutritional and Anthropometric Epidemiology in Thailand

Thailand has historically represented one of the healthiest populations in the world, with a low incidence of cardiovascular disease, diabetes and cancer. The geography in Thailand has played an important role in maintaining this healthy Thai lifestyle, because the environment is characterized by riverine and lacustrine features which have shaped both Thai culture and traditional Thai dietary preferences (Wyatt 1984). Since the inception of the first Thai kingdom of *Sukhothai* in the 13<sup>th</sup> century A.D., Thailand has had its foundations in river-based communities, with the economy and dietary staples influenced by wet-based agriculture and marine resources (Kosulwat 2002; Vallibhotama 1992). A significant wealth of archaeological data has demonstrated the importance of rice, fish, plants and herbs to the traditional Thai diet. Aside from palynological and taphonomic evidence for these components of the Thai diet, written records from the period, such as King Ramkhamhaeng's stone inscription from 1292 AD, demonstrate the long history of the traditional Thai diet (Higham and Kijngam 1984; Wyatt 1984; Kosulwat 2002).

For the past seven hundred years, the traditional Thai food pattern has been characterized by a diet rich in legumes, fruits and vegetables, low in animal foods, and moderate in low-fat meats such as fish (Kosulwat 2002). These diverse sources of animal protein and complex carbohydrates represent a dietary regime which closely resembles several of the main components of the Paleolithic diet. In addition, the cooking practices utilized within traditional Thai cuisine emphasize stewing, baking and boiling- methods

which tend to introduce fewer carcinogens via the cooking process than techniques such as frying or grilling (Kosulwat 2002). Perhaps just as importantly, the diverse plants utilized by Thais include fruits, vegetables and spices with known anticarcinogenic and antioxidant properties which form the basis for both the traditional Thai diet and traditional Thai medicine (Itharat 2004; Kosulwat 2002; Malin 2003; Clinton 1997).

Similar to many Asian populations which utilize holistic or traditional medicinal practices, Thailand possesses a long history of ethnopharmacological practices, in which plant foods have served as both medicines and as sources of nutrition. Since the 13<sup>th</sup> century AD, many of the plant species which comprise part of the traditional Thai diet have been incorporated into traditional Thai medicine, with many plants utilized in either dried or extract form for the treatment of both chronic and terminal illnesses (Saralamp et al 1996)

Consumption of dark green and yellow vegetables from the cruciferous family, for example, represents an important component of the traditional Thai diet which is consistently associated with a low incidence of breast cancer (Kusamran et al 1998; Malin et al 2003; Moongkarndi et al 2004; Murakami et al 1995). Cruciferous vegetables inhibit chemically induced carcinogens and induce the activity of phase II detoxifying enzymes such as glutathione S-transferase (GST), which play an important role in neutralizing and removing carcinogens from the body (Kusamran et al 1998). Specific vegetables in the cruciferous family, such as cabbage, broccoli and turnip greens have also been shown to contain isothiocyanates which inhibit phase I carcinogen-activating enzymes (Kusamran et al 1998; Murakami et al 1995). Significantly, these same cruciferous vegetables are utilized within traditional Thai medicine, either in powder

form or in tonics produced with alcohol-derived bases, to treat not only cancer, but also a variety of gastrointestinal and circulatory conditions (Saralamp et al 1996).

Other components of the traditional Thai diet, including bitter melon, turmeric, ginger, ivy gourd leaves, sesbania flowers, sweet basil leaves, holy basil leaves, citrus leaves and lemongrass, have also been demonstrated to significantly induce GST activity in rats with DMBA-induced mammary gland carcinogenesis (Kusamran et al 1998; Moongkarndi et al 2004). These same herbs and spices also form an integral component of traditional Thai medicine, where they are utilized as poultices, tinctures and salves to treat a wide range of illnesses, such as dermatitis, gastroenteritis, hypertension, diabetes and breast cancer (Saralamp et al 1996).

Over the past two decades, however, Thailand has experienced an economic and health transition characterized by the shift from traditional Thai dietary practices to a more Westernized diet. Concomitant with these changes in dietary behaviors, both obesity and the diseases associated with weight gain have increased in prevalence, especially among the urban residents of Bangkok. Nationally, breast cancer represents the second-most diagnosed cancer in Thai women (NCI Thailand 2005). But in urban areas like Bangkok, where populations are more affluent and influenced by urbanized dietary habits, breast cancer represents the most commonly diagnosed cancer in Thai women (NCI Thailand 2005).

Perhaps even more importantly, breast cancer risk among Thai women in Bangkok dramatically increases by age 35, peaks by age 45, then declines after age 50 (NCI Thailand 2005). This pattern contrasts with the data from Western nations, where breast cancer is less common among women in their 30s, and where incidence peaks at

the age of 50 before gradually declining (NCI Thailand 2005) (Table 1.1). One of the interesting patterns to emerge from this examination of breast cancer incidence in Thai women is the age-related difference in the expression of breast cancer, in which younger Thai women are at a higher risk for developing the disease when compared to their older counterparts.

**Table 1.1 Incidence per 100,000 population of breast cancer in Thailand and the US by age group.**

	20-29	30-39	40-49	50-59	60-69
<b>Thailand</b>	3.18	21.99	46.94	36.69	30.95
<b>United States</b>	5.20	41.87	152.54	287.26	397.46

Data derived from IARC and Cancer Mondial.

This age-related difference in breast cancer incidence in urban areas like Bangkok follows a parallel income-related trend in the consumption of animal products, dietary fats and refined carbohydrates beginning in the mid-1980s (Kosulwat 2002). As affluence and personal wealth have grown over the past two decades, urban Thai populations centered in Bangkok, Phuket and Chiang Mai have increasingly replaced healthier, traditional meals with ready-to-eat foods high in fats and refined carbohydrates (MOPH 2005). Furthermore, younger, affluent Thais living in these urban centers have accounted for the largest proportion of overweight/obesity due to these changes in dietary preferences.

In Bangkok alone, over 50% of food expenditure is spent on purchased, ready-to-eat meals, compared to only 12.2% in rural, less affluent regions of Thailand (Kosulwat 2002; Sachamuneewongse 2008). Moreover, the proportion of calories derived from

animal protein and fat intake has increased in the past twenty years, with 51% of the total caloric intake derived from animal products, and with 30% of calories coming from fats (Kosulwat 2002). Only twenty-five years ago, the ordinary level of fat intake among Thais was comparatively low, at roughly 10% (Sachamuneewongse 2008). Along with the increased consumption of animal products and fats, refined carbohydrates have increasingly replaced traditional Thai fruit and vegetable staples. Over a twenty year period from 1983 to 2003, refined sugar intake increased from less than 5% to roughly 20% of an individual's daily energy intake (Sachamuneewongse 2008).

Concomitant with this shift towards urbanized dietary practices, obesity has dramatically increased. In middle to high-income families, the prevalence of obesity is 32.3%, compared to the significantly lower rate of 14.6% in low-income families (Kosulwat 2002; MOPH 2004). Interestingly, as with breast cancer, the prevalence of overweight/obesity also demonstrates age-related differences, with younger Thais experiencing a greater overall increase in chronic conditions when compared to older Thais (>55 years in age) (Sachamuneewongse 2008; MOPH 2005).

This recent increase in overweight/obesity follows a parallel trend in the increase of NCCDs and breast cancer incidence in the Thai population, due in large part to the replacement of traditional Thai dietary practices by a more Western-style diet. By substituting components of the traditional Thai diet with foods high in animal protein, refined carbohydrates and trans-fatty acids, current dietary practices among urban, affluent Thai populations essentially replaces those aspects of traditional Thai medicine which provide protective antioxidants and phytochemicals when used within a dietary perspective. These observations suggest that the recent increase in breast cancer

incidence in Thailand may be related to the decreased dependence of Thai women upon traditional medicinal plants for the main source of their energy intake. Moreover, from an evolutionary perspective, these lines of evidence suggest that the incorporation of medicinal plants into the traditional Thai diet represents a cultural adaptation which enables the Thai population to maintain a balanced relationship between essential nutrient intake and its positive effects upon overall health.

### Goals of the Study and Hypotheses

While many epidemiological studies have examined breast cancer risk in terms of diet and energy intake, these studies have failed to establish a consistent trend between specific dietary variables and breast cancer incidence. In addition, previous research has approached the question of nutrition and breast cancer risk from the perspective that dietary intake creates a negative, rather than a positive, impact upon human health. In other words, foods contribute to negative health consequences by increasing energy intake and by providing the body with unhealthy levels of dietary lipids and unrefined carbohydrates. This study instead utilizes a new approach- by analyzing breast cancer risk from a nutritional ecological perspective, in which optimal nutrient intake promotes human health. In this study, it is hypothesized that the incorporation of Thai medicinal plants into the traditional Thai diet provides protective health benefits by facilitating the intake of essential micronutrients in proportions comparable to the Paleolithic dietary pattern.

This study specifically examines how the nutritional use of Thai medicinal plants mediates breast cancer risk among urban Thai women by evaluating the Thai dietary pattern within the context of the Paleolithic model via the following methods: (1) an

evaluation of the micronutrient content of Thai medicinal plants using known reference values for Thai foods from the Faculty of Nutrition, Mahidol University; (2) an evaluation of the traditional Thai diet to determine the proportion of micronutrients and substrates provided by complex carbohydrates and diverse animal protein sources using metadata analysis; (3) an evaluation of the traditional Thai diet to determine parallels with the early *Homo* dietary pattern in terms of micronutrient and substrate bioavailability; and (4) an analysis of the degree of adherence to the traditional Thai diet and the nutritive use of Thai medicinal plants via data collected through a case-control study of Thai women residing in Bangkok. The following hypotheses were tested using data collected by this study, as well as known reference values for Thai foods as determined by Mahidol University:

- H<sub>1</sub>: The traditional Thai diet will provide Thai women with a ratio of micronutrients and substrates comparable to the Paleolithic dietary pattern.
- H<sub>2</sub>: The ratio of micronutrient and substrate intake in Thai women without breast cancer will more closely reflect the Paleolithic dietary pattern than Thai women with breast cancer.
- H<sub>3</sub>: Thai women without breast cancer will have a greater adherence to the traditional Thai diet and the dietary utilization of Thai medicinal plants than Thai women with breast cancer.
- H<sub>4</sub>: Thai women without breast cancer will have a higher intake of Thai medicinal plants as part of their diet than Thai women with breast cancer.

## **Chapter 2**

### **Literature Review and Theoretical Basis**

#### Introduction

Biological anthropology provides an alternative way to examine the distribution of breast cancer incidence in developing nations by examining the beneficial, instead of the negative, aspects of nutrition in the role of cancer prevention. Rather than focusing exclusively upon dietary quality and body composition, biological anthropology offers researchers with the unique opportunity to explore differences in the expression of breast cancer at the population level through a combination of: (1) an evolutionary perspective- which looks at the role of nutritional changes from the Paleolithic diet upon breast cancer incidence; and (2) a cultural perspective- which assesses the adaptations made by populations in response to the contemporary human diet. Indeed, numerous populations from developing nations in Asia, Latin America and Africa have utilized cultural adaptations within their main subsistence base in order to derive health benefits with the potential to significantly impact disease expression. In particular, the use of traditional medicinal plants in both indigenous medicine and in the regular diet represents an important biocultural adaptation with the potential to reduce the incidence of non-communicable chronic diseases. This chapter presents an interdisciplinary literature review covering evolutionary, ethnopharmacological, physiological and nutritional ecological approaches to a holistic understanding of the role of traditional medicinal plants in providing important health benefits to developing populations.

## An Overview of the Paleolithic Diet

In anatomically modern humans (AMH), the digestive tract reflects adaptations made to the Paleolithic diet, which many anthropologists consider to represent the pattern of subsistence utilized by our hominid ancestors. Although disagreement exists over the exact proportion of nutrients provided by the Paleolithic diet, anthropologists tend to concur that the Paleolithic diet is characterized by low saturated fat and high fiber intake. Because the archaeological record is limited in terms of providing an accurate picture of the early human diet, anthropologists have relied upon two main lines of evidence to ascertain the rough macronutrient content of the Paleolithic diet- isotope analyses and the nutritional patterns of contemporary hunter-gatherer populations.

Isotope studies provide an indication of specific dietary components by utilizing the relationship between the four-carbon mode of photosynthesis found in C<sub>4</sub> plants and the high content of <sup>13</sup>C found in animals which feed upon these plants (Peters and Vogel 2005). Isotope studies carried out on hominid skeletal remains show <sup>13</sup>C values between that of C<sub>3</sub> and C<sub>4</sub>, suggesting that C<sub>4</sub> grass-eating vertebrates were incorporated into the diet (Peters and Vogel 2005; Cordain et al 2002). This evidence concurs with recent models developed by anthropologists which suggest that increases in stature and brain size in *Homo habilis* and *Homo erectus* and reduced gut size in anatomically modern humans (AMH) followed dietary changes emphasizing the inclusion of animal protein, either via scavenging or hunting, as a major component of the hominid diet (Ungar et al 2006; Aiello and Wheeler 1995; Cordain et al 2002). Moreover, microwear analysis of animal bones found at sites associated with *Homo habilis* and *Homo erectus* show evidence of cut marks and striations consistent with butchery (Ungar et al 2006).

Aside from suggesting the clear importance of animal protein to the hominid diet, isotope studies also indicate the potential role of xeric plants, or underground storage organs (USOs), in the Paleolithic diet. Isotope studies conducted on the skeletal remains of *Homo erectus* show elevated levels of Sr/Ca, reflecting the possible use of USOs, which are high in strontium, as part of the hominid diet (Ungar et al 2006). USOs may have represented useful dietary resources during periods of food shortage in dry, savannah environments and have been implicated by anthropologists as one stimulus for the development of tools among hominid species (Hernandez-Aguilar et al 2007; Ungar et al 2006).

Studies of chimpanzees (*Pan troglodytes*) living in Ugalla, Tanzania provide further evidence for the potential role of USOs in the Paleolithic diet. Hernandez-Aguilar et al (2007) report that chimpanzees utilizing this seasonal habitat employ digging technology in order to obtain USOs as part of their diet. Furthermore, the chimpanzees at Ugalla regularly utilize USOs during the rainy season, well after the period of likely food-shortage. This comparative primate evidence further supports the idea that the Paleolithic diet of hominids emphasized the incorporation of both animal protein and plant resources (Hernandez-Aguilar et al 2007; Ungar et al 2006).

Studies of contemporary hunter-gatherer populations provide additional evidence for the unique role played by plant resources as an integral part of the Paleolithic diet. Although animal products provide a higher energy return per unit of weight when compared to plant resources, gathered plant foods represent a more stable source of energy intake when compared to hunted game. Ethnographic analyses of the extant contemporary hunter-gatherer populations of North America, South America and Africa

demonstrate that less than 20% of hunter-gatherer groups derive their caloric intake from the exclusive consumption of animal protein (Cordain et al 2002; Jenkins et al 1998). Research by Richard Lee (1968) and Cordain et al (2002) also show that hunter-gatherers derive roughly equal portions of their energy intake from animal products and complex carbohydrates (with variations in this proportion occurring in response to the availability of specific plant and animal species).

In terms of the specific macronutrient content of the contemporary hunter-gatherer diet, the most salient differences with regards to the Western diet exist in the dietary lipid and fiber content. According to numerous ethnographic studies, saturated fats derived from animal products represent less than 10% of food energy in the hunter-gatherer diet, compared with 35% in the modern Western diet (Garn 1997; Smil 2002; Cordain et al 2002). Moreover, the complex carbohydrates utilized by hunter-gatherers contain high phytochemical and fiber contents, resulting in slow digestion and reduced postprandial insulin surges (Jenkins et al 1998; Cordain et al 2002).

Therefore, by utilizing a combination of isotope analyses and studies of hunter-gatherer populations, the Paleolithic diet has been described by anthropologists as a pattern of subsistence characterized by: (1) low levels of saturated fats and cholesterol; (2) significantly higher levels of fiber intake (when compared to the contemporary Western diet); (3) the use of complex carbohydrates containing high levels of antioxidants and phytochemicals; and (4) the use of wild, minimally processed foods (Cordain et al 2002). As a result, the Paleolithic diet reflects a pattern of food intake which is comparatively healthy when evaluated against the contemporary Western diet, in that saturated fat intake represents less than 10% of daily caloric intake and unrefined

carbohydrates constitute anywhere from 41-67% of average daily sustenance (Cordain et al 2002; Smil 2002; Garn 1997).

The overall proportion of macronutrient content is especially salient given the fact that the Paleolithic diet still incorporates animal products as a significant contributor to daily energy intake, while at the same time resulting in healthy serum cholesterol profiles and a reduced risk for cardiovascular disease and cancer (Jenkins et al 1998; Cordain et al 2002). Studies show that the contribution of unsaturated fats and precursors to omega-3 fatty acids via complex carbohydrate intake shifts the polyunsaturated:saturated fat (P:S) and omega-6:omega-3 fatty acid ratios towards healthy profiles capable of reducing the risk of many non-communicable chronic diseases (Jenkins et al 1998; Cordain et al 2002). Moreover, the incorporation of complex carbohydrates into the Paleolithic diet further balances animal product intake by providing the body with phytochemicals such as plant sterols, flavonoids, phenolics and phytates which are natural anticholesterolemic and antilipidemic agents (Jenkins et al 1998).

When examining the relevance of the Paleolithic diet to Asian countries in particular, epidemiological studies show that populations living in Thailand, China and Japan have utilized a pattern of dietary subsistence similar to aspects of the Paleolithic diet for at least the past 500-2000 years (Smil 2002). This observation is particularly significant, since Asian populations have been characterized by the lowest levels of mortality due to NCCDs. Moreover, when compared to Western nations, which have experienced the nutrition transition (i.e. the Western diet, or the shift towards refined carbohydrate and high saturated fat intake) since the mid-1800s, many Asian populations only recently have been exposed to this phenomenon within the past 20-30 years (Smil

2002). Because this recent transition in Asia has been accompanied by a significant increase in the prevalence of NCCDs within the past decade, the data suggests that contemporary human populations are maladapted to diets of domesticated and processed plant foods due to adaptations made by our digestive system in response to the Paleolithic diet (Richards 2002).

### Comparative Hominid Digestive Morphology

All hominoids possess similar digestive morphology- a simple acid stomach, small intestine, a small caecum terminating in the appendix, and a markedly sacculated colon; however, humans differ from other apes in terms of gut proportions (Milton 2003; Aiello and Wheeler 1995). In contrast with other apes, which are hindgut dominated due to the large proportion of total gut volume in the colon, humans are foregut dominant, with nearly 56% of total gut volume found in the small intestine (Milton 2003). The proportion of small intestine volume to colon size reflects different dietary adaptations, with hindgut dominance in apes suggesting an adaptation to a low quality diet, and with foregut dominance in AMH reflecting a high quality diet (Milton 2003). This pattern of foregut dominance reflects both the importance of animal protein and energy-rich complex carbohydrates to the hominid diet, two elements which are essential to the Paleolithic model.

Indeed, the switch to the Paleolithic diet has been used by anthropologists to explain the hominid trend towards gracilization of the mandible, increases in brain size, and reduction in gut volume (Richards 2002; Milton 2003). Rather than suggesting an exclusive dependence upon animal products, human digestive morphology reflects the need for dietary diversity, or a joint dependence upon plant and animal foods (Stiner et al

2000; Ungar et al 2006; Isaac 1971). A dependence upon both plant and animal products provides several important adaptations in terms of the human digestive system. First of all, the Paleolithic diet tends to be processed more slowly in the human digestive tract, resulting in stable blood glucose levels and a reduction in postprandial insulin surges (Jenkins et al 1998; Milton 2003). In addition, the high fiber content of the Paleolithic diet reduces cholesterol levels by downregulating bile acid uptake in the ileum, resulting in the increased removal of dietary cholesterol via fecal bile acid loss (Jenkins et al 1998). Finally, the importance of dietary diversity within the Paleolithic model ensures that the human body acquires all of the essential nutrients needed for health and survival, including vital antioxidants and phytochemicals which reduce oxidative damage from free radicals and carcinogens introduced by both the environment and the cooking process (Johns 1999).

#### A Comparison of the Paleolithic and Modern Western Diets in Terms of Human Digestive Morphology

The digestive morphology of anatomically modern humans reflects adaptations made to the Paleolithic diet; therefore switching from the Paleolithic model to the contemporary Western diet results in several negative consequences in terms of dietary quality and digestive efficiency. The contemporary human diet differs considerably from the Paleolithic diet in terms of several key aspects:

1. Early hominid populations relied upon animal food products for an important source of dietary protein; however hominids derived their protein from diverse sources such as terrestrial mammals, fish, shellfish and birds. Using the hunter-gatherer model as a proxy for the Paleolithic diet, as much as 33-59% of the dietary intake was derived from animal sources (Mann 2000; Cordain et al 2002).
2. Although early hominids derived almost 35% of their calories from fat intake (due to the consumption of all the edible components in hunted game), the fat

content of wild game contains considerably higher amounts of mono- and polyunsaturated fats (Eaton 2006; Hockett and Haws 2003).

3. Early hominids supplemented their diet with previously-little-utilized plant resources (i.e. tubers, fruits and vegetables) in order to provide a source of dietary fiber necessary to balance the high intake of animal food sources (Eaton 2007; Hermann et al 2002). This source of dietary fiber aided in detoxifying the colon from the metabolites formed through the digestion of animal meat, in addition to providing an important source of antioxidants to the diet (Hockett and Haws 2003; Hermann et al 2002).

By contrast:

1. The contemporary human diet is characterized by a greater contribution of refined carbohydrates to the overall diet. Between 50-60% of the daily caloric intake is derived from refined carbohydrates, which are energy dense, but low in micronutrient content (Eaton 2007).
2. The carbohydrates consumed in the modern human diet are significantly lower in dietary fiber content than the fruits and vegetables consumed by hunter-gatherer populations. The process of plant domestication and agriculture has reduced the fiber content of fruits and vegetables consumed by modern human populations by nearly 60% (Hermann et al 2002).
3. The remaining calories in the modern human diet are derived from animal sources, which have higher saturated fat content than wild game due to the use of grain-fed, commercialized stock (Eaton 2006). Moreover, the protein derived from animal sources is less diverse, with a majority of calories coming from red meat (Hockett and Haws 2003). This contributes to the greater intake of saturated fats when compared to the Paleolithic diet. It is estimated that the contemporary Western diet contains nearly twice the levels of saturated fat as the early human diet, while at the same time providing only half the polyunsaturated fat content as found in the diets of our hominid ancestors (Eaton 2007).

Although the digestive morphology in contemporary humans (shorter colon and longer small intestine) has evolved to accommodate the omnivorous diet practiced by early hominids, those adaptations favor high fiber, lean protein sources of nutrient intake, rather than the refined carbohydrate-rich diets practiced by modern human populations which have experienced the nutrition transition (Hermann et al 2002; Hockett and Haws 2003; Jonnson 2005). This mismatch between the Paleolithic diet and the contemporary

Westernized diet has contributed to the recent increase in NCCDs such as overweight/obesity, diabetes mellitus, hypertension, cardiovascular disease and breast cancer. The negative health consequences of the Westernized diet are further compounded by specific cooking practices, including grilling and frying, which introduce additional carcinogens and nutrient metabolites capable of inducing tumorigenesis in human tissue (Cho et al 2003; Dai et al 2002).

When examining the effects of cooking and the nutrition transition upon breast cancer incidence in particular, women with the highest intake of animal meat and refined carbohydrates have nearly a three-fold increase in the risk for developing breast cancer when compared to women with a more diverse diet incorporating complex carbohydrates (Dai et al 2002). This increased risk for breast cancer is due to several important factors:

1. Although the morphology of the human digestive tract is adapted to digest animal food products, the human gut is primed to more efficiently extract nutrients from lean game animal, rather than domesticated cattle (Hermann et al 2002).
2. Contemporary sources of animal meat, when compared to wild-game, contain significantly higher levels of saturated fat, which have been demonstrated in case-control studies to induce mammary tumorigenesis (Cho et al 2003). Moreover, imbalances in the ratio of saturated to unsaturated fats, and omega-6 to omega-3 fatty acids contribute to the etiology of chronic disease. The Paleolithic diet provides a 1:1 ratio compared with the 10-20:1 modern ratio, reflecting a shortage of n-3 fatty acids and a concomitant increase in the risk of tumorigenesis and cardiovascular disease (Johns 1999)
3. Cooking animal meat results in the production of numerous mutagenic chemicals, including heterocyclic amines and polycyclic aromatic hydrocarbons such as 1,3- butadiene, benzene, acrolein, and formaldehyde, in addition to the generation of non-volatile hazardous compounds, such as hydroperoxides, trans-fatty acids, and aldehydes (Dai et al 2002). These mutagenic and non-volatile compounds introduced by the cooking process are endogenous reactive chemicals capable of inducing carcinogenesis within mammary cells (Hermann et al 2002).

4. Refined carbohydrates contain fewer micronutrients and phytochemicals than complex carbohydrates, thus reducing the bioavailability of antioxidants and dietary fiber necessary for neutralizing carcinogens and free radical damage to cells (Hermann et al 2002).

Populations from developing countries such as Thailand tend to practice traditional diets which more closely resemble the Paleolithic dietary model, in that protein is derived from non-commercialized animal resources and complex carbohydrates provide sufficient micronutrients, phytochemicals and dietary fiber to the diet (Hockett and Haws 2003). In populations which have maintained a connection between the traditional diet and the nutritive use of medicinal plants, studies further show that an adherence to this eating pattern seems to forestall the development of chronic illnesses while positively enhancing human health (Hermann et al 2002). These beneficial health consequences arise in part due to the efficiency with which the human digestive tract extracts the micronutrients contained in these traditional diets, and to the nutritive use of medicinal plants, which provide the body with essential micronutrients (Johns 1999; Hockett and Haws 2003).

### Nutritional Ecology

One of the fundamental principles of human health and nutrition- that diverse diets increase overall human health by lowering infant mortality rates and increasing average life expectancy, represents a central key to the theoretical model of nutritional ecology. Broadly stated, the core theoretical model of nutritional ecology maintains that the interaction of diet, somatic maintenance, physical activity, and pathogenic agents are constrained by the relationship between the environment, essential nutrient intake and their effects upon overall human health (Hockett and Haws 2003; Johns 1999). When examining human health from a nutritional ecological perspective, studies show that

humans require a diverse suite of over fifty essential nutrients in order to effectively maintain somatic homeostasis and physical health. In most cases, these diverse nutrients can rarely be found in one food group and must instead be obtained by a diverse diet (Hockett and Haws 2003). In this sense, nutritional ecology does not focus upon net energy intake, but rather upon the ability to consume a diversity of foods in order to obtain the full suite of essential nutrients necessary to optimize health (Hockett and Haws 2003).

Because these essential nutrients are required to sustain both vital organ function and normal levels of physical activity in humans, the synergy between diverse subsistence practices and essential nutrient intake represents a dietary adaptation made relatively early in hominid history (Johns 1999; Hockett and Haws 2003). For example, studies of contemporary hunter-gatherers and the Paleolithic diet suggest that early populations of AMH relied upon five primary food groups which supplied the essential nutrients: terrestrial mammals, fish, shellfish, birds and plants (Hockett and Haws 2003). A significant aspect of examining the animal food sources is that each animal food group, for example terrestrial versus marine animals, provides different levels of the essential nutrients (Johns 1999; Hockett and Haws 2003). Moreover, due to the importance of animal protein to early humans, plants represented a necessity in the Paleolithic diet because they supplied the body with additional essential nutrients, such as carotenoids and other antioxidants, not found in animal foods (Hockett and Haws 2003).

The crucial incorporation of plant foods into the Paleolithic diet is especially relevant since many anthropological models suggest that long-chain polyunsaturated fats (LC-PUFAs) derived from animal meat may have played an important role in the origin

of the genus *Homo* and in hominid encephalization. While LC-PUFAs such as arachidonic, eicosapentanoic and docosahexanoic acids are necessary to central nervous development and are best acquired from terrestrial and marine animals, LC-PUFAs are also associated with oxidative damage (Johns 1999; Hockett and Haws 2003).

In humans, the oxygen required for respiration results in free radicals and other reactive oxygen species which are catalyzed by LC-PUFAs and cholesterol due to their degree of unsaturation (Johns 1999). Incidentally, oxidative stress and damage to biological tissues represent the first steps in the development of cancer. Therefore, naturally occurring antioxidants from complex carbohydrates would have represented a necessary part of the Paleolithic diet in order to counteract the effects of lipid peroxides (Johns 1999; Etkin 1988).

#### Ethnopharmacology from a Nutritional Ecological Perspective

Due to the necessity of acquiring antioxidants and phytochemicals via plant food intake, co-evolutionary models suggest that particular plants became domesticated or genetically altered by humans based on their secondary metabolite content (Etkin 1988). Secondary metabolites refer to phytochemicals such as alkaloids, phenols and mycotoxins which provide plants with defense mechanisms against microbial and insect infestation, and which have unique antioxidant, hypocholesterolemic and hypolipidemic effects in humans (Etkin 1988; Johns 1999). Ethnographic studies of the human consumption of plant foods with therapeutic properties suggest that plants may have been initially domesticated for use first as medicines and later expanded to be used as foods (Etkin 1988). For example, soybeans were first cultivated in China for use as a medicine and only later became used as a food. Similarly, in the Andes, coca primarily served as a

medicine, but later came to be used as a food during times of nutritional stress (Etkin 1988). Moreover, the Amazonian Kayapo regularly transplant medicinal plants to „forest fields“ so that the population can ensure access to a wide-range of useful therapeutic species, further suggesting that the first domesticated plants represented medicinal plants rather than staple food crops (Etkin 1988).

This evidence suggests that the origins of ethnopharmacology may have had its roots in the Paleolithic diet and the need for essential antioxidants and additional phytochemicals found only in plant food sources. Indeed, a majority of traditional and developing societies utilize ethnopharmacological practices in which nutritive and therapeutic plant sources overlap. The notion that medicines and foods are derived from the same origin represents a key component of traditional medicinal ideologies among developing populations (Huffman 1997; Etkin 1988). Interestingly, when examining the incidence of non-communicable chronic diseases in developing populations which still maintain their traditional dietary and ethnopharmacological practices, studies show that these populations have the lowest rates of cancer, heart disease and diabetes in the world.

In many of the developing nations of Asia, Latin America and Africa, populations have utilized indigenous plants for the treatment of chronic illnesses and conditions for centuries. The indigenous populations of Mexico and Central America, for example, have relied upon *chaya*, a tree spinach, and prickly pear cactus as both foods and as traditional medicine in the treatment of diabetes and gastrointestinal ailments. Similarly, the Maasai of East Africa incorporate wild plant material (*Acacia* sp. and *Salvadora* sp.) into their traditional diet of meat, blood and milk as a digestive aid and to counteract the high saturated fat content of their diet (Johns 1999). While original anthropological studies

conducted in the 1960s and 1970s concluded that high levels of physical activity counteracted the effects of the Maasai diet, which is high in animal meat and animal products, more recent studies show that the Maasai incorporate over thirty plant additives to their meat based soups, milks and beverages (Johns 1999). These plants, in addition to providing characteristic flavors to meals and beverages, also contain high levels of antioxidants, hypocholesterolemic agents and other phytochemicals when utilized from a nutritional perspective (Johns 1999).

In Thailand, much like other developing societies, traditional medicines are often derived from plants which are regularly utilized as part of the diet. Among the common plant species used within Thai cuisine, including chilis, garlic, onion, anise, cinnamon, coriander, cumin, ginger, lemongrass and turmeric, many are often incorporated into traditional Thai medicine for their natural antibacterial and anticarcinogenic properties (Sherman and Billing 1999; Johns 1999). Interestingly, when examining the common herbs, fruits and vegetables used in Thai cooking with natural pharmacologic properties, many of these species contain secondary metabolites which provide them with a strong or bitter taste associated with their natural medicinal characteristics (Manosroi et al 2007; Kusamran et al 1998; Saralamp et al 1996).

For example, cruciferous vegetables represent a common component in the traditional Thai diet with important applications in Thai ethnopharmacology. Cruciferous vegetables, such as broccoli, brussel sprouts and bitter melon, possess sulfur-containing indoles and isothiocyanates which act as inducers of detoxification enzymes, specifically glutathione-S-transferase (Johns 1999). Moreover, the saponin and phytosterol content of cruciferous vegetables lowers blood lipid levels, inhibits platelet aggregation, and

regulates insulin action (Johns 1999). Cruciferous vegetables also provide exogenous antioxidants, such as vitamins A, C and E, which protect PUFAs from attack by oxygen radicals via the donation of an electron to the peroxy radical in order to produce the alpha-tocopherol radical- an unreactive species which naturally degrades with no harmful effects in the human body (Johns 1999). Many of these cruciferous vegetables, in addition to other fruit, vegetable and herb species used in traditional Thai medicine and cooking, are often cultivated in small patio gardens by Thais, who often utilize home-grown plant species to treat their own common illnesses, such as stomach discomfort, allergies, or infection (Saralamp et al 1996).

#### Primate Medicinal Plant Use

In humans, as with other primates, bitter taste is often associated with medicinal use, which suggests a deep history for self-medicative behaviors within our primate evolutionary roots (Johns 1999; Huffman 2001). In recent decades, research into the self-medicative behaviors of the African great apes has provided evidence for a common primate cultural adaptation involving the use of plants with known medicinal properties to treat common illnesses. However one distinction which separates non-human primate zoopharmacognosy from human ethnopharmacology is that traditional human societies often use plant species as both foods and medicines, while non-human primates seek out non-food plant species to treat their illnesses.

Much of this research into the self-medicative behavior of primates has focused upon chimpanzees (*Pan troglodytes*) due to their close genetic relationship with humans. Primatologists have noted for decades in anecdotal stories that chimpanzees regularly engage in zoopharmacognosy based on three main lines of evidence: (1) the use of plant

species not regularly associated with their diet; (2) the restriction of plant use to seasons with an increased risk for parasitic infection; and (3) the individual ingestion of a plant followed by improvement in condition (Huffman 1997).

For example, the chimpanzees of Mahale have been observed to utilize non-food plants to treat a variety of digestive ailments. Bark of the *Pycnanthus angolensis* and *Grewia platyclada* plants are chewed by residents of Mahale for the relief of stomach aches and diarrhea (Huffman 1997). Chimpanzees also selectively use several plant species in the treatment of parasitic infection. During the rainy season, when the risk of nematode infection increases, chimpanzees have been observed to use the leaves of *Aspilia mossambicensis* and the pith of *Vernonia amygdalina* to reduce helminthic load (Etkin 1988; Huffman 1997). Researchers have noted that the leaves of *Aspilia mossambicensis* are often swallowed whole by chimpanzees suffering from intestinal parasites; the undigested leaves emerge in the feces, containing the ova and nematodes associated with infection (Huffman 1997). Another chimpanzee population living in Kibale has been noted to consume the berries of *Phytolacca dodecandra*; these berries contain triterpenoid saponins which have both antiviral and antibacterial activity (Huffman 1997). In many cases, primatologists note that the use of these particular plant species requires a detour from the group's regular travel route, suggesting at least an awareness of the association between consumption of the plant and a reduction in illness (Etkin 1988; Huffman 1997).

Aside from chimpanzees, other non-human primate species have been observed to engage in self-medicative behavior. Gorillas at Kahuzi-Biega, for instance, consume the fruits of *Aframomum sanguineum*, a wild ginger species which possesses antimicrobial

and antibacterial activity (Huffman 1997). Western lowland gorillas also eat on occasion the tips of the young leaves of *Thomandersia laurifolia* in order to reduce nematode load (Huffman 1997).

Interestingly, many of the plant species utilized by non-human primates for the self-treatment of common helminthic or infectious illnesses are also used by humans to prevent or treat similar conditions. For example, the bark of the *Pycnanthus angolensis* plant is also utilized by West Africans as a laxative, purgative, digestive tonic, emetic and reliever of toothaches (Huffman 1997). Moreover, various African ethnic groups utilize a concoction made from *Vernonia amygdalina* to treat malarial fever, intestinal parasites and gastrointestinal discomfort (Huffman 1997). Finally, the fruits of *Fromomum sanguineum* are also sold locally in the Bwindi area for the treatment of bacterial and fungal infections in humans (Huffman 1997).

### Summary

This study represents a unique approach to the study of non-communicable chronic diseases (NCCDs) such as breast cancer in its utilization of an anthropological, rather than an epidemiological, perspective on disease etiology. In particular, while epidemiology has focused narrowly upon the nutrition transition as a causative factor for the recent increases in NCCDs worldwide, these approaches have been limited to examinations of the role of dietary quantity and body composition upon breast cancer incidence. Instead, this study introduces a number of new ways in which researchers can address the problem of NCCDs like breast cancer through the use of several anthropological methods.

Rather than utilizing a standard epidemiological approach to the study of breast cancer, in which cases and controls are evaluated with regards to body composition and dietary intake, this study examines breast cancer incidence in terms of the nutritive use of traditional medicinal plants. Ethnopharmacology has its evolutionary roots in both early *Homo* populations and in non-human primates such as chimpanzees. The plants utilized within traditional medicine represent complex carbohydrates with natural anti-carcinogenic and antioxidant properties capable of reducing the risk for developing non-communicable chronic diseases such as breast cancer. Just as importantly, when looking at differences in breast cancer distribution between Western and developing populations, in which traditional societies have only recently experienced an increase in cancer incidence, the use of ethnopharmacology has represented a key distinction. While traditional societies frequently utilize medicinal plants as a central component of their diets, Western populations are characterized by the loss of this cultural adaptation. Only recently have developing nations begun to replace traditional dietary components with Western-style food options, perhaps contributing to the increase in NCCDs like breast cancer over the past two decades.

In addition, this study analyzes the nutritive use of traditional medicinal plants within an evolutionary perspective- first by comparing and contrasting the dietary adaptations of early *Homo* and contemporary human populations, and second by evaluating the efficiency with which the human digestive system can extract beneficial micronutrients from both dietary patterns. These approaches provide an evolutionary context in which the etiology of breast cancer can be evaluated. By providing baseline markers for optimal dietary intake utilizing a combination of paleoanthropological and

medical sources, this study assesses the relevance of changing from traditional dietary patterns incorporating medicinal plants towards Western models lacking these natural anticarcinogenic and antioxidant properties upon disease profiles. By likewise analyzing the significance of these dietary and cultural changes within a nutritional ecological model, this study further deviates from the typical epidemiological approach. In particular, the interdisciplinary approach utilized within this study enhances our understanding of how population differences in the utilization of ethnopharmacology, and the concomitant differences in the degree of deviation from the Paleolithic dietary model, impacts the phenotypic expression of diseases like breast cancer at the population level.

### **Chapter 3**

## **Research Design and Methods**

### Site Location

This study was conducted at Ramathibodi Hospital, Mahidol University in Bangkok, Thailand, which has a regionally recognized center for cancer research and treatment. Ramathibodi Hospital is located in the heart of downtown Bangkok and serves patients with diverse socioeconomic and educational backgrounds. This key demographic characteristic of Ramathibodi Hospital was essential in order to recruit subjects for this study who represented the different levels of income and education found within the broader Thai population.

Subjects were specifically recruited from the outpatient ward of the Breast Care Center at Ramathibodi Hospital. Physicians associated with the Breast Care Center provided recently diagnosed breast cancer patients utilizing the clinic with general information pertaining to the research objectives and goals of this study. Patients interested in participating in this study were forwarded to the principal investigator, who provided each potential subject with an informed consent form documenting the expectations for their participation in the study. The informed consent form was formatted in accordance with the Ethics Committee of Ramathibodi Hospital, Mahidol University and the University of Michigan IRB Human Subjects Board and was translated into Thai by the principal investigator for the purposes of this study. Subjects agreeing to enroll in this study after a review of the informed consent form were asked to

sign the document and were provided with the option of declining to participate in any aspect of data collection (including anthropometric assessment and administration of the Enrollment Questionnaire) during the enrollment process. Completion of the Enrollment Questionnaire (Appendix A) and data collection process occurred over a two hour period after the patient's clinic appointment. Due to the study design, subjects were not required to return to the clinic for follow-up visits with the principal investigator.

This study was conducted in accordance with the Ethics Committee of Ramathibodi Hospital, Mahidol University Protocol Number 09-49-22 (Appendix B) and with the University of Michigan IRB Human Subjects Board Study Number HUM00001149 (Appendix C).

#### Sample Size and Criteria for Sample Recruitment

This population-based case-control study uses a sample of 129 subjects ranging from 15 to 75 years of age which were classified into two groups: (1) 51 Thai women with breast cancer- hereafter referred to as the case group, case subjects or breast cancer cases; and (2) 78 Thai women without breast cancer- also referred to as the control group, control subjects, or healthy subjects for this study.

The following criteria were used to determine the eligibility of subjects to participate as cases in the study:

- a) Thai women between 15-75 years of age;
- b) Residence in the Bangkok Metropolitan area;
- c) Histologically confirmed diagnosis of breast cancer;
- d) Diagnosis of breast cancer within two months prior to enrollment in the study in order to control for changes in body composition due to medical treatment;
- e) No history of using hormone replacement therapy (HRT).

The enrollment of subjects as study controls was determined by the following criteria:

- a) Thai women between 15-75 years of age;
- b) Residence in the Bangkok Metropolitan area;
- c) No previous history of breast cancer diagnosis;
- d) No history of using hormone replacement therapy (HRT).

### Study Design

This research was carried out as a case-control study, which represents the most salient and cost-effective method for measuring the relationship between breast cancer and dietary intake, as well as for collecting large quantities of data pertaining to the nutritional use of traditional medicinal plants (Freudenheim 1999). The case-control method was chosen for this project because it allows for the cost-effective collection of in-depth data on lifestyle and dietary behaviors through a combination of interviews and questionnaires which are related to the disease of interest- in this case breast cancer, as well as to the exposure variable- the nutritive use of Thai medicinal plants (Freudenheim 1999). Moreover, because dietary analysis represents an important component of this project, the use of a case-control method provides more comprehensive data pertaining to dietary and nutrient intake than other study methods (Freudenheim 1999). The data collected as part of this case-control study, when used in conjunction with multivariate analysis, allows for multiple questions to be addressed within the context of dietary and anthropometric data collected at the individual level (Freudenheim 1999).

### Data Collection and Analysis

**Development of the Enrollment Questionnaire.** Each subject participating in the study was required to complete an Enrollment Questionnaire (Appendix A), which contained three main parts: (1) a demographic history questionnaire; (2) a medical history questionnaire; and (3) a food frequency questionnaire. The Enrollment Questionnaire was developed and translated into Thai by the principal investigator after conducting an

extensive literature review on the demographic factors contributing to breast cancer incidence and the main dietary constituents of the traditional Thai diet. Moreover, previous clinical research experience provided by employment with an NIH funded clinical trial and enrollment in epidemiological coursework provided the additional professional and academic background necessary for the development of the Enrollment Questionnaire. The principal investigator, who is fluent in written and spoken Thai, administered the Enrollment Questionnaire to each subject during the enrollment and data collection process.

**Demographic, Medical History and Anthropometric Data.** Data regarding the demographic and medical history was based in part on recall by the subject, but also included a review of the patient's medical records through the cooperation of Ramathibodi Hospital. The demographic and medical history components of the Enrollment Questionnaire examined the following information: (a) age at first menstruation; (b) number of live births; (c) age at first pregnancy; (d) length of breastfeeding; (e) number of first-degree relatives with breast cancer; (f) smoking status; (g) age at menopause (for post-menopausal subjects); (h) length of residence in Bangkok; (i) education level; (j) mean monthly income; and (k) occupation. The demographic data and medical history were used to control for the known effects of parity and family history upon breast cancer risk. In addition, in order to test for the role of affluence and Westernization on the pattern of breast cancer incidence in Thailand, monthly income, education and occupation were utilized in order to assess the relative socioeconomic status of each subject participating in the study.

Body composition was determined through a variety of anthropometric measurements using methods standardized by the NIH, including: (a) weight (kg); (b) height (m); (c) BMI ( $\text{kg}/\text{m}^2$ ); (d) waist-to-hip ratio, as determined by waist and hip circumference; and (e) triceps skinfold thicknesses and upper arm circumference. The principal investigator conducted the anthropometric assessment utilizing standard anthropometric equipment, including a calibrated stadiometer and weight scale (for height and weight) provided by the Breast Care Center and Lange skinfold calipers for the skinfold measurements. As with the data on medical history and demographics, these anthropometric markers were used to control for the known effects of overweight/obesity upon breast cancer risk.

Analysis of the demographic data, medical history, and anthropometric measurements included the use of odds ratio analysis in order to assess the role of each specific variable upon breast cancer risk. The odds ratio represents a descriptive statistic which compares whether the probability of a certain event is the same for two populations. Odds ratios are calculated as the probability of the event occurring divided by the probability of the event *not* occurring. An odds ratio of 1 implies that the event is equally likely in both groups. By contrast, an odds ratio below 1 implies protection against a certain event, while an odds ratio over 1 suggests an increased probability of the event occurring within the population based on exposure to specific variables.

Odds ratio analysis was conducted for both continuous and categorical predictors of breast cancer in the study population. The continuous variables included age (years), age at menarche (years), age at first pregnancy (years), height (cm), weight (kg), BMI, waist circumference (cm), and hip circumference (cm). The categorical variables

included waist-to-hip ratio, family history of breast cancer, obesity (as defined by a BMI cutoff of 25+ and 30+), parity (yes/no), lactation history (yes/no), and socioeconomic status (low-middle/middle-upper). 95% confidence intervals were calculated for each odds ratio in order to estimate the amount of error involved with the data collected for this study. In addition, regression analyses were conducted in order to assess the potential role of socio-economic status upon several demographic and anthropometric variables in this study, including reproductive history and measures of body composition.

**Nutritional Data.** Data regarding the dietary intake of subjects was collected via the food frequency component of the Enrollment Questionnaire. As with the data on medical history, the dietary intake of each subject was based in part on recall. The food frequency questionnaire (FFQ) for this project was used in conjunction with supplemental references that included photographs of the food items of interest in the study, as well as drawings to assist in the determination of serving sizes (in order to increase the validity of the data). This questionnaire focused on the frequency of intake for 120 of the most commonly consumed Thai foods, beverages and spices, as determined by a literature review of traditional Thai dietary practices. The FFQ included the following food groups: (a) animal protein; (b) fish and shellfish; (c) unrefined carbohydrates, including fruits, vegetables and spices used in both traditional Thai medicine and the traditional Thai diet; (d) refined carbohydrates, i.e. sodas, cakes, cookies, etc.; (e) dairy products; and (f) nuts and legumes.

The data derived from this FFQ allowed the investigator to quantify the bioavailability of macro- and micronutrients using the known values for Thai foods and ingredients as determined by recently published studies conducted by the Faculty of

Nutrition, Mahidol University (Judprasong et al 2006; Sirichakwal et al 2005; Kamchan et al 2004; Charoenkiatkul et al 2003). The specific dietary variables examined as part of this study included: (1) antimutagenic and anticarcinogenic micronutrients; (2) antioxidants; (3) carbohydrates, fats and proteins; and (4) vitamins and minerals. In addition, the data derived from the FFQ were utilized to quantify the average daily energy intake for each subject.

Odds ratio analysis based on quintile of intake for each macro- and micronutrient, as well as specific Thai fruits, vegetables and spices, was utilized in order to determine the effect of these nutritional variables upon breast cancer risk. For the purposes of this study, the first quintile is associated with the lowest intake of each nutrient, while the fifth quintile is associated with the highest intake. When interpreting odds ratios based upon quintiles of exposure, the first quintile is used as the baseline value, from which increasing levels of intake are compared for their relative contribution to breast cancer risk. As with the odds ratio analyses conducted on the medical, demographic and anthropometric data, an odds ratio below 1 implies protection against a certain event, while an odds ratio over 1 suggests an increased probability of the event occurring within the population based on exposure to the variable of interest. Regression analyses were also conducted in order to determine the effects of socioeconomic status upon the intake levels of each nutrient and food item analyzed as part of this study.

**Hypothesis Testing.** In order to examine the data in terms of the specific hypotheses postulated at the beginning of this study, *Chi-square* statistics and *t-student* tests were utilized to determine whether the data supported the assumptions made with regards to the following: (1) comparing the micronutrient and fiber content of Thai

medicinal plants with the Paleolithic diet; (2) comparing the ratio of macro- and micro-nutrients found in the traditional Thai diet with the Paleolithic dietary pattern; (3) comparing adherence to the traditional Thai diet among the cases and controls; and (4) comparing the socioeconomic status of the cases and controls. The results of these analyses enabled the Principle Investigator to determine the specific role of the nutritional use of Thai medicinal plants in mediating breast cancer risk, and to evaluate the nutritional quality of the Thai traditional diet within the framework of the Paleolithic dietary model.

## **Chapter 4**

### **Descriptive Statistics**

#### Introduction

This chapter provides a brief overview of the descriptive statistics for the control and case populations recruited for this study. The first section covers the general characteristics associated with the controls, or healthy Thai women recruited for this study, while the second section focuses on the general traits associated with the breast cancer cases. A summary chart of the descriptive statistics for both populations is included for reference and consists of mean values and standard deviations for several demographic and anthropometric variables.

#### Descriptive Statistics: Controls

The control population consists of 78 subjects recruited from the Phrakhanong District, Bangkok, Thailand and Ramathibodi Hospital, Mahidol University. The average age for the control population was 42.4 years ( $\sigma = 12.5$ ), with a range from 16 years to 70 years of age (Table 4.1). Based on the demographic information provided by subjects during the data collection process, 84.6% of the respondents reported having steady employment or self-employment, with an average income of 16,184 Baht (485USD) per month. The control subjects typically possess a high school education, with approximately 14 years of schooling.

The average age at menarche for the control subjects was 13.6 years ( $\sigma = 1.72$  years). Approximately one-quarter (25.6%) of the control population were

postmenopausal, with an average age of 47.6 years ( $\sigma = 6.3$  years) at the time of onset. Nearly 62% of the control population reported having experienced at least one live birth. The mean age at first pregnancy was 23.2 years ( $\sigma = 4.4$  years), with an average of 2.4 children per subject. Out of these 50 parous control subjects, 98% of the women had breastfed their children, with an average duration of breastfeeding at 7.9 months.

The anthropometric and nutritional data suggests that the control population exhibits both healthy body composition levels and dietary intake. The mean body mass index (BMI) for the control subjects was 21.9 ( $\text{kg}/\text{m}^2$ ), well within the „normal“ weight status category as determined by CDC standards. In terms of the distribution of fat mass, the use of the waist-to-hip ratio (WHR), a highly reliable measure of abdominal fat storage, indicates that a majority of the control subjects have a low degree of abdominal fat storage, with a mean WHR of 0.82. The overall body composition values for the control subjects suggest that both weight and adipose tissue levels fall within the healthy range, reflecting an optimal nutritional intake.

For the control population, the mean energy intake of 2062 kcal/day reflects the USDA Recommended Daily Allowance for energy density based on age and gender. A majority of the caloric intake for the control population was derived from carbohydrates (approximately 66.1% of the diet), with the remainder coming from proteins (19.3%) and fats (14.6%).

#### Descriptive Statistics: Cases

The case population consists of 51 breast cancer patients recruited from the Breast Care Center of Ramathibodi Hospital, Mahidol University in Bangkok, Thailand. The subjects consisted of patients diagnosed with Stage 1 and Stage 2 breast cancer within the

two months prior to enrollment in this study. The two month cutoff was utilized in this study in order to control for changes in diet and body composition due to pharmaceutical and palliative treatment.

The mean age for the case population was 52.1 years ( $\sigma = 8.5$ ), with a range of 33 to 73 years of age (Table 4.1). While the average age for the case population, and thus the average age at breast cancer diagnosis for this population, reflects the typical age for breast cancer cases in Western nations (post-menopausal women over 50 years of age), almost 44% of the cases (22 out of 51) were below 50 years of age, reflecting the high prevalence of younger women who are diagnosed with breast cancer in developing nations like Thailand. When compared to the control populations, the cases tend to have some level of college education, with an average of 14.4 years of schooling. Almost 62% of the case subjects (61.5%) reported having either steady employment or self-employment with a mean income of 26,573 Baht (796USD) per month.

The average age at menarche for the case population was 14.7 years ( $\sigma = 1.79$  years). Over two-thirds (67.3%) of the case subjects reported having had at least one live birth, with an average age at first pregnancy of 27.1 years (5.79 years) and 2.03 children per subject. Out of these 37 parous cases, 88.6% reported having breastfed their children, with an average duration of 11.3 months. Nearly 75% of the cases reported having undergone menopause, with a mean age of 46.9 years at the time of onset.

Just as the control subjects possessed normal body composition and energy intake levels, the case population exhibited both healthy anthropometric and dietary markers. The average body mass index for the case subjects was  $23.01 \text{ kg/m}^2$ , well within the CDC standards for „normal“ weight status. When compared to the control population, the cases

possessed a higher average waist-to-hip ratio of 0.857, suggesting a greater degree of abdominal fat storage. These healthy anthropometric markers reflect the significantly lower levels of energy intake found in the cases when compared to the control population. The average energy intake for the case subjects was 1422 kcal/day, with a majority of the diet coming from carbohydrates (63.6% of the diet), followed by lesser amounts of protein (20.4%) and fat (16%).

**Table 4.1 Descriptive statistics for the controls and cases.**

<b>Variable</b>	<b>Controls (n = 78)</b>	<b>Cases (n = 51)</b>
Age (years)	42.4±12.5	52.1±8.5
Education (years)	13.7±1.8	14.4±2.2
Percent Employed	84.6%	61.5%
Income (per month)	16184 Baht/485USD	26573 Baht/796USD
Age at Menarche (years)	13.6±1.7	14.7±1.8
Percent Menopause	25.6%	75%
Age at Menopause (years)	47.6±6.3	46.9±5.8
Percent Pregnant	61.5%	67.3%
Age at First Pregnancy (years)	23.2±4.4	27.1±5.8
Number of Children	2.4±1.6	2.03±1.2
Percent Breastfeed		
Parous Subjects	97.9 %	88.6 %
Total Subjects	60.3%	59.6%
Months Breastfeeding (months)	7.85±5.2	11.3±7
Weight (kg)	55.55± 8.7	56.4± 8.5
Height (cm)	158.5±6.6	155.2±6.1
BMI (kg/m <sup>2</sup> )	21.9± 4.1	23.01± 4.5
Kcal Intake (Kcal/day)	2062±802	1422±419
Macronutrient Proportion (% of total daily energy intake)		
Protein	19.3%	20.4%
Fat	14.6%	16%
Carbohydrate	66.1%	63.6%

## **Chapter 5**

### **Data Analysis: Comparative Statistics**

#### Introduction

For the past two decades, epidemiological studies examining the role of demographic and anthropometric variables upon breast cancer risk have demonstrated a positive relationship between increasing age and measures of body composition with the risk for breast cancer. While increasing age and postmenopausal status are widely regarded by scientists as accepted risk factors for breast cancer, the use of anthropometric measures to predict breast cancer risk has been less conclusive. Although some studies suggest a strong relationship between measures of adiposity, such as body mass index (BMI) and waist-to-hip ratio (WHR), and breast cancer incidence, other studies have highlighted the potential confounding effects of ethnicity and diet upon body composition as a predictor for the risk of breast cancer (Harvie et al 2003; Baumgartner et al 2004; Lahmann et al 2004). As with previous epidemiological studies, an analysis of the data from this case-control study provided results which confirm the role of age, parity and some measures of adiposity upon breast cancer risk, while at the same time providing some interesting departures from the expected relationships between the exposures and outcomes.

This chapter will provide an analysis and discussion of the impact of demography and body composition upon breast cancer risk in the case and control populations recruited for this study. Specifically, age at first pregnancy and parity, age at menarche

and adult height, and measures of body composition will be analyzed within the context of breast cancer risk. Statistical methods used to compare risk factors based upon exposure to different demographic and anthropometric variables include standard regression statistics and odds ratio analysis. Tables highlighting the continuous and categorical variables discussed in this chapter, as well as the results of the statistical analysis, are included for reference purposes.

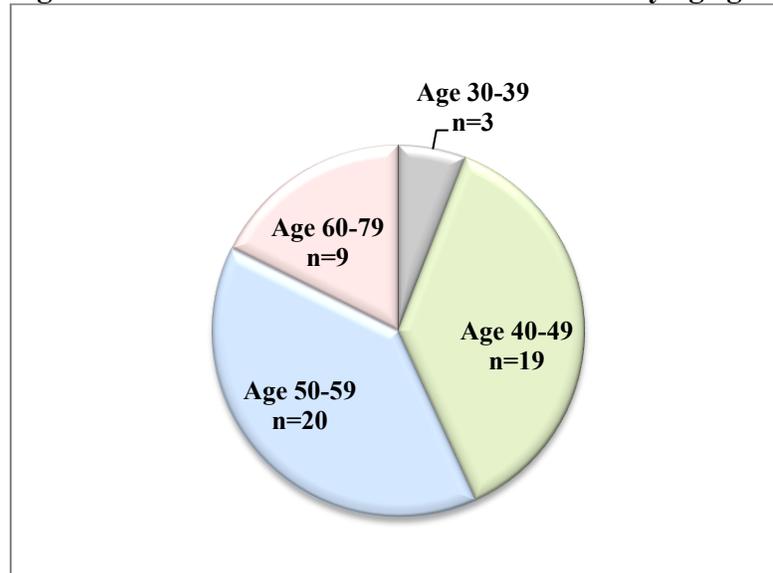
#### Age at First Pregnancy, Parity and Breast Cancer Risk

As expected, both older age and a later age at first pregnancy in this population demonstrated a positive association with breast cancer risk as determined via odds ratio analysis. The case population possessed a higher average age (52.1 years) when compared to the controls (42.4 years), which resulted in an 8% increase in breast cancer risk (OR = 1.08,  $p = 0.0001$ ) (Table 5.1). In much the same manner, a later mean age at first pregnancy (27.1 years) for the cases when compared to the controls (23.2 years) likewise produced a 15% increase in breast cancer risk (OR = 1.15,  $p = 0.002$ ) (Table 5.1). Although the average age of the case population suggests that a majority of breast cancer cases are associated with Thai women over the age of 50, examining the distribution of the cases by age group shows that nearly half of the patients are below age 50 (Figure 5.1). This pattern in age distribution may account for the relatively low contribution of overall age to breast cancer risk observed in this sample.

Over sixty percent of the case and control subjects were parous (72.5% and 60.3% respectively) and reported having breastfed their infants (64.7% and 62.5% respectively). Although there was a general trend towards decreases in breast cancer risk for both parity

and a history of lactation (OR = 0.57,  $p = 0.1$ ; OR = 0.92,  $p = 0.4$ ), neither variable was statistically significant (Table 5.2).

**Figure 5.1 Distribution of breast cancer cases by age group.**



**Table 5.1 Continuous predictors of breast cancer among Thai women.**

Predictor	Cases mean ( $\sigma$ )	Controls mean ( $\sigma$ )	Odds Ratio	95% confidence interval	$P$ value
Age (years)	52.1 (8.50)	42.4 (12.5)	1.08	1.04 – 1.13	0.0001
Age at 1 <sup>st</sup> pregnancy	27.1 (5.79)	23.2 (4.4)	1.15	1.05 – 1.27	0.002
Age at menarche	14.7 (1.79)	13.6 (1.72)	1.35	1.09 – 1.67	0.0037
Height (cm)	155.22 (6.11)	158.53 (6.63)	0.92	0.86 – 0.98	0.0041
Weight (kg)	56.41 (8.52)	55.55 (8.70)	1.01	0.97 – 1.05	0.58
BMI (kg/m <sup>2</sup> )	23.02 (4.53)	21.89 (4.07)	1.07	0.98 – 1.17	0.15
Waist circumference (cm)	84.76 (7.96)	76.73 (9.98)	1.10	1.05 – 1.15	0.00001
Hip circumference (cm)	98.86 (7.93)	93.32 (8.08)	1.09	1.04 – 1.14	0.0002

Data presented as mean (standard deviation), 95% confidence interval, and  $P$  value for cases and controls.

**Table 5.2 Categorical Predictors of Breast Cancer in Thai Women.**

Predictor	Cases n (%)	Controls n (%)	Odds ratio	95% confidence interval	P value
<b>Waist-to-hip-ratio</b>					
≤ 0.77	4 (7.8)	22 (28.2)	1.00		
> 0.77 to ≤ 0.85	18 (35.3)	30 (38.5)	3.3		
> 0.85	29 (56.9)	26 (33.3)	6.13		0.002
<b>Waist-to-hip-ratio (Premenopausal Subjects)</b>					
≤ 0.77	2 (15.3)	21 (35)	1.00		
> 0.77 to ≤ 0.85	6 (46.2)	21 (35)	3.00		
> 0.85	5 (38.5)	18 (30)	2.92		0.33
<b>Waist-to-hip-ratio (Postmenopausal Subjects)</b>					
≤ 0.77	2 (5.3)	1 (5.6)	1.00		
> 0.77 to ≤ 0.85	12 (31.6)	10 (55.6)	0.60		
> 0.85	24 (63.1)	7 (38.8)	1.71		0.24
<b>Family history of breast cancer</b>					
Yes	7 (13.7)	2 (2.6)	6.05	1.203 - 30.38	0.007
No	44 (86.3)	76 (97.4)			
<b>Obesity (BMI ≥ 25)</b>					
Yes	15 (28.8)	17 (21.8)	1.50	0.67 – 3.35	0.44
No	36 (71.2)	61 (78.2)			
<b>Obesity (BMI ≥ 30)</b>					
Yes	1 (2)	2 (2.6)	0.76	0.067 - 8.604	0.4
No	50 (98)	76 (97.4)			
<b>Have ever been pregnant</b>					
Yes	37 (72.5)	47 (60.3)	0.57	0.267 - 1.232	0.1
No	14 (27.5)	31 (39.7)			
<b>Have ever breastfed</b>					
Yes	33 (64.7)	49 (62.8)	0.92	0.441 - 1.923	0.4
No	18 (35.3)	29 (37.2)			
<b>Socioeconomic status</b>					
Low-middle	30 (58.8)	54 (69.2)	1.58	0.754 - 3.289	0.2
Middle-upper	21 (41.2)	24 (30.8)			

Data presented as number (%), age-adjusted odds ratio, 95% confidence interval and *P* value for cases and controls.

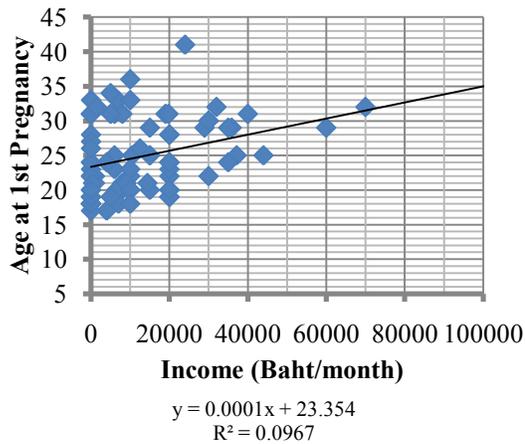
Regression analysis was used in order to assess the role of socioeconomic status upon parity and lactation. In general, the cases possessed higher levels of income and education when compared to the controls. However, neither measure produced any statistically significant results in terms of the impact of socioeconomic status upon the

variables associated with parity. Instead, the regression analyses suggested general trends in which increases in socioeconomic status impacted the reproductive variables measured in this study.

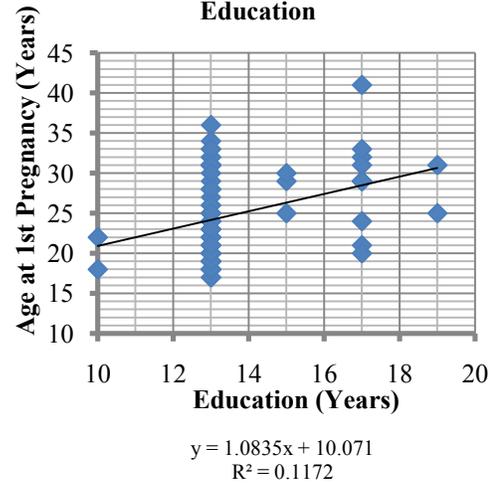
Using income as a measure of socioeconomic status, a weak positive trend existed between age at first pregnancy and income level ( $y = 0.0001x + 23.354$ ,  $r^2 = 0.0967$ ), with higher levels of monthly salary associated with an older age at first pregnancy (Figure 5.2). Substituting years of education for income resulted in a similarly weak, yet positive, trend in which an older age at first pregnancy was positively associated with higher levels of education ( $y = 1.0835x + 10.071$ ,  $r^2 = 0.1172$ ) (Figure 5.3). Years of education also demonstrated a weak inverse association with the number of live births ( $y = 0.1968x + 4.1456$ ,  $r^2 = 0.0712$ ) (Figure 5.4) and months of lactation ( $y = 0.1011x + 10.571$ ,  $r^2 = 0.0359$ ) (Figure 5.5).

Although the regression analyses were not statistically significant, the overall trends suggest that increases in socioeconomic status result in changes in reproduction consistent with the demographic transition. This implies that higher levels of both income and education represent two demographic features which result in an increased risk of breast cancer due to their effects upon parity. Specifically, both income and education appear to influence reproduction by increasing the age at first pregnancy and by reducing the number of live births and months of lactation. As demonstrated by previous epidemiological studies, a delayed age at first pregnancy, in addition to reductions in parity and breastfeeding, all play important roles in increasing breast cancer risk for several important hormonal and immunological reasons.

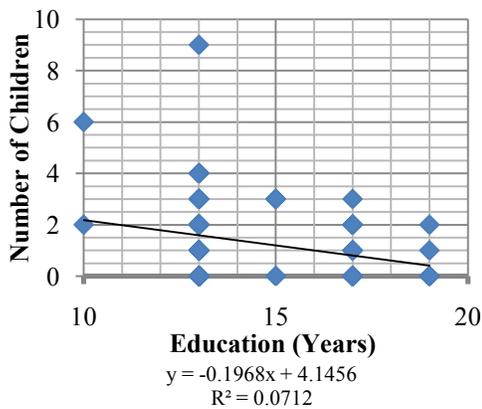
**Figure 5.2 Age at 1st Pregnancy and Income**



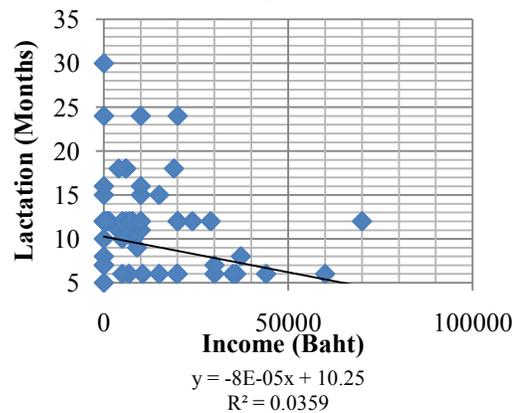
**Figure 5.3 Age at 1st Pregnancy and Education**



**Figure 5.4 Number of Live Births and Education**



**Figure 5.5 Lactation in Months and Income**



## Discussion

In general, age at first pregnancy plays a significant role in determining breast cancer risk throughout the human lifecycle, with studies demonstrating a decreased risk of cancer for first pregnancies prior to age 25 (OR = 0.5) and an increased risk of cancer for first pregnancies occurring after age 35 (OR = 1.5 to 5.4) (Lee et al 2003; Britt et al. 2007). Because over seventy percent of breast cancer cases are estrogen receptor positive (ER+), meaning that tumorigenesis occurs in the presence of a high-estrogen

environment, age at first pregnancy represents a significant factor in breast cancer epidemiology, since parity protects against ER+ breast cancer (Britt et al 2007).

Parity represents a particularly salient factor in breast cancer risk as both pregnancy and lactation produce structural and hormonal changes in breast tissue which result in the complete maturation of the mammary gland and in long-term protection against carcinogenesis. In humans, the mammary epithelium possesses two main stages of postnatal development. During puberty, or the first stage of postnatal mammary development, the ductal network responsible for milk production and lactation, spreads out from the nipple through specialized growth structures known as terminal end buds (TEBs) (Britt et al 2007; Schneider and Bocker 2006). The TEBs consist of two main cell types- an inner luminal layer of body cells and an outer layer of cap cells which are driven by stem cell activity (Britt et al 2007; Schneider and Bocker 2006).

Stem cell activity during puberty initiates increases in breast size in order to provide the mammary tissue with its adult complement of stem cells. During this process of pubertal stem cell division, any DNA damage or mutations which occur will become fixed and maintained within the stem cell compartment throughout adulthood, thereby increasing the risk for breast cancer (Britt et al 2007). The ultimate result of ductal elongation and stem cell activity during adolescence is the production of the future milk-producing structures of the breast known as the terminal ductal lobuloalveolar units (TDLUs) (Britt et al 2007; Schneider and Bocker 2006). The TDLUs are clustered in the human breast into larger units called lobules, which are classified by their degree of complexity, as determined by the number of ductal clusters per lobule.

From menarche to first pregnancy, human breast tissue consists of Type 1 lobules, which are less complex and consist only of Type 1 stem cells- the main stem cell class responsible for the initiation of mammary growth during adolescence. Type 1 lobules are the most common sites of breast cancer in humans, because the Type 1 stem cells tend to foster more rapid cell duplication and DNA mutations, especially in the presence of estrogen, and therefore have a higher degree of association with breast cancer risk (Britt et al 2007; Schneider and Bocker 2006). During menstrual cycles occurring between menarche and first pregnancy, the normal breast epithelium undergoes cyclic variations in DNA synthesis in order to maintain the proliferation of new cells necessary for tissue integrity. This form of mammary development initiated by ovarian estrogen production never returns to the starting point of the preceding cycle, resulting in slightly more tissue growth than the previous cycle, a process which continues to age 35 (Schneider and Bocker 2006). The accumulation of epithelial cells in nonparous women largely consists of Type 1 stem cells, which are more carcinogenic and therefore more susceptible to breast cancer.

While puberty initiates changes and growth in breast tissue geared towards tumorigenesis due to the presence of relatively immature lobules containing Type 1 stem cells, pregnancy initiates the completion of breast maturation and produces both structural and hormonal changes which provide long-term protection against breast cancer. During pregnancy, the presence of human chorionic gonadotropin (hCG) initiates the expansion of the TDLUs in preparation for lactation. An important consequence of hCG initiated breast maturation during pregnancy is the development of Types 2 and 3 lobules, which are more complex, because they contain a greater number of TDLUs, and

which are characterized by the predominance of Type 2 stem cells (Russo et al 2007; Schneider and Bocker 2006). In parous women, Type 2 stem cells are responsible for coding the genes associated with DNA transcription, RNA processing, immune response, apoptosis, and DNA repair and recombination; as a result, Type 2 stem cells possess a higher degree of resistance to carcinogenic transformation (Russo et al 2007; Schneider and Bocker 2006).

In addition to increasing the structural complexity of the mammary gland, pregnancy results in immunological responses which also reduce the risk for breast carcinogenesis. Due to the high proportion of Type 2 stem cells in parous women, the resulting epithelial cells found in Types 2 and 3 lobules have the capacity to upregulate several important DNA repair genes. For example, both RPS3 (ribosomal protein S3) and RADS1 (radiation sensitive related-like 1 homologous recombination repair gene) are capable of inducing either apoptosis or the repair of carcinogen-induced cell damage by unscheduled DNA synthesis (Russo et al 2006). Moreover, the hormonal changes associated with pregnancy induce the upregulation of four specific genes associated with increases in immune surveillance: (1) the cytoplasmic toll/interleukin-1 receptor, which induces a signaling cascade that enhances innate immune responses associated with passive immunity; (2) T-cell receptor V-beta 1, which recognizes and deletes cytotoxic T-cell lymphocytes; (3) HLA-A24 (human leukocyte antigen A24), which produces anticytotoxic T-cells; and (4) the interleukin-22 receptor, which activates the STAT3 pathway (signal transducer and activator of transcription 3) responsible for downregulating epithelial cell growth factors (Lee et al 2003; Britt et al 2007).

In women with an earlier age at first pregnancy, these genomic changes are more permanently imprinted into the mammary tissue, producing increases in immune surveillance and decreases in the number of potentially transformative epithelial and stem cells, a protective pattern which continues into menopause (Russo et al 2007; Schneider and Bocker 2006). Even after menopause, when breast tissue involutes by returning to Type 1 lobules, the epithelial cells of postmenopausal women with a history of parity show structural differences from postmenopausal, nonparous women, in that Type 2 stem cells still predominate. Therefore, parity produces lifetime protection against breast cancer via three main mechanisms: (1) an increase in immune surveillance; (2) decreases in the overall number of epithelial and stem cells; and (3) a reduction in the responsiveness of breast tissue to estrogen. This effect appears to be age dependent, with an earlier age at first pregnancy resulting in a stronger protection against breast cancer risk.

Because the control population in this study consists of women with a significantly earlier age at first pregnancy when compared to the controls, the findings in this study support the notion that decreases in breast cancer risk are associated with a younger age at first pregnancy. For the control population, an earlier age at first pregnancy most likely produces this protective effect through the initiation of hormonal and immunological changes which accompany the complete maturation of the mammary tissue during pregnancy and lactation. Furthermore, the regression analyses suggest that the demographic transition has altered patterns of reproduction among Thai women towards a more Westernized pattern in which women give birth to fewer numbers of offspring later in life. Although the regression analyses lacked statistical significance, the

trends indicate that as income and education levels increase, women tend to have fewer children at a later age. Therefore the increased risk for breast cancer observed in the case population may be related in part to the loss of the traditional Thai reproductive pattern, which is characterized by larger family sizes and an earlier age at first pregnancy.

#### Age at Menarche, Adult Height and Breast Cancer Risk

Just as parity protects against breast cancer through beneficial hormonal and immunological changes, researchers have suggested for similar reasons that a later age at menarche is protective against breast cancer, by reducing lifetime exposure to estrogen (Adebamowo et al 2003; Harvie et al 2003). However, in this study, a later age at menarche for the cases (14.4 years) when compared to the controls (13.7 years) was associated with a 35% increased risk for breast cancer (OR = 1.35,  $p = 0.004$ ) (Table 3). This statistically significant finding contradicts the expected relationship between age at menarche and breast cancer risk.

Another unexpected finding in terms of the demographic and anthropometric data is the unusual relationship between age at menarche and adult height. Age at menarche has widely been used by researchers as a predictor of adult height, with a later age at first menstruation typically associated with increases in stature. However, in this study the cases possessed a lower average adult height (155.22 cm) when compared to the controls (158.53), despite having a later age at menarche. One unique statistical result from this unexpected relationship is the association between taller adult statures and a reduction in breast cancer risk.

Although previous studies have suggested that taller women possess an increased risk of breast cancer, due to the effects of growth hormones upon the development of the

mammary stem cell compartment, the findings in this study contradict the expected relationship between height and breast cancer risk. Rather than shorter stature contributing to a reduction in breast cancer risk, taller adult height among Thai women was associated with a protective effect. When compared to the case population (155.22cm), the controls were significantly taller (158.53 cm), a variable which was associated with an 8% decreased risk for breast cancer (OR = 0.92,  $p = 0.004$ ) (Table 5.1).

Through the use of regression analysis to examine the relationship between socioeconomic factors and age at menarche, neither income nor education yielded a statistically significant effect upon the timing of first menstruation. In much the same manner, an analysis of adult height in relationship to both income and education failed to provide any statistically relevant explanations for the observed differences in adult height between the cases and the controls. In contrast with the measures of parity, for which socioeconomic status weakly predicted the timing of first pregnancy, the data for adult height and age at first menstruation lack a clear association with income and education levels. This suggests that ethnicity may account for: (1) the unexpected differences in the relationship between menarche and adult stature among Thai women; and (2) the divergent pattern in the association between breast cancer risk and the timing of menarche in Thai women.

### Discussion

Both age at menarche and adult height represent two variables which possess the most consistent associations with breast cancer risk in studies conducted among Western populations over the past half century. In a meta-analysis of these data, researchers have

determined that an approximate 4% reduction in breast cancer risk occurs for each year of delayed menarche (Onland-Moret et al 2005; Ziegler 1997; Ballard-Barbash 1994). By contrast, the highest quartile of height is associated with a 90% increase in the risk for breast cancer (Onland-Moret et al 2005; Ziegler 1997; Ballard-Barbash 1994). Age at menarche and adult height are closely related in their impact upon breast cancer risk because puberty is initiated by increases in sex hormones, which in turn control the release of growth factors responsible for the adolescent growth spurt. Because adult height in females is usually reached within two years after menarche, adult height reflects an individual's cumulative exposure to endogenous hormones and growth factors which control skeletal maturation and increases in both mammary mass and the number of ductal stem cells attained prior to epiphyseal closure (Baer et al 2006; DeStavola et al 2004; Ruder et al 2008; Ziegler 1997; Ballard-Barbash 1994).

Human breast tissue possesses the most susceptibility to malignant transformation during adolescence, when endogenous sex hormones initiate the rapid proliferation of stem cells and the increased complexity of the ductal network (Baer et al 2006). These same hormonal changes which lead to the growth of breast tissue during puberty likewise initiate the adolescent growth spurt, a rapid increase in stature which typically occurs two to three years after menarche. Longitudinal studies examining the relationship between age at menarche and adult height show that girls from Western nations with an earlier onset of puberty tend to have a more intense, but shorter, period of growth (Baer et al 2006; DeStavola et al 2004). As a result, in developed countries, females with an earlier age at menarche tend to be shorter on average when compared to girls with a later age at menarche due to a shorter pubertal growth period (Baer et al 2006; DeStavola et al 2004).

By contrast, a delayed age at menarche in females from Western populations results in greater adult stature by allowing for a longer period of skeletal growth prior to the closure of the epiphyseal growth plates (Onland-Moret et al 2005). Although breast cancer risk is reduced by lower exposure to endogenous ovarian estrogen due to an extended period of growth, long bone growth is concomitantly associated with higher levels of insulin-like growth factor 1 (IGF-1), which contributes to breast cancer risk by enhancing stem cell differentiation within the developing breast (Beaber et al 2008; Onland-Moret et al 2005).

While this relationship between age at menarche and adult height appears to provide a clear-cut explanation for the differences in breast cancer risk associated with the timing of adolescence, more recent studies suggest that the timing of menarche and the attainment of adult height in Western nations can be influenced by pre-pubertal weight and nutrition status. Among girls with a high proportion of body fat, adipose tissue produces higher pre-pubertal levels of estrogen due to the conversion of adrenal androgens into estradiol via the aromatization of body fat (DeStavola et al 2004). These high levels of endogenous estrogen in turn facilitate an earlier onset of puberty, which produces a longer, more intense, period of skeletal growth resulting in greater adult stature (DeStavola et al 2004; Baer et al 2006). Under the dual influences of high adipose tissue and an early onset of puberty, girls are exposed to high levels of both estrogen and IGF-1 during breast development, thus increasing the potential for genetic mutations to accumulate within the rapidly developing stem cell compartment (DeStavola et al 2004; Baer et al 2006). Among females which experience an earlier age at menarche due to overweight/obesity, the attainment of maximal adult height occurs prior

to age 14, an age-dependent relationship which possesses a particularly strong correlation with breast cancer risk. By contrast, acquiring maximum adult stature after age 14 (typically due to the absence of excess body fat during childhood) produces less of an impact upon breast cancer risk (Ahlgren et al 2006).

Therefore, the timing of the maximal growth spurt in conjunction with age at menarche seems to play a more important role in determining breast cancer risk than each of these variables in isolation. In turn, the combination of these two factors among developed populations seems to be strongly influenced by childhood weight and nutrition status, with heavier girls attaining their adult height and menarche at an earlier age, and with leaner girls attaining their adult height and menarche at a later age.

Within the context of this framework, the association between a later age at menarche and shorter adult stature with an increased risk of breast cancer represents a unique disease pattern found within developing nations, and the Thai population in particular. Specifically, a later age at menarche among Thai women results in both shorter stature and an increased risk of breast cancer for the following reasons: (1) decreases in the amount of available nutrients during childhood necessitates a delayed onset of menarche in order to conserve energy vital to skeletal growth; (2) a later age at menarche produces a longer period of skeletal maturation associated with a slower rate of growth, thus resulting in a shorter adult stature; and (3) a longer period of skeletal maturation raises the risk of breast cancer by increasing the exposure of the developing breast to endogenous estrogen and growth factors which contribute to mutagenesis and the hyper-proliferation of breast stem cells.

### Anthropometric Measures of Body Composition and Breast Cancer Risk

As expected, based on previous epidemiological studies evaluating breast cancer risk in terms of body composition, increases in fat storage elevate breast cancer risk for both pre- and postmenopausal case subjects. Looking at general measures of body composition, including weight and BMI, both the cases and controls were relatively similar in their measurements, with a mean weight of 56.41 kg and 55.55 kg and a mean BMI of 23.02 and 21.89 for the cases and controls respectively. Evaluating the cases and controls for the effects of obesity as measured by BMI cutoff values  $\geq 30$  resulted in no statistically significant impact upon cancer risk. When lowering the BMI to cutoff values  $\geq 25$ , the common threshold for obesity used within Asian populations, there was a trend towards an increased risk for breast cancer with higher BMI values; however, this relationship was not statistically significant (Table 5.2).

While both the case and control populations would be considered within the normal range for both weight and BMI based upon CDC standards, significant differences exist in the pattern of fat storage. In general, the cases tend to have significantly higher waist (84.76 cm) and hip (98.86 cm) circumference measures when compared to the controls (76.74 and 93.32 cm respectively). Higher waist and hip circumference values were associated with an approximate 10% increase in the risk for breast cancer (OR = 1.10,  $p = 0.00001$ ; OR = 1.09,  $p = 0.0002$ ) (Table 5.1). When using this data to calculate the waist-to-hip ratio (WHR), the cases also possess higher average WHR measurements than the controls, suggesting that despite the similarities in weight and BMI status, the breast cancer cases tend to have a higher proportion of fat stored abdominally.

With increases in WHR, a concomitant increase in breast cancer risk was observed, with the highest WHR tertile associated with over a six-fold increase in breast cancer risk (OR = 6.13,  $p = 0.002$ ) (Table 5.2). Looking at the risk patterns associated with WHR by menopausal status shows a general trend towards increasing risk with higher WHR values for both pre- and postmenopausal Thai women. Although this trend was not statistically significant, one important observation from this analysis of WHR by menopausal status is the stronger effect of abdominal fat storage upon breast cancer risk among the premenopausal subjects.

This observation is especially salient, given that previous researchers have only observed a significant effect of central fat storage upon breast cancer risk among postmenopausal women from Western nations. In women from developing populations, central fat storage produces an elevation in breast cancer premenopausally. Therefore, the results from this study, similar to the outcomes found in other research populations from developing nations, demonstrates that abdominal fat distribution has the most profound impact upon breast cancer risk among younger women. These findings further suggest that ethnic differences may exist in the timing of the metabolic syndrome, with developing populations possessing a younger age at which abdominal fat storage produces insulin resistance and its synergistic effects upon breast cancer risk.

### Discussion

Measures of body composition associated with overweight and obesity have been implicated in the increased risk for breast cancer due to the impact of adipose tissue upon alterations in hormone metabolism and immune function. However, the relationship between overweight/obesity and breast cancer risk is dependent upon the reproductive

lifecycle, with a distinctive risk pattern occurring in developed or Westernized populations, in which premenopausal obesity is associated with a reduction in breast cancer risk, due to more anovulatory cycles, while postmenopausal obesity is associated with an increase in breast cancer risk (Ziegler 1997; Ballard-Barbash 1994).

In premenopausal women, endogenous estrogen is largely produced by the ovaries, which outweighs the production of estrogen from adipose tissue; therefore premenopausal obesity contributes very little to overall endogenous estrogen levels and insulin response (Ballard-Barbash 1994). By contrast, in postmenopausal women, ovarian estrogen production ceases, and endogenous estrogen production occurs via the conversion of androgens in adipose tissue (Ballard-Barbash 1994). As a result, circulating levels of estrogen in blood plasma are dependent upon the amount of fat stores within the individual, with higher levels of adipose tissue correlated with higher circulating levels of estrogen. In addition, menopause results in changes in hormonal metabolism which result in reduced hepatic clearance of insulin and therefore higher insulin levels, thus contributing to insulin resistance, especially in the presence of excessive fat stores (Ballard-Barbash 1994; Ziegler 1997).

In particular, abdominal, or central, fat appears to exert an even greater influence upon insulin resistance and the metabolic syndrome, and is associated with hormonal and immunological changes which directly increase the risk for breast cancer. One specific hormonal change associated with central obesity is the direct increase in estrogen via aromatase activity, leading to a decreased ratio of 2-hydroxy estradiol when compared to 16 $\alpha$ -hydroxy estradiol (Ballard-Barbash 1994; Sauter and Scott 2008).

In humans, estrogen is metabolized via two main pathways: the 2-hydroxyestrone (2-OHE) and 16 $\alpha$ -hydroxyestrone (16 $\alpha$ -OHE) pathways. The 2-OHE product is associated with a reduced risk for breast cancer because it is anti-estrogenic, and therefore markedly suppresses the growth and proliferation of mammary stem cells (Jernstrom et al 2003). By contrast 16 $\alpha$ -OHE is associated with an increased risk of breast cancer due to its effect upon mammary stem cell differentiation, which mimics the action of 7, 12-dimethylbenz[ $\alpha$ ]anthracene (DMBA), a chemical used to produce cancer in research settings. 16 $\alpha$ -OHE not only initiates breast stem cell differentiation and the process of carcinogenesis in vivo, but also stimulates the growth of established breast cancer cells (Telang et al 1992; Gupta et al 1998).

In addition to increasing the ratio of 2-OHE to 16 $\alpha$ -OHE, central adiposity likewise results in the increase of several adipocytokines associated with the regulation of insulin and estrogen action. Leptin, an adipocytokine with important functions in both metabolism and hormone regulation, controls energy intake, energy expenditure, and appetite in humans. With increases in abdominal fat, leptin signaling is upregulated and this augmentation of leptin production results in interference with insulin signaling (Rose 2004). The effect of leptin upregulation appears to be dose dependent, with the degree of insulin resistance directly correlated with plasma leptin levels (Rose 2004).

With increases in plasma leptin levels, estrogen biosynthesis is stimulated via the induction of aromatase activity in adipose tissue and the concomitant downregulation of sex hormone binding globulin (SHBG) (Rose 2004; Ballard-Barbash 1994). Estrogen synthesis stimulated via leptin signaling tends to increase the ratio of 16 $\alpha$ -OHE to 2-OHE, and in the absence of sufficient amounts of SHBG necessary to reduce plasma

hormone levels, increases the bioavailability of sex hormones (Rose 2004; Harvie 2003). The simultaneous increase in leptin and estrogen levels, in association with the downregulation of SHBG, synergistically promotes both tumorigenesis and angiogenesis via decreases in cell-mediated immunity (NK cells, leukocytes and T helper cells), increases in inflammatory mediators associated with tumor production (prostaglandins, C-reactive proteins), and increases in reactive oxygen species (Sauter and Scott 2008).

Augmentation of leptin production due to central fat storage also results in the over-production of insulin-like growth factors (IGFs), which stimulate cell turnover in the majority of body tissues, and therefore increase breast cancer risk via the initiation of mammary cell differentiation and carcinogenesis. IGF is usually removed from the body by insulin-like growth factor binding protein 1 (IGFBP-1); however, abdominal fat downregulates IGFBP-1 production, resulting in increased plasma concentrations of IGF (McTiernan 2000). The presence of insulin-like growth factor and higher circulating levels of insulin in women with abdominal fat stimulates insulin receptors in breast tissue, making stem cells in the breast more responsive to estrogen and impairing the effectiveness of apoptosis in removing damaged or precancerous cells from the mammary tissue (Lajous et al 2008).

When combined, the effects of the metabolic syndrome due to central fat storage in postmenopausal women from developed nations results in concomitant decreases in the production of binding proteins responsible for removing estrogen and insulin-like growth factor from the body, as well as increases in the synthesis of estrogen, insulin, and IGF. The elevation of these hormones initiates several key responses associated with an elevated risk of breast cancer, including: (1) decreases in cell-mediated immunity; (2) the

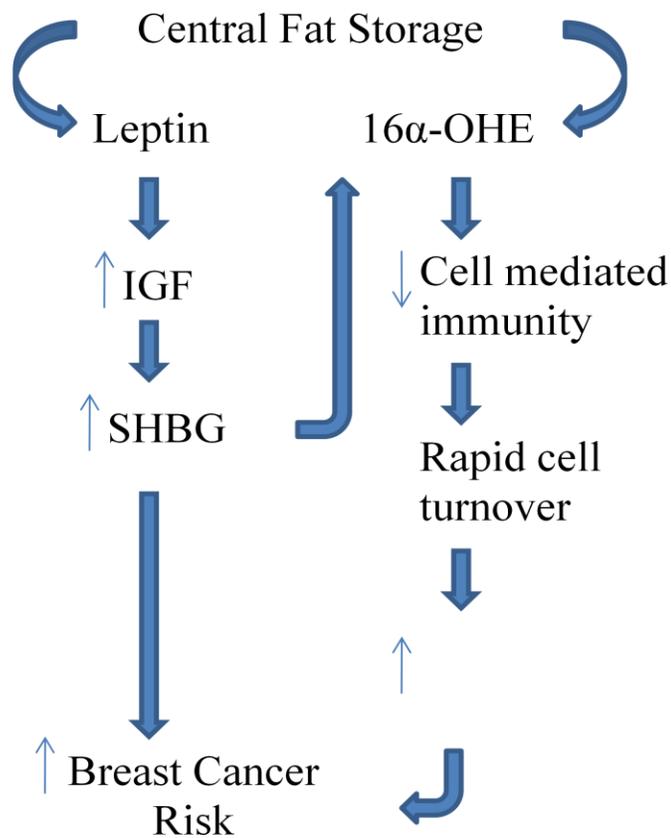
stimulation of rapid cell turnover; and (3) increases in angiogenesis and tumorigenesis (Figure 5.6). While this pattern holds true for postmenopausal women in Western nations, the dominant pattern among Asian women is increasing risk with increasing adiposity, regardless of menopausal status (Ziegler 1997). In women from developing populations in Asia, the positive association between fat storage and breast cancer risk is stronger and more apparent at younger ages (Ziegler 1997).

Indeed, the findings from this study suggest that Thai women with central adiposity experience the negative effects of the metabolic syndrome regardless of menopausal status. Although classification by menopausal status demonstrated only a weak trend towards increased breast cancer risk in association with higher waist-to-hip-ratio values, the presence of an elevated risk for both pre- and postmenopausal Thai women suggests that the metabolic syndrome exerts an influence upon hormone and immune function throughout the lifecycle. Moreover, the increase in breast cancer risk appears to be greater in premenopausal women with higher WHR values than among postmenopausal Thai women.

This finding confirms earlier epidemiological studies suggesting that fat storage elevates cancer risk in younger Asian women when compared to the pattern found in Western nations, where only postmenopausal women experience increases in risk due to obesity. Among Thai women, central adiposity, regardless of age, may result in key changes to immune function and hormone levels which predispose these individuals to a greater risk for breast cancer. Some of these potential changes include elevations in estrogen and insulin-like growth factors and decreases in sex-hormone binding globulin and cell-mediated immunity due to increased aromatization of adipose tissue and elevated

plasma leptin levels. While these characteristics are typically found only in postmenopausal women with high levels of abdominal fat storage living in Western countries, the same pattern seems to predict breast cancer risk in both pre- and postmenopausal women from Thailand. Some of the statistics suggest that the effects of central obesity may even exert a stronger influence upon breast cancer risk for premenopausal Thai women.

**Figure 5.6 Schematic Diagram of Breast Cancer Risk Due to Central Fat Storage**



Summary

Overall, the analysis of breast cancer risk via exposure to standard anthropometric and demographic variables both confirms and diverges from the expected patterns found

in previous epidemiological studies. While the demographic transition, as characterized by a later age at first pregnancy and fewer numbers of live births, helps to explain the increased risk of breast cancer among the case subjects, other demographic and anthropometric variables contradict their expected effects upon cancer risk. Specifically, age at menarche and adult height both display unusual risk patterns with regards to breast cancer. In Western populations, a later age at first menstruation and shorter adult stature both protect against breast cancer risk. However, among Thai women, a later age at menarche, rather than predicting a taller adult stature, is not only associated with a shorter adult stature, but also with an increased risk of breast cancer.

In much the same manner, waist and hip circumference, as well as waist-to-hip ratio, are all associated with significant increases in the risk of breast cancer among Thai women due in part to the hormonal and immunological changes associated with the metabolic syndrome. However, when examining the impact of central fat storage upon breast cancer risk by menopausal status, the Thai subjects in this study also demonstrate a divergent pattern when compared to Western populations. In particular, while studies have consistently demonstrated a strong correlation between abdominal fat and breast cancer risk among postmenopausal women in Western nations, central fat distribution among Thai women exerts a stronger influence upon cancer risk in premenopausal women.

Together, these observations substantiate the presence of ethnic differences in the role of traditional demographic and anthropometric variables upon breast cancer risk. In this study, only age at first pregnancy and overall age followed the expected patterns for breast cancer risk as defined by previous studies. The remaining variables, including age

at menarche, adult height, and WHR, which have demonstrated a consistent relationship with breast cancer risk in research conducted on Western populations, seem to possess a different relationship with breast cancer etiology in Thai women. Some of these ethnic differences may relate to how the timing of the adolescent growth spurt and the onset of menarche impacts breast maturation, as well as the non-age-dependent role of central fat storage in producing the negative effects associated with the metabolic syndrome.

## **Chapter 6**

### **Data Analysis: General Dietary Measures**

#### Introduction

Although numerous epidemiological studies have demonstrated an association between diet and cancer risk, controversy still exists over the exact relationship between specific micro/macronutrients and breast cancer etiology. For example, while some studies show a link between overall fat intake and breast cancer risk, other studies suggest that the intake of animal protein possesses a stronger correlation with cancer risk. These conflicting results have prompted many researchers to argue that most published evidence on dietary factors and breast cancer prevention is insufficient, and that more research needs to be conducted in order to elucidate the role between dietary factors and breast cancer etiology. In spite of this lack of a clear association between diet and cancer etiology, research still suggests that nearly 50% of all breast cancer cases could be prevented via modification of dietary choices (Milner 2006). This approach to cancer prevention is especially salient, given the fact that nearly 90% of all breast cancer cases are related to environmental and /or lifestyle factors, rather than genetics (Milner 2006).

Perhaps the greatest difficulty in clarifying the role of specific nutrients in breast cancer etiology occurs due to the synergistic effect that foods have upon nutrient bioavailability. Nonetheless, meta-analyses of the available research data highlights three important relationships between dietary intake and breast cancer risk: (1) overall fat intake increases breast cancer risk by raising endogenous estrogen levels; (2) high levels

of fruit and vegetable consumption reduce breast cancer risk by introducing antioxidants into the body capable of neutralizing free radical damage; (3) animal protein intake is positively associated with breast cancer risk (Michels et al 2007).

In particular, studies have highlighted the strong association between the Westernized diet practiced in many developed nations, which include higher amounts of animal products, fats and sugars, and the high incidence rates of cancers found in these populations. By contrast in developing populations prior to the introduction of a more globalized food economy, incidence rates of cancer remained relatively low. Only within the past decade, with the advent of the nutrition transition, has the rate of breast cancer incidence increased in developing populations such as Thailand (Key et al 2004). Diet represents a key factor in explaining the recent increase in breast cancer incidence in developing populations like Thailand because the observable changes in cancer risk demonstrate a clear association with the introduction of Western dietary influences and with the departure from traditional food choices.

This chapter will provide a discussion of the role of dietary practices upon breast cancer risk in Thailand. General dietary measures, including macro- and micronutrient intake will be evaluated for their impact upon breast cancer risk in Thai women. Standard regression analysis and odds ratio analysis will be used to evaluate the role of macro- and micronutrient intake upon breast cancer risk.

#### Caloric Intake and Breast Cancer Risk

The use of the food frequency component of the Enrollment Questionnaire developed by the principal investigator provided both the intake frequency and portion sizes for select foods. The FFQ was used in order to calculate the consumption rate for

both Western and Thai traditional foods for the subjects recruited as part of this study. In addition to providing information pertaining to differences in dietary habits between the cases and controls, the data from the FFQs also supplied mean intake values for specific foods and micronutrients, as calculated through the use of dietary data tables produced by the Thai Ministry of Public Health and the United States Food and Drug Administration.

The overall macro- and micronutrient intake values were calculated for each subject based on the information from the FFQs and the dietary data tables, and were analyzed through the use of odds ratio statistics based on quintile of intake. The first quintile is associated with the lowest level of intake for a specific nutrient and is utilized as the baseline value from which each successive quintile of increasing intake is evaluated for its impact upon breast cancer incidence. Odds ratios are interpreted based on whether the exposure exerts a protective effect upon disease expression (values less than 1.00), or increases the risk of a specific condition (values greater than 1.00).

Based on the data from the FFQs, the controls (2062 kcal/day) possessed a significantly higher energy intake when compared to the cases (1421 kcal/day) (Table 6.1). In some instances, breast cancer patients had a lower overall caloric intake due to the side-effects associated with chemotherapy; however, in those cases where subjects reported a reduction in food intake due to chemotherapy, patients were instead asked to report about their typical food intake prior to their cancer diagnosis. Even after controlling for this effect, cases still reported a lower caloric intake than the controls.

When analyzing the role of total caloric intake upon breast cancer risk in this study, no discernible effects were observed with increases in energy intake. Previous epidemiological studies have reported that breast cancer risk in developed populations is

positively correlated with higher levels of caloric intake due to the role of excessive energy consumption in producing the metabolic symptoms associated with overweight/obesity. However, as discussed in the previous chapter, both the Thai cases and controls possessed healthy BMI values which were not statistically associated with breast cancer risk. Therefore, as expected, the use of regression analysis to further evaluate the role of energy intake upon body composition produced no statistically relevant associations with either BMI or weight. Furthermore, WHR, which was associated with an increased risk of breast cancer in this population, only demonstrated a weak, non-significant correlation with energy intake. Despite a lack of statistical significance, this positive trend between increasing WHR values due to higher levels of energy intake suggests that in Thai women excessive energy intake is associated with abdominal fat storage, rather than overall increases in weight or BMI status.

Using regression analysis to examine the effects of socioeconomic status upon energy intake likewise revealed no statistically significant trends. Although the data suggest that energy intake decreases as income and education levels increase, the trends were too small to account for the observed differences in caloric intake between the cases and the controls.

#### Macronutrient and Micronutrient Intake and Breast Cancer Risk

When analyzing energy intake based on the breakdown of specific macronutrients, controls possessed a higher intake of both refined (218g/day) and unrefined carbohydrates (269g/day) and proteins (81g/day) when compared to the controls (138g/day refined CHO; 194g/day unrefined CHO; 79g/day PRO) (Table 6.1).

Refined carbohydrate consumption and high protein intake are typically associated with increases in breast cancer risk in Western nations for two main reasons:

- (1) Refined carbohydrates typically consist of processed foods which affect insulin resistance and plasma levels of insulin and glucose. Insulin resistance, one of the main characteristics of the metabolic syndrome, results in the upregulation of estrogen synthesis and the downregulation of sex hormone binding globulin, the molecule responsible for removing plasma estrogen from circulation. Increased bioavailability of estrogen as a byproduct of refined carbohydrate consumption increases the risk of developing mammary carcinogenesis (Key et al 2004; Gonzalez and Riboli 2006; Michels et al 2007).
- (2) High protein intake is usually associated with the consumption of animal meat in developed populations. The method of preparing animal meat in Western nations typically involves frying or grilling, which converts the protein into heterocyclic aromatic amines. Heterocyclic aromatic amines represent metabolites with strong carcinogenic properties that have an association with increases in the risk for breast cancer (Michels et al 2007; Cordain et al 2005).

However, in the Thai population recruited for this study, neither refined carbohydrates nor protein intake were associated with breast cancer risk, as determined by odds ratio analysis of the dietary data. Moreover, regression analyses revealed no significant associations between refined carbohydrate consumption, protein intake, and measures of body composition.

By contrast, a high intake of unrefined carbohydrates was associated with a 49% reduction in breast cancer risk (OR = 0.51,  $p = 0.00001$ ) (Table 6.2). The reduction in breast cancer risk due to complex carbohydrate consumption can be explained in part by the incorporation of fruits, vegetables and spices with known anticarcinogenic properties into the traditional Thai diet. As discussed in the next chapter on specific Thai traditional medicinal plants and breast cancer risk, these species, when analyzed individually, are associated with a significant reduction in breast cancer risk in Thai women. Therefore, the protective effect of complex carbohydrates may be specifically related to the

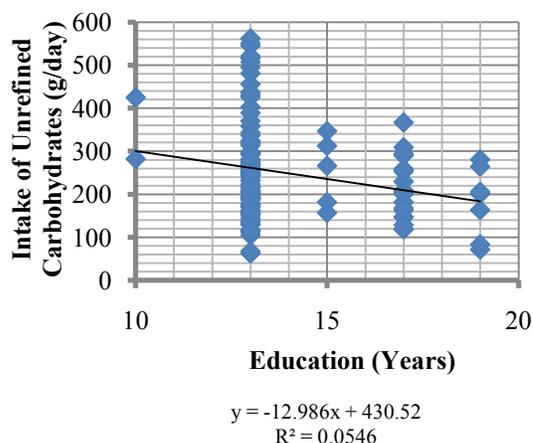
cumulative and synergistic effects of the anticarcinogenic phytochemicals found in Thai fruits, vegetables and spices.

**Table 6.1 Comparison of Macro- and Micronutrient Intake between Cases and Controls.**

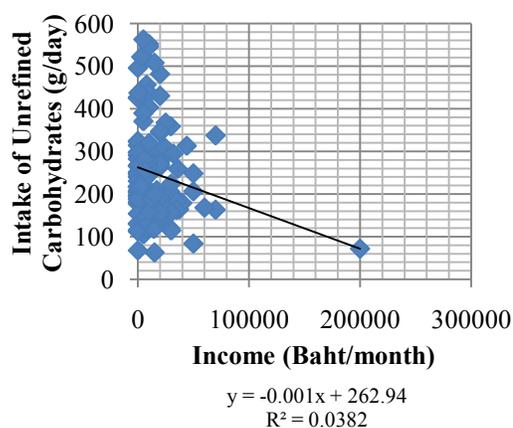
	<b>Cases mean±SD</b>	<b>Controls mean±SD</b>
<b>Total Caloric Intake (Kcal/day)</b>	1421±419	2062±802
<b>Macronutrients (g/day)</b>		
<b>Carbohydrates</b>	356±95	463±171
<b>Refined</b>	138±38.4	218±70.0
<b>Unrefined</b>	194.3±66.6	269±123.7
<b>Proteins</b>	79±34	81±53
<b>Fats (Total)</b>	55±35	42±14
<b>Saturated</b>	38.8±30.4	12.1±5.5
<b>Monounsaturated</b>	9.38±3.9	17.9±5.4
<b>Polyunsaturated</b>	6.9±2.8	11.6±4.2
<b>Proportion of Caloric Intake Derived from:</b>		
<b>Carbohydrates</b>	63.6%	66.0%
<b>Proteins</b>	20.4%	19.3%
<b>Fats</b>	16.0%	14.6%
<b>Micronutrients</b>		
<b>Vitamin A (IU/day)</b>	7105±4430	9319±5943
<b>Vitamin B1 (mg/day)</b>	52.3±26.8	121.1±60.7
<b>Vitamin C (mg/day)</b>	329.7±134.4	346.1±147
<b>Vitamin E (mg/day)</b>	8.4±2.8	15.3±6.4
<b>Vitamin B2 (mg/day)</b>	1.9±0.8	5.4±4.5

Although unrefined carbohydrates are associated with a significant reduction in breast cancer risk, their intake does not significantly influence measures of body composition according to regression analysis. However, when evaluating the intake of unrefined carbohydrates in terms of socioeconomic status, lower levels of education and income were weakly associated with higher levels of complex carbohydrate consumption ( $y = -12.986x + 430.52, r^2 = 0.054$ ;  $y = -0.001x + 262.9, r^2 = 0.038$ ) (Figures 6.1 and 6.2). This suggests that healthy Thai women tend to eat unrefined carbohydrates in greater quantities due to the lower cost of purchasing these foods from neighborhood markets, where fruits, vegetables and spices are sold directly from the farmers themselves.

**Figure 6.1 Education and Unrefined Carbohydrate Intake**



**Figure 6.2 Income and Unrefined Carbohydrate Intake**



**Table 6.2 Age-Adjusted Odds Ratios for the Association of Breast Cancer Risk with the Intake Level of Selected Macronutrients and Micronutrients.**

	Q1(low)	Q2	Q3	Q4	Q5	p-value
<b>Total Caloric Intake (Kcal/day)</b>	1.00	1.08	0.91	0.98	0.99	0.51
<b>Macronutrients (g/day)</b>						
<b>Carbohydrates</b>	1.00	1.18	1.14	1.04	1.15	0.37
<b>Refined</b>	1.00	0.84	1.00	0.91	0.96	0.94
<b>Unrefined</b>	1.00	1.71	1.66	0.82	0.51	0.00001
<b>Proteins</b>	1.00	1.09	0.91	1.02	0.97	0.51
<b>Fats</b>	1.00	1.01	1.03	0.96	1.11	0.39
<b>Saturated</b>	1.00	1.07	0.87	0.93	1.01	0.82
<b>Monounsaturated</b>	1.00	0.93	0.87	1.05	0.94	0.94
<b>Polyunsaturated</b>	1.00	1.31	0.82	1.10	0.98	0.33
<b>Micronutrients</b>						
<b>Vitamin A (IU/day)</b>	1.00	0.92	0.96	0.91	0.99	0.83
<b>Vitamin B1 (mg/day)</b>	1.00	0.88	0.95	0.96	0.90	0.44
<b>Vitamin C (mg/day)</b>	1.00	0.90	1.06	0.84	1.01	0.78
<b>Vitamin E (mg/day)</b>	1.00	0.88	0.89	0.95	0.88	0.14
<b>Vitamin B2 (mg/day)</b>	1.00	0.61	0.74	1.03	0.55	0.00004

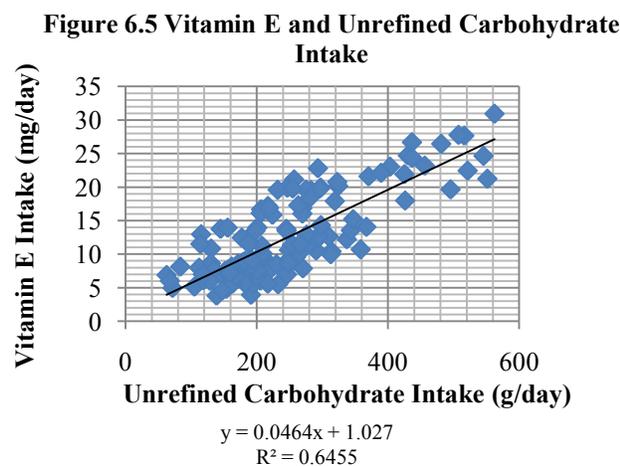
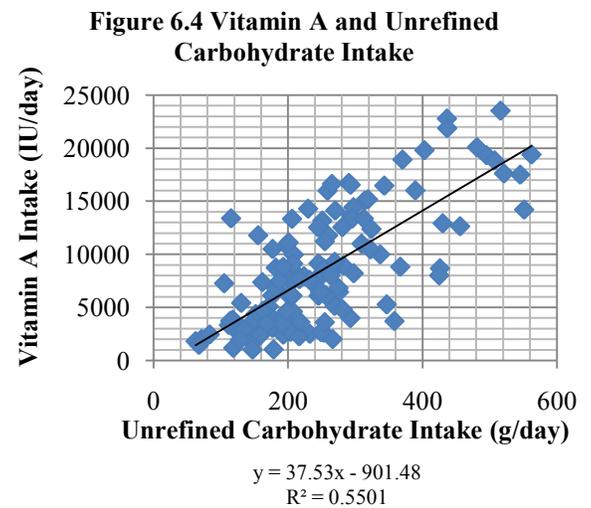
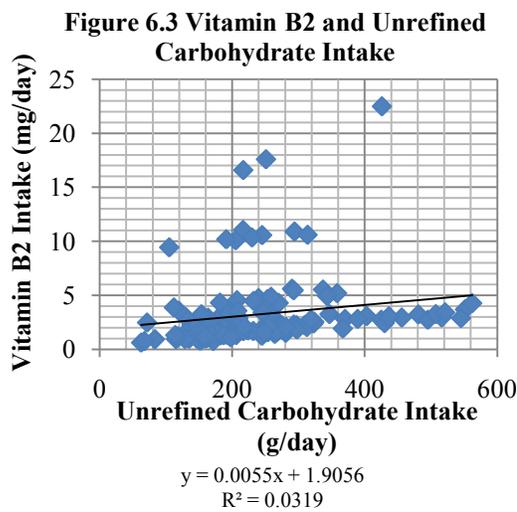
Aside from carbohydrate and protein consumption, overall fat intake has also traditionally represented a variable of interest for researchers examining breast cancer

incidence since saturated and trans-fatty acids have been historically implicated in the etiology of mammary carcinogenesis. Fat intake specifically contributes to breast cancer etiology by increasing plasma estrogen levels through the aromatase conversion of dietary lipids into estrogen (Michels et al 2007; Gonzalez and Riboli 2006). Saturated and trans-fatty acids are particularly implicated in mammary carcinogenesis on the basis of epidemiological studies, which show a 19% increased risk of cancer at the highest levels of intake (Key et al 2004).

Among the subjects recruited for this study, the controls possessed lower overall levels of total fat intake (42g/day) when compared to the cases (55g/day) (Table 6.1). When examining dietary lipid intake in terms of saturated versus unsaturated fats, the two populations also exhibited several key differences, with cases possessing a higher intake of saturated fats and a lower intake of unsaturated fats when compared to the controls. However, these differences in fat intake did not significantly alter the risk patterns for breast cancer incidence in this population. Overall levels of saturated and unsaturated fat intake were not significantly correlated with either measures of body composition or socioeconomic status using regression analysis.

General levels of vitamin intake also differed between the cases and controls, with the healthy Thai subjects having higher levels of antioxidant and B-complex vitamins when compared to the breast cancer patients. Dietary antioxidants are believed to protect against mammary carcinogenesis by neutralizing free radical species formed through the body's natural metabolic processes, but to date epidemiological studies have found only weak evidence for a protective effect associated with high levels of vitamin intake. Consistent with previous epidemiological research, odds ratio analysis of vitamin intake

for the Thai population used in this study suggests that dietary antioxidant intake is associated with a moderate reduction in breast cancer risk (Table 6.2). However, only thiamine exhibited a statistically significant reduction in breast cancer risk at the highest quintile of intake (OR = 0.55,  $p = 0.00004$ ). The intake of thiamine also possesses a weak positive association with the consumption of unrefined carbohydrates based on regression analysis ( $y = 0.0055x + 1.9056$ ,  $r^2 = 0.0319$ ) (Figure 6.3).



Interestingly, while the other vitamins examined as part of this study only had a moderate impact on breast cancer risk, their intake possessed a much stronger correlation with complex carbohydrate intake. High levels of vitamins A and E, for example, possessed a strong positive correlation with increases in the consumption of unrefined carbohydrates ( $y = 37.53x - 901.48, r^2 = 0.5501$ ;  $y = 0.0464x + 1.027, r^2 = 0.6455$ ) (Figures 6.4 and 6.5). This suggests that diets containing higher amounts of fruits and vegetables provide the richest source of micronutrients necessary for optimal human health. While these vitamins individually did not account for a significant modification in breast cancer risk, with the exception of thiamine, their synergistic activity may account for the statistically relevant reduction in cancer risk associated with the incorporation of high levels of complex carbohydrates into the traditional Thai diet.

For instance, the traditional medicinal plants incorporated into the traditional Thai diet derive a majority of their bioactive properties from their high micronutrient content. As will be discussed in the following chapter, which analyzes the role of specific medicinal plants in reducing breast cancer risk, Thai fruits, vegetables, herbs and spices consumed as a regular part of the diet contain high levels of phytochemicals and vitamins with strong anticancer properties. Because unrefined carbohydrates represented the only general dietary component with a significant impact upon breast cancer risk, the specific action of this macronutrient may be derived from the combined activity of the phytochemical constituents from these various Thai medicinal plants.

## **Chapter 7**

### **Data Analysis: Thai Traditional Medicinal Plant Intake**

#### Introduction

This chapter will analyze the role of specific Thai traditional medicinal plants regularly consumed as part of the Thai diet upon breast cancer risk using odds ratio analysis based on quintile of intake. The first quintile is associated with the lowest level of intake for a specific plant species and is utilized as the baseline value from which each successive quintile of increasing intake is evaluated for its impact upon breast cancer incidence. Odds ratios are interpreted based on whether the exposure exerts a protective effect upon disease expression (values less than 1.00), or increases the risk of a specific condition (values greater than 1.00). Although additional specific food items were analyzed in this chapter for their effect upon breast cancer risk, the foods which reduced the risk of mammary carcinogenesis consisted largely of plant species utilized within Thai traditional medicine.

#### An Introduction to Thai Medicinal Plants

Traditional Thai medicine utilizes nearly 500 different plant species with bioactive properties capable of reducing symptoms related to a variety of chronic conditions, including high blood pressure, diabetes mellitus and hypercholesterolemia (Kusamran et al 1998b; Tepsuwan et al 1999; Saralamp et al 1996). Out of this diverse complement of plants, almost 150 species are also utilized within the traditional Thai diet, and include a variety of fruits, vegetables, herbs and spices with strong natural anti-

cancer properties derived from their plant secondary metabolite content (Hutadilok-Towatana 2006; Saralamp et al 1996). The use of these plant species as both medicines and foods dates back nearly 900 years to the Dvaravati Period, with the introduction of Buddhism to Thailand via travelling monk-scholars and merchants from India and Sri Lanka (Saralamp et al 1996).

According to stone tablets from the Khon Empire in the 12<sup>th</sup> century AD, which provide some of the earliest evidence for the establishment of a Thai traditional medicinal epistemology, during a four year period from 1182-1186AD, King Chaiworaman VII made merit by mandating the establishment of over 100 hospitals and the cultivation of a medicinal plant garden for the benefit of his subjects (Saralamp et al 1996). The establishment of this ethnobotanical garden coincided with the incorporation of Buddhism into one of the earliest theocratic kingdoms in Thai history, and reflects the importance of travelling scholars and merchants in the propagation of Ayurvedic and Chinese medical modalities into mainland Southeast Asia (Saralamp et al 1996). For nearly six hundred years, subsequent Thai monarchs of the Sukhothai and Ayuthaya kingdoms built upon this foundation of ethnobotanical knowledge, which culminated with the renovation of Wat Pho in the late 1780s (Saralamp et al 1996).

Located in the heart of Bangkok, Wat Pho represents Thailand's major repository for traditional Thai medical texts and medicinal formulas, and is also the location for the kingdom's most extensive medicinal botanical garden (Saralamp et al 1996). One of the main functions of Wat Pho, even into the 21<sup>st</sup> century, is to catalog the plant species and medicinal recipes used in traditional Thai medicine, as well as to educate individuals in the techniques and methodologies integral to the practice of Thai homeopathy (Saralamp

et al 1996). Because of the importance of preserving traditional Thai medicinal knowledge in the face of strong Westernizing influences in the fields of science and public health, Wat Pho essentially represented the first open university in Thailand, and continues to function as a respected institution for the dissemination of Thai traditional medicine (Saralamp et al 1996).

Within the past ten years, researchers from the Faculty of Nutrition at Mahidol University have studied the plant species at Wat Pho from a Western biomedical perspective in order to learn more about the micronutrient content and bioactive properties of these ethnobotanicals. This body of research has culminated in the production of food composition tables which provide both the macro- and micronutrient content of the specific plant species examined in this study, as well as a series of journal articles highlighting the main bioactive properties found in Thai medicinal plants which contribute to cancer prevention (Kusamran et al 1998b; Maisuthisakul et al 2007; Manosroi 2007; Laohavechvanich et al 2006).

In traditional Thai medicine, various parts of plant species are utilized within powders, tinctures, salves and tonics in order to treat a variety of conditions. Some of the specific plant foods used in Thailand to treat cancer include the rhizomes of ginger, krachai, cumin, galangal and turmeric, which are ground into powders and mixed with an alcohol or water base for ingestion or direct application to tumorous growths (Lee and Houghton 2005). These plant species were included in the FFQ used for this study in order to determine whether the intake of these spices contributed to a reduction in breast cancer risk. Additional Thai medicinal plant foods incorporated into the FFQ include fruits such as mangosteen and krathon, and vegetables like bitter melon, ivy gourd and

smooth loofah, which are primarily used as antifungal and antihyperglycemic agents (Murakami et al 1995; Jiwajinda et al 2002). However, these Thai fruits and vegetables also contain high levels of phytochemicals with the potential to inhibit carcinogenesis according to recent studies conducted on these plant species. The specific anticarcinogenic mechanisms found in Thai medicinal plant foods appears to be directly related their plant secondary metabolite content, which has the capacity to serve an anti-initiators and anti-promoters of cancer activity (Table 7.1).

**Table 7.1 Anticarcinogenic Activity of Common Thai Medicinal Plants.**

Common Name	Scientific Name	Uses in Thai Traditional Medicine	Method of Anti-Cancer Activity
<b>Fruits:</b>			
<b>Mangosteen</b>	<i>Garcinia mangostana</i>	Antifungal; Antibacterial	Anti-promotion; Flavones
<b>Mango</b>	<i>Mangifera indica</i>	Antibacterial	Anti-initiation; FRSA
<b>Krathon</b>	<i>Sandoricum koetjape</i>	Anti-inflammatory	Anti-promotion; Flavones
<b>Vegetables:</b>			
<b>Bitter Melon</b>	<i>Momordica charantia</i>	Antihyperglycemic	Anti-initiation; GST
<b>Ivy Gourd</b>	<i>Coccinia indica</i>	Antihyperglycemic	Anti-initiation; GST
<b>Smooth Loofah</b>	<i>Luffa cylindrica</i>	Antihyperglycemic	Anti-initiation; FRSA
<b>Angled Loofah</b>	<i>Luffa acutangula</i>	Antihyperglycemic	Anti-initiation; FRSA
<b>Herbs and Spices:</b>			
<b>Turmeric</b>	<i>Curcuma longa</i>	Relieve Stomach Ache	Anti-promotion; Flavones
<b>Cumin</b>	<i>Cuminum cyminum</i>	Carminative; Antimicrobial	Anti-promotion; Flavones
<b>Galangal</b>	<i>Kaempferia galangal</i>	Carminative, Relieve Stomach Ache	Anti-promotion; Flavones
<b>Ginger</b>	<i>Zingiber officinale</i>	Carminative, Relieve Stomach Ache	Anti-promotion; Flavones
<b>Holy Basil</b>	<i>Occimum sanctum</i>	Expectorant, carminative	Anti-promotion; Flavones
<b>Lemongrass</b>	<i>Cymbopogon citrates</i>	Antifungal; Antibacterial	Anti-initiation; GST
<b>Krachai</b>	<i>Boesenbergia pandurata</i>	Antihypertensive	Anti-promotion; Flavones

### A Discussion of the Adaptive Mechanisms found in Thai Medicinal Plants and Their Role in Cancer Prevention

Oncologists have identified three distinct stages in the etiology of cancer development: (1) initiation; (2) promotion; and (3) progression. Cancer initiation occurs

when viral, chemical or other mutagenic agents produce irreversible mutations to cell DNA which affect the function of genes involved in cell differentiation, such as suppressor genes, proto-oncogenes, or transcription factors (Murillo and Mehta 2001). Alterations in the function of genes associated with cell differentiation produce dysplastic cells in which the nuclear and cell membranes are visibly altered when compared to healthy cells. The promotion step of cancer development occurs when an initiated cell is transformed into a neoplastic cell, which possesses a higher degree of susceptibility to endogenous and exogenous factors such as the activation of proto-oncogenes or the inactivation of suppressor genes (Murillo and Mehta 2001). During the promotion stage, neoplastic cells usually undergo uncontrolled differentiation due to the loss of normal cell growth control mechanisms and resistance to host immune responses (Murillo and Mehta 2001). With increases in the accumulation of cellular genetic abnormalities, malignant cells become increasingly invasive and metastatic, leading to the final stage of carcinogenesis known as progression.

Chemopreventive agents derived from food sources prevent carcinogenesis via two main pathways: (1) anti-initiation, in which blocking agents found in foods prevent the initiation of dysplastic cell development; or (2) anti-promotion, in which phytochemicals suppress abnormal and uncontrolled cell differentiation in neoplastic cells (Murillo and Mehta 2001). Common blocking agents found in foods include indoles, isothiocyanates and dithiolethiones found in cruciferous vegetable; terpenes in citrus fruits; and organosulfurs from garlic; while common suppressor agents include retinols, vitamin D analogs, monoterpenes and selenium (Murillo and Mehta 2001).

The traditional Thai diet is unique in that it incorporates fruits, vegetables, spices and herbs which contain a combination of both blocking and suppressing agents, and which form an important component of traditional Thai medicine. These Thai medicinal plant foods derive their anticancer bioactivity from plant secondary metabolites (PSM), or phytochemicals, a diverse group of natural products used primarily for plant defense and protection from pathogens, parasites and predators (Acamovic and Brooker 2005; Liu 2004). While PSMs are not essential for plant tissue synthesis or energy production and storage, their manufacture is essential to the viability of the plant, due to their ability to protect the plant from xenobiotic pathogens and environmental pollutants (Lampe 2003; Acamovic and Brooker 2005). Over the course of millions of years, plants have evolved the capacity to synthesize diverse chemicals with a variety of functions, including: (1) defense against grazing herbivores and insects; (2) defense against micro-organisms, including bacteria, fungi and viruses; (3) defense against other plants competing for nutrients and light; and (4) protection against the damaging effects of UV radiation (Lampe 2003; Acamovic and Brooker 2005).

The major classes of phytochemicals found in plants with health benefits for humans can be broadly classified as carotenoids, phenolics, alkaloids, and organosulfur compounds (Liu 2004). Each of these plant secondary metabolites possesses a specific function within plants. For example, carotenoids represent nature's most widespread pigment, with strong provitamin and antioxidant properties which play an important role in photosynthesis and plant tissue photoprotection (Liu 2004; Dillard and German 2000). In a similar capacity, phenolic compounds aid in plant reproduction, act as defense mechanisms against pathogens and predators, and provide color to plants (Liu 2004).

Alkaloids, the third type of PSM, function as phytoalexins- glycoside substances which render plants unpalatable and thereby reduce their intake by animals (Lampe 2003).

Finally, organosulfur compounds such as glucosinolates aid plants by functioning either as repellents of harmful organisms or as attractants for beneficial insect or animal species necessary to propagate pollen (Dillard and German 2000).

Just as plant secondary metabolites enhance the viability of diverse plant species, PSMs also benefit human health through their capacity to function within the body in a manner similar to their method of bioactivity in plants. Humans have specifically developed the ability to utilize these compounds for hormonal and growth regulatory functions, with the presence of these molecules in mammalian tissues providing a measure of protection from certain diseases, especially those related to chronic damage and growth dysregulation (Dillard and German 2000; Liu 2004).

Indigenous cultures from Asia have long recognized the health benefits derived from PSMs, and this knowledge of utilizing specific plant parts with high concentrations of plant secondary metabolites have formed the basis of traditional ethnopharmacology in Asia (Acamovic and Brooker 2005; Dillard and German 2000). In Thailand, this understanding of the relationship between human health and the use of plant foods containing high levels of PSMs has contributed to the development of traditional Thai ethnobotany, which consists of hundreds of different fruit and vegetable species.

For the purposes of this study, however, the FFQ included only those Thai pharmaceutical plants which are commonly consumed as part of the traditional Thai diet and which are readily available in Thai markets, as determined by an extensive literature review. Some of the common foods which overlap in their function as foods and as

medicines include fruits such as mangosteen, mango, krathon and coconut, vegetables like bitter melon, ivy gourd and angled loofah, and spices such as ginger, galangal, turmeric, cumin and holy basil. An analysis of these specific medicinal plant foods, in addition to other commonly consumed foods, via data from the food frequency questionnaire highlights four main classes of phytochemicals which contribute to both the anti-initiation and anti-promotion of breast cancer, including glucosinolates, free radical scavenging activity of antioxidants, flavonoids, and phytoestrogens. Each of these plant foods will be discussed below in terms of the main bioactive functions of their phytochemical content which contribute to the chemoprevention of breast carcinogenesis.

**Table 7.2 Phytochemical Content and Method of Cancer Prevention in Thai Food Sources.**

<b>Phytochemical</b>	<b>Thai Food Sources</b>	<b>Method of Cancer Prevention</b>
<b>Glucosinolate</b>	Lemongrass, Bitter melon Ivy gourd, Chinese mustard cabbage	Induction of Phase II detoxification enzymes
<b>Antioxidants</b>	Tomatoes, Mango, Angled Loofah, Smooth Loofah	Free radical scavenging activity; Inhibition of DNA mutagenesis
<b>Flavones</b>	Holy basil, Santol, Mangosteen, Cumin, Turmeric, Galangal, Krachai, Ginger	Inhibition of cell proliferation; Induction of cell cycle arrest and apoptosis; Enhances immune function
<b>Phytoestrogens</b>	Soy	Alters estrogen metabolism

### Glucosinolates and Breast Cancer Risk

Among the subjects recruited for this study, the controls consumed higher overall levels of Asian foods containing glucosinolates- cruciferous vegetables, lemongrass, bitter melon and ivy gourd- than the cases as measured by both frequency of consumption and quantity of intake by grams per day (Table 7.3). For each of these food items, as measured by odds ratio per quintile of consumption, increases in intake were associated

with a reduced risk of breast cancer. For the two cruciferous vegetables measured in this study- pickled mustard cabbage and Chinese mustard cabbage, the risk of breast cancer was reduced by 50% and 82%, respectively (OR = 0.50,  $p = 0.0000001$ ; OR = 0.18,  $p = 0.0000001$ ). Among members of the Cucurbitaceae family- ivy gourd and bitter melon- consumption of both plants in the highest quintile was likewise associated with a respective 90% and 97% reduction in breast cancer risk (OR = 0.10,  $p = 0.0000001$ ; OR = 0.03,  $p = 0.0000001$ ). Finally, the consumption of lemongrass in the highest quartile resulted in a 72% reduction in the risk of breast cancer (OR = 0.28,  $p = 0.0000001$ ) (Table 7.4).

Because of the high glucosinolate content of cruciferous vegetables, ivy gourd, bitter melon, and lemongrass, consumption of these foods potentially reduces breast cancer risk in Thai women through the activation of the phase II detoxification system. In animal and microbiological studies in which these foods have been administered to *in vivo* tumors and cancer cell cultures, these vegetables have shown the ability to initiate and enhance the activity of the phase II enzyme system via their glucosinolate content. Moreover, in models which have specifically tested the effects of ivy gourd and bitter melon consumption upon GST activity, both vegetables increased phase II reactions by approximately 63% compared to basal levels for indirect-acting mutagens at relatively low dose rates (Kusamran et al 1998; Kusamran et al 1998b). Similarly, administration of lemongrass extracts to rodents treated with carcinogenic toxins demonstrated a 1.5 fold increase in GST and phase II enzyme activity (Nakamura et al 2003). The results from this case-control study confirm these findings, suggesting that the main bioactive role of

these plant foods in reducing breast cancer risk among Thai women is due in large part to their activation of the phase II enzyme detoxification system.

**Table 7.3 Intake of Selected Animal Products, Fruits and Vegetables among Cases and Controls.**

Item (weight g/day)	Cases Mean $\pm$ SD (weight g/day)	Controls Mean $\pm$ SD
<b>Animal Products (Total)</b>	165.32 $\pm$ 79.04	258.78 $\pm$ 142.22
<b>Seafood</b>	104.44 $\pm$ 72.46	173.72 $\pm$ 135.54
<b>Poultry</b>	17.05 $\pm$ 15.3	29.35 $\pm$ 22.75
<b>Red Meat</b>	43.84 $\pm$ 27.09	55.71 $\pm$ 25.62
<b>Vegetables (Total)</b>	390.98 $\pm$ 177.55	549.08 $\pm$ 256.69
<b>Cruciferous</b>	83.93 $\pm$ 58.10	138.66 $\pm$ 75.71
<b>Chinese broccoli</b>	21.51 $\pm$ 17.55	39.11 $\pm$ 26.70
<b>Chinese water spinach</b>	12.08 $\pm$ 8.42	9.46 $\pm$ 8.53
<b>Chinese mustard cabbage</b>	42.22 $\pm$ 41.8	80.5 $\pm$ 48.96
<b>Pickled mustard</b>	8.11 $\pm$ 12.53	9.59 $\pm$ 12.20
<b>Dark yellow</b>	45.31 $\pm$ 43.52	39.73 $\pm$ 38.00
<b>Legumes</b>	38.07 $\pm$ 25.23	56.69 $\pm$ 38.74
<b>Bitter melon</b>	11.92 $\pm$ 12.51	14.67 $\pm$ 15.63
<b>TumLeung</b>	19.06 $\pm$ 16.56	24.62 $\pm$ 18.50
<b>Thai chili peppers</b>	29.29 $\pm$ 13.69	29.62 $\pm$ 14.60
<b>Smooth Loofah</b>	5.18 $\pm$ 8.09	14.10 $\pm$ 16.52
<b>Angled Loofah</b>	10.35 $\pm$ 12.16	17.64 $\pm$ 18.10
<b>Tomato</b>	18.68 $\pm$ 18.82	20.46 $\pm$ 18.14
<b>Others</b>	129.22 $\pm$ 66.91	190.90 $\pm$ 112.76
<b>Fruits (Total)</b>	336.24 $\pm$ 193.60	551.34 $\pm$ 308.64
<b>Pomegranate</b>	3.73 $\pm$ 9.37	3.85 $\pm$ 6.92
<b>Mangosteen</b>	18.29 $\pm$ 23.10	27.45 $\pm$ 26.06
<b>Krathon</b>	5.70 $\pm$ 10.49	8.67 $\pm$ 12.30
<b>Mango</b>	26.66 $\pm$ 27.52	41.20 $\pm$ 32.93
<b>Coconut</b>	8.59 $\pm$ 10.08	12.86 $\pm$ 12.98
<b>Citrus</b>	68.13 $\pm$ 51.92	92.34 $\pm$ 52.87
<b>Others</b>	205.146 $\pm$ 112.03	364.95 $\pm$ 215.02
<b>Spices/Herbs (Total)</b>	16.87 $\pm$ 8.55	23.68 $\pm$ 14.15
<b>Galangal</b>	1.49 $\pm$ 1.47	1.47 $\pm$ 1.60
<b>Ginger</b>	1.88 $\pm$ 2.03	3.14 $\pm$ 3.12
<b>Turmeric</b>	0.38 $\pm$ 0.77	0.45 $\pm$ 0.75
<b>Krachai</b>	0.84 $\pm$ 0.98	0.85 $\pm$ 1.09
<b>Sacred Basil</b>	1.69 $\pm$ 1.32	2.32 $\pm$ 1.67
<b>Sweet Basil</b>	1.51 $\pm$ 1.38	1.85 $\pm$ 1.65
<b>Cumin</b>	0.19 $\pm$ 0.47	0.31 $\pm$ 0.63
<b>Lemon Grass</b>	3.78 $\pm$ 2.95	4.39 $\pm$ 3.73
<b>Others</b>	5.12 $\pm$ 2.15	6.24 $\pm$ 2.33
<b>Soy Products (Total)</b>	117.02 $\pm$ 105.29	126.16 $\pm$ 102.77

**Table 7.4 Age-Adjusted Odds Ratios for the Association of Breast Cancer Risk with the Intake Level of Selected Food Groups.**

	Q1(low)	Q2	Q3	Q4	Q5 (high)	<i>p</i> -value
<b>Animal protein (Total)</b>	1.00	0.97	0.87	0.97	0.95	0.6
<b>Seafood</b>	1.00	1.03	1.09	0.89	0.95	0.1
<b>Poultry</b>	1.00	0.21	0.90	0.82	0.71	0.8
<b>Red Meat</b>	1.00	7.94	1.20	1.37	1.44	0.6
<b>Vegetables (Total)</b>	1.00	0.85	0.95	0.96	0.97	0.8
<b>Cruciferous</b>	1.00	0.96	1.09	0.93	0.95	0.45
<b>Pickled mustard cabbage</b>	1.00	0.54	0.27	0.55	0.50	<0.0000
<b>Chinese mustard cabbage</b>	1.00	0.42	0.28	0.08	0.18	<0.0000
<b>Legumes</b>	1.00	0.96	1.02	0.94	0.95	0.56
<b>Bitter Melon</b>	1.00	0.39	0.27	0.08	0.03	<0.0000
<b>TumLeung, Ivy Gourd</b>	1.00	0.42	0.65	0.44	0.10	<0.0000
<b>Thai chili peppers</b>	1.00	0.50	2.26	1.12	1.00	0.57
<b>Smooth Loofah</b>	1.00	0.82	0.86	0.76	0.53	<0.0000
<b>Angled Loofah</b>	1.00	0.37	0.35	0.10	0.17	<0.0000
<b>Tomato</b>	1.00	0.61	0.33	0.81	0.43	<0.0000
<b>Others</b>	1.00	0.92	0.96	0.83	0.93	0.15
<b>Fruits (Total)</b>	1.00	1.18	1.04	1.09	1.05	0.89
<b>Mangosteen</b>	1.00	0.13	0.59	0.15	0.30	<0.0000
<b>Krathon/Santol</b>	1.00	0.94	0.47	0.25	0.36	<0.0000
<b>Mango</b>	1.00	0.37	0.52	0.22	0.15	<0.0000
<b>Coconut</b>	1.00	1.00	0.25	0.67	0.20	<0.0000
<b>Others</b>	1.00	0.92	1.03	0.95	1.00	0.87
<b>Spices/Herbs (Total)</b>	1.00	1.06	1.10	1.06	1.07	0.43
<b>Galangal</b>	1.00	0.71	1.34	0.64	0.59	<0.0000
<b>Ginger</b>	1.00	0.53	0.72	0.11	0.11	<0.0000
<b>Turmeric</b>	1.00	0.06	0.19	0.14	0.10	<0.0000
<b>Sacred Basil</b>	1.00	0.69	1.14	0.51	0.14	0.02
<b>Krachai</b>	1.00	1.11	0.91	1.04	0.71	0.006
<b>Sweet Basil</b>	1.00	1.15	1.64	0.67	0.34	0.26
<b>Cumin</b>	1.00	2.65	0.39	0.71	0.19	<0.0000
<b>Lemon Grass</b>	1.00	0.79	1.02	0.54	0.28	<0.0000
<b>Others</b>	1.00	0.82	1.18	0.82	1.27	0.06
<b>Soy Products (Total)</b>	1.00	0.81	0.48	0.74	0.67	0.00002

## Discussion

Glucosinolates represent a class of plant secondary metabolites which possess strong anticarcinogenic and cancer-blocking activity when metabolized via enzymatic hydrolysis into biologically active isothiocyanates and indoles (Higdon et al 2007).

Cruciferous vegetables constitute an especially important source of glucosinolates, and

include many commonly consumed vegetables from the *Brassica* genus such as broccoli, Brussels sprouts, cabbage, cauliflower, collard greens, kohlrabi, bok choy and Chinese cabbage (Higdon et al 2007). In addition to several Asian species of these cruciferous vegetables regularly consumed as part of the Thai diet, traditional Thai cuisine also incorporates several additional plant species which contain high amounts of glucosinolates, including lemongrass, bitter melon and ivy gourd. As an essential component of cruciferous vegetables, lemongrass, bitter melon and ivy gourd, glucosinolates provide these plants with their distinctively pungent aroma and bitter flavor due to a high content of sulfur-containing compounds (Higdon et al 2007; Greenwald et al 2001).

Upon exposure to gastrointestinal microflora, glucosinolates are converted to isothiocyanates and indole-3 carbinol, compounds which possess strong anticancer properties. One important mechanism resulting from the ingestion of glucosinolates is the upregulation of cytochrome P450 (CYP) 1A2, which aids in both cell-mediated death via apoptosis and in the shift of estradiol metabolism to favor the 2-OHE pathway (Greenwald et al 2001). As previously discussed above in context of the metabolic syndrome, endogenous estradiol can be metabolized in the human body via two main pathways- the 2-OHE and 16 $\alpha$ -OHE pathways. While the 16 $\alpha$ -OHE pathway enhances the proliferation of estrogen sensitive breast cancer cells, the 2-OHE product reduces breast cancer risk by decreasing the sensitivity of mammary stem cells to estrogen. In numerous studies evaluating the intake of glucosinolate-containing vegetables with regards to breast cancer risk, researchers have consistently found that a high intake of

these foods can shift estrogen metabolism towards the 2-OHE pathway (Higdon et al 2007; Lee et al 2008; Murillo and Mehta 2001).

In addition to catalyzing apoptosis and the metabolism of estrogen via the 2-OHE pathway, glucosinolates, when converted to isothiocyanates, specifically upregulate the detoxification of oxidative damaging genotoxic and carcinogenic chemicals by the induction of phase II enzymes (Lee et al 2008). In humans, when potentially toxic chemicals and compounds are either ingested or absorbed into the body, these products can be eliminated from the body through one of two pathways- the phase I or phase II enzyme systems. The phase I enzyme system carries potential toxins to the liver, where they are metabolized in a manner favoring carcinogenesis via the conversion of toxins into cancer-promoting compounds. These compounds produced by phase I enzymes remain in the liver for a short period of time before being released into the body's general circulation. Due to the sequestration of these cancer-promoting compounds within the body, the byproducts of phase I enzymes come into close contact with a number of important organs and stem cell compartments within the human body, where they have the potential to initiate and promote carcinogenesis via mutagenesis.

By contrast, the phase II enzyme system, when activated by isothiocyanates, blocks tumorigenesis by the induction of glutathione s-transferases (GSTs). GSTs are part of a family of soluble proteins which conjugate xenobiotics with glutathione; after glutathionylation, metabolites become more hydrophilic, and therefore biologically inactive (Lee et al 2008; Nakamura et al 2003). Unlike the products of the phase I enzyme system, which remain sequestered in the body, the metabolites neutralized by GSTs are readily excreted in bile or in urine as biologically inactive conjugates

(Nakamura et al 2003). In humans, the susceptibility to carcinogenesis is mediated by the balance between the phase I and phase II enzyme systems. When the phase I system is favored during detoxification, there is an increased risk of cancer initiation; by contrast, the predominance of the phase II system results in a reduced risk of carcinogenesis (Kusamran et al 1998).

Asian cruciferous vegetables, lemongrass, bitter melon and ivy gourd represent integral components of the traditional Thai diet which contain high concentrations of glucosinolates. In numerous studies evaluating the impact of these specific foods upon cancer risk, researchers have demonstrated the strong cancer-preventive role for these plant species. Bitter melon and ivy gourd, for example, aside from their nutritional importance in the Thai diet, have been regularly used in Thai traditional medicine as an antipoison and antipyretic agent (Nagasawa et al 2004; Grover and Yadav 2004; Kusamran et al 1998). In much the same manner, lemongrass, while representing an essential herb in Thai cuisine, has also been utilized as an anti-bacterial and anti-fungal agent in traditional medicine (Nakamura et al 2003).

Each of these vegetables, when examined from an epidemiological perspective in previous studies, has been associated with a significantly reduced risk of cancer. As suggested by both the results from this study and from previous epidemiological research, these plant species derive their main anticarcinogenic properties due in large part to their high glucosinolate content, which upregulates phase II detoxification enzymes in the body. The induction of phase II detoxification enzymes through the ingestion of Thai vegetables containing glucosinolates aids in the removal of potential xenobiotic and endogenous carcinogens which contribute to breast carcinogenesis.

### Antioxidants and Breast Cancer Risk

In this study, four specific plant species with high levels of free radical scavenging activity were examined for their effects upon breast cancer risk- tomatoes, mango, angular loofah and smooth loofah. Within the sample population recruited for this study, the controls possessed a higher overall intake of these plant species when compared to the cases, as measured by grams of intake per day and frequency of intake (Table 7.3). The higher intake of each plant species was associated with a reduced risk for breast cancer, as determined by odds ratio analysis per quintile of consumption. Smooth loofah and tomatoes were associated with only a moderate reduction in breast cancer risk, at 47% and 57 % respectively (OR = 0.53,  $p = 0.0000001$ ; OR = 0.43,  $p = 0.0000001$ ). By far, the most powerful effect upon breast cancer incidence was exerted by the consumption of mangoes and angular loofah, which at the highest rate of intake reduced cancer risk by 85% and 83% respectively (OR = 0.15,  $p = 0.0000001$ ; OR = 0.17,  $p = 0.0000001$ ) (Table 7.4).

Each of these four plant foods derives their main anti-cancer activity from high concentrations of antioxidant compounds. In particular, both tomatoes and mango represent rich sources of carotenoids- pro-vitamin A compounds which inhibit oxidative damage to lipid molecules. Angular and smooth loofah, on the otherhand, contain high levels of vitamins C and E, which work to reduce oxidative damage in both hydrophilic and hydrophobic environments. In studies of the free radical scavenging activity of each of these four plant species, research has suggested that tomatoes, mangos and loofah species possess especially high capacities to reverse oxidative damage brought about by the body's regular metabolic processes.

## Discussion

Oxidative stress produced by reactive oxygen species induces damage to critical cellular biomolecules such as lipids, proteins and DNA, and therefore has been implicated in the etiology of various human diseases, including cancer (Agarwal et al 2000; Rao and Agarwal 2000). Reactive oxygen species (ROS) are created endogenously via regular metabolic activity, lifestyle factors and dietary choices, and possess both beneficial and harmful effects in living organisms (Liu 2004). Beneficial effects of ROS include cellular defense against infectious agents and enhancements in the function of cellular signaling systems at low concentrations (Valko et al 2006). However, at high concentrations, ROS contribute to carcinogenesis by producing base mutations, single- and double-strand breaks, DNA cross-linking, and chromosomal breakage and rearrangement (Liu 2004). Because oxidative damage due to ROS accumulates throughout the lifecycle, ROS play a key role in the development of age-dependent diseases such as cancer, atherosclerosis, and arthritis (Valko et al 2006). ROS are derived from free radicals, which are defined as molecules containing one or more unpaired electrons; the presence of unpaired electrons accounts for the high degree of ROS reactivity in biological organisms (Valko et al 2006).

In order to neutralize the effects of oxidative stress, antioxidants derived from plant phytochemicals need to be consumed in sufficient quantities to significantly delay and/or prevent damage to critical biomolecular components within the human body. The most common sources of antioxidants are fruits and vegetables, which contain thousands of phytochemicals, or bioactive, non-nutrient plant compounds with complementary and overlapping mechanisms of action (Liu 2004; Agarwal et al 2000). Approximately 5000

individual phytochemicals have been identified to date in fruits, vegetables and grains, with a larger percentage still unidentified (Liu 2004). In humans, the most effective non-enzymatic antioxidants include Vitamin C, Vitamin E and  $\beta$ -carotene, which represent the only three nutrients capable of directly scavenging free radicals (Valko et al 2006; Machlin and Bendich 1987). While some antioxidants react in hydrophilic environments- such as Vitamin C, others only act in hydrophobic phases- Vitamin E for example (Valko et al 2006; Machlin and Bendich 1987).

Vitamin E, also known as  $\alpha$ -tocopherol, represents the most important lipid-soluble antioxidant found in all living organisms. By reacting directly with a variety of oxy radicals present in lipid molecules, including the peroxy radical (ROO) and the superoxide radical (Or), Vitamin E protects against lipid peroxidation, an important source of oxidative damage in overweight and obese individuals (Machlin and Bendich 1987). In addition to directly reducing lipid peroxidation, Vitamin E also exerts  $\beta$ -carotene sparing action by preventing the oxidation of the conjugated double bonds of  $\beta$ -carotene (Machlin and Bendich 1987). Because the oxidation of these double bonds effectively eliminates the free radical scavenging ability of  $\beta$ -carotene, Vitamin E acts synergistically with  $\beta$ -carotene in order to protect against lipid peroxidation (Machlin and Bendich 1987). In addition to its capacity to work synergistically with other antioxidants in the prevention of oxidative damage to lipids, Vitamin E also has been demonstrated *in vivo* to induce the activity of p21waf1/cip1, a powerful cell cycle inhibitor which triggers apoptosis in cancer cells (Machlin and Bendich 1987).

Ascorbic acid, or Vitamin C, represents the most significant water-soluble antioxidant, which possesses the capability to scavenge a variety of free radicals,

including superoxide, hydroxyl radicals, and singlet oxygen species (Machlin and Bendich 1987; Valko et al 2006). Like Vitamin E, ascorbic acid has the ability to work synergistically with other antioxidants, such as  $\alpha$ -tocopherol and  $\beta$ -carotene. For example, when Vitamin E neutralizes a free radical molecule, it is converted to an  $\alpha$ -tocopherol radical which lacks the ability to further neutralize oxidative damage; Vitamin C cooperates with Vitamin E by regenerating the useful  $\alpha$ -tocopherol molecule after it has been depleted by its reaction with free radicals (Machlin and Bendich 1987; Valko et al 2006). Although Vitamin C in isolation prevents free radical damage in aqueous environments, when working synergistically with  $\alpha$ -tocopherol, ascorbic acid can increase resistance to lipid peroxidation in a dose-dependent manner (Machlin and Bendich 1987; Valko et al 2006). In a variety of *in vivo* studies, administration of Vitamin C supplements results in a marked reduction in the markers of oxidative DNA, lipid and protein damage, due in large part to its ability to partner with other antioxidants (Machlin and Bendich 1987; Valko et al 2006).

$\beta$ -carotene, a pigment found in all plants, constitutes one class of carotenoids—nature's most widespread lipophilic pigment with pro-Vitamin A activity (Garcia-Solis et al 2008; Liu 2004). In general, carotenoids provide numerous cancer-preventive mechanisms, including inhibition of cell proliferation, inhibition of oncogene expression, and modulation of carcinogen metabolism (Greenwald et al 2001).  $\beta$ -carotene, unlike other carotenoids, represents the most efficient quencher of singlet oxygen species in nature, in addition to its ability to function as an antioxidant (Valko et al 2006).

$\beta$ -carotene constitutes the major carotenoid precursor to Vitamin A. Although Vitamin A lacks the ability to scavenge free radicals,  $\beta$ -carotenes have antioxidant

capacities due to the ability of the conjugated double-bonded structure to delocalize unpaired electrons (Valko et al 2006). In high doses, carotenoids can protect lipid molecules from peroxidative damage, and has been demonstrated to inhibit cell cycle progression in numerous cancer cell lines as a result of its capacity to bind and reduce insulin-like growth factor production (Valko et al 2006; Agarwal et al 2000; Machlin and Bendich 1987).

Although each of the plant species examined in this section (tomato, mango, angular and smooth loofah) is high in important phytochemical antioxidants, such as Vitamin C, Vitamin E and  $\beta$ -carotene, when these individual micronutrients were examined for their role in breast cancer protection, no significant effects were found. Mango and tomatoes, for example, possess high amounts of  $\beta$ -carotene and lycopene, two carotenoids which accumulate in breast adipose tissue and which reduce cancer risk by reducing oxidative damage to lipid molecules (Giovannucci 1999; Garcia-Solis et al 2008). Moreover, angular loofah and smooth loofah possess high levels of ascorbic acid and  $\alpha$ -tocopherol, which in a combination of aqueous and hydrophobic environments possess strong free radical scavenging activity (Pedraza- Chaverri et al 2008; Moongkarndi 2004). However, the determination that each food item possesses a strong, significant impact upon reducing breast cancer risk, particularly in the highest quintile of intake, further supports the notion that antioxidants work synergistically, rather than in isolation in order to prevent tumor development (Machlin and Bendich 1987; Liu 2004). Furthermore, the main benefit of eating a diet rich in fruits and vegetables may be attributed to the complex mixture of antioxidants provided by these whole foods, an effect which may account for the inability of previous epidemiological studies to explain

the benefits of individual micronutrients in reducing breast cancer risk (Machlin and Bendich 1987; Liu 2004).

### Flavonoids and Breast Cancer Risk

Many of the traditional medicinal plants examined as part of this study represent species with high levels of flavonoid content, including sacred basil, santol, mangosteen, cumin, turmeric, galangal, krachai and ginger. The majority of these plant species, when examined by odds ratio analysis, provided some of the most potent and consistent inhibitors of breast cancer risk within this sample population.

**Weak Flavonoid Inhibitors.** Among the weak flavonoid inhibitors of breast cancer risk among these plant species, galangal and krachai represent two wild varieties of ginger commonly used in traditional Asian medicine and in spicy Thai curries. In this study, consumption of galangal and ginger at the highest levels of intake reduced breast cancer risk by 41% and 29% respectively (OR = 0.59,  $p = 0.000005$ ; OR = 0.71,  $p = 0.006$ ).

**Intermediate Flavonoid Inhibitors.** Intermediate inhibitors of breast cancer risk include mangosteen and santol, two fruits which have been used in traditional Thai medicine as antibacterial, antifungal, antiviral and antiinflammatory agents, and which have constituted popular fruits consumed as a regular part of the traditional Thai diet (Chaverri et al 2008; Kaneda et al 1992). Controls possessed a higher overall intake of both mangosteen and santol, resulting in a 70% and 64% reduction in breast cancer risk respectively (OR = 0.30,  $p = 0.0000001$ ; OR = 0.36,  $p = 0.0000001$ ).

Mangosteen, in addition to possessing a high flavonoid content, also contains an additional class of plant secondary metabolites known as xanthone, a group of chemical

compounds which contribute to the color and flavor of this fruit (Chaverri et al 2008). One specific xanthone found in mangosteen,  $\alpha$ -mangostin, also possesses strong antioxidant activity, with the capacity to both scavenge free radicals and to induce apoptotic activity in breast cancer cells in a dose-dependent manner (Chaverri et al 2008).

In much the same manner, santol contains a bioactive compound which works synergistically with flavonoids- triterpene. Triterpenes represent one of the largest classes of phytonutrients found in green plants, where they play an essential role in plant metabolism by fixing carbon through photosynthetic reactions using photosensitizing pigments (Dillard and German 2000; Kaneda et al 1992). In humans, consumption of triterpene-containing plants such as santol aids in cancer inhibition by protecting against cancer caused by chronic oxidative cell damage and cell growth dysregulation (Dillard and German 2000).

**Strong Flavonoid Inhibitors.** Flavonoid-containing plants with the strongest and most consistent inhibitors of breast cancer activity in this study include turmeric, ginger and cumin, all closely related species belonging to the Zingiberaceae family, as well as sacred basil, a member of the *Ocimum* genus. These four plant species, in addition to their utility within traditional medicine, are regularly utilized as spices in the traditional Thai diet, and derive their bioactivity from curcumin, a naturally occurring phenolic phytochemical responsible for the yellow coloring found in many Asian curries.

The control sample studied as part of this project consumed turmeric, ginger and cumin in greater quantities than the case population. Intake of these spices was associated with up to a 90% reduction in breast cancer risk at the highest quintile of intake (OR = 0.10,  $p = 0.0000001$ ; OR = 0.11,  $p = 0.0000001$ ; OR = 0.19,  $p =$

0.0000001). This reduction in cancer risk is produced by the capacity of curcumin to act synergistically with flavonoids as free radical scavengers within the body (Choudhuri et al 2002; Duvoix et al 2005). In addition, curcumin inhibits the COX-2 (cyclo-oxygenase 2) and LOX (lipoxygenase) inflammatory molecules, both associated with tumors which proliferate during inflammatory stress (Choudhuri et al 2002; Duvoix et al 2005; Kirana et al 2003). Curcumin likewise enables cells to perform critical repair functions before progressing through the cell cycle (Choudhuri et al 2002; Duvoix et al 2005; Kirana et al 2003).

Sacred basil, like turmeric, ginger and cumin, was also consumed in greater quantities by the control population when compared to the cases, and was associated with an 86% reduction in breast cancer risk (OR = 0.14,  $p = 0.02$ ). In addition to being used as a spice in traditional Thai cooking, sacred basil has also been utilized in traditional Thai medicine as an analgesic and in the treatment of diabetes and infections (Prakash and Gupta 2000). Sacred basil provides high flavonoid content to the traditional Thai diet, thereby enhancing ROS neutralization and inducing GST activity *in vivo* (Geetha et al 2004; Trevisan et al 2006). Aside from their chemopreventive qualities, additional health benefits of sacred basil essential oil include the ability to: (1) serve as a smooth muscle relaxant; (2) promote lower blood pressure; and (3) act as a cardiac depressant during high stress activities (Geetha et al 2004).

### Discussion

Flavonoids represent a class of plant secondary metabolites characterized by a common phenylbenzopyrone structure consisting of one or more hydroxyl groups (Liu 2004; Birt et al 2001; Ren et al 2003). Phenolics play several essential roles in plants,

including the reproduction and growth of plants; acting as defense mechanisms against pathogens, parasites and predators; and contributing to plant color (Liu 2004). Phenolic acids are subdivided into two main groups: hydroxybenzoic acids and hydroxycinnamic acids, and based on their degree of saturation can be further categorized into several flavonoid forms, including flavones, flavanols, isoflavones, flavanones and flavanonols (Ren et al 2003; Liu 2004). Because they are only found in terrestrial plants like fruits and vegetables and cannot be synthesized within mammalian organisms, flavonoids can only be obtained from the intake of foods (Birt et al 2001; Liu 2004). The flavonoids derived from teas, fruits and vegetables reduce carcinogen by blocking initiation through a variety of mechanisms, including the inhibition of cell proliferation, the induction of cell cycle arrest and apoptosis, and the improvement of immune function (Greenwald et al 2001).

Reactive oxygen species promote tumor growth through the activation of endogenous tumor-promoting enzymes such as cyclo-oxygenases (COX), and lipoxygenases (LOX). Flavonoids represent a particularly powerful inhibitor of xanthine oxidase, a form of ROS which is specifically associated with tumor cell proliferation (Ren et al 2003). In plants, flavonoids provide resistance to fungal and bacterial growth through the inhibition of the cell cycle, a characteristic which aids in tumor prevention by reversing or retarding cellular hyper-proliferation and by inducing apoptosis (Birt et al 2001). Flavonoids further prevent tumor growth by inhibiting angiogenesis, or the development of blood vessels necessary for tumor progression. In healthy humans, angiogenesis is strictly regulated by a variety of endogenous angiogenic and angiostatic factors; when the balance between blood vessel growth and destruction encourages the

over-proliferation of blood vessels, tumor growth can occur (Ren et al 2003). Flavonoids interfere with various steps of angiogenesis, such as basement destruction of blood vessels or inhibition of the proliferation and migration of endothelial cells, thus depriving tumors of the nutrients and oxygen necessary for their survival (Ren et al 2003). In the absence of proper vascularization, tumors undergo apoptosis.

Flavonoids also prevent carcinogenesis by inhibiting oxidative damage to lipid molecules. Because ROS consist of free radicals with unpaired electrons, flavonoids reduce oxidative damage by several mechanisms, including: (1) serving as one-donor electrons to free radicals; (2) stabilizing free radicals through hydrogenation; and (3) complexing with oxidizing species (Birt et al 2001). In particular, flavonoids possess significant antioxidizing activity with regards to low-density lipoproteins (LDL) and adipose aromatase activity. Flavonoids constitute strong metal chelators which inhibit both aromatase activity and LDL oxidation (Geetha et al 2004). Because LDL oxidation can produce inflammatory responses responsible for atherosclerosis and tumor formation, while aromatase activity produces estrogen from adipose stores, the metal chelating activity of flavonoids can significantly reduce both the inflammatory responses associated with cytochrome P450 and the production of estrogen from fat stores (Ren et al 2003; Geetha et al 2004). Therefore, flavonoids further reduce the risk of carcinogenesis by inhibiting both the cellular damage and cell proliferation associated with LDL oxidation and aromatase activity.

Similar to the effects of glucosinolates upon the induction of cancer preventing detoxification pathways, flavonoids also act as potent inducers of the phase II enzyme system. When ingested in sufficient quantities, flavonoids inhibit the activity of certain

P450 isozymes associated with phase I metabolism of xenobiotics and xenotoxins, resulting in the preferential detoxification of carcinogens which enter the body via the phase II enzyme system (Ren et al 2003; Birt et al 2001). In addition to blocking the activity of the phase I pathway, flavonoids also work in concert with glucosinolates to enhance the action of glutathione s-transferase, thus enabling carcinogens to be more readily detoxified and eliminated from the body (Ren et al 2003; Birt et al 2001).

Because a high intake of sacred basil, santol, mangosteen, cumin, turmeric, galangal, krachai and ginger is associated with significant reductions in breast cancer risk, the flavonoid content of these plant species represents the most likely mechanism contributing to anticarcinogenesis. In Thai women with a higher consumption of these plant foods, flavonoids reduce mammary carcinogenesis through a variety of methods, including: (1) free radical scavenging activity; (2) enhancing overall immune function; (3) inducing the phase II detoxification pathway; and (4) inhibiting the growth of established cancer cells through apoptosis and antiangiogenesis.

#### Phytoestrogens and Breast Cancer Risk

In this study, the controls consumed approximately 8% more soy products than the cases; however, this modest increase in soy consumption resulted in a 33% reduction in breast cancer risk (OR = 0.67,  $p = 0.00002$ ). In Thailand, soy products are consumed in the form of both beverages and in main dishes or desserts beginning at younger ages than in Western nations, and their intake appears to reduce breast cancer incidence through their ability to reduce overall estrogen levels and to increase the level of mammary tissue maturation. Apart from these estrogenic mechanisms, soy foods and their genistein component have also been demonstrated to inhibit the growth of both

hormone-dependent and hormone-independent breast cancer cells through several mechanisms.

First of all, genistein inhibits the production of tyrosine protein kinase, an enzyme which is over-expressed in cancer cells and which codes for the proteins found in many cancer causing genes (Messina and Loprinza 2001). In the absence of tyrosine protein kinase, regular mechanisms of cell regulation and control can remove cancerous cells via apoptosis, a form of regulated cell death (Messina and Loprinza 2001). In addition, modest consumption of soy products inhibits angiogenesis, or the growth of blood vessels necessary to provide nutrients and oxygenation to tumors (Messina and Loprinza 2001). In much the same manner that genistein protects against mammary cell proliferation during adulthood due to endothelial growth factor (EGF) downregulation, genistein's ability to exert a strong inhibitory response to EGF production also prevents the proliferation of endothelial cells necessary for angiogenesis (Messina and Loprinza 2001). The combination of these two additional effects upon cell regulation and angiogenesis may help to explain the modest decrease in breast cancer incidence among Thai women with higher levels of soy intake.

### Discussion

Phytoestrogens have interested researchers for several decades in terms of their role in breast cancer prevention due to the observation that Asian women tend to have a higher intake of soy products, the main dietary provider of phytoestrogens, in conjunction with significantly lower incidence rates of breast cancer when compared to women from Western nations. Phytoestrogens represent plant secondary metabolites with bioactive properties similar to endogenous estrogen, and constitute a specific class of flavonoids known as isoflavones. When compared to other flavonoids, isoflavones have a very

limited distribution in nature, with the only natural dietary source of these compounds derived from soybeans (Messina and Loprinza 2001). Within soybeans, there are twelve different isoflavone subclasses, including glucosides, daidzin and genistein; of these twelve subclasses, genistein represents the most predominant form found in soy beans (Messina and Loprinza 2001).

Genistein possesses a spatial conformation similar to that of mammalian estrogen. Because of this structural similarity, genistein binds to estrogen receptors and affects estrogen-regulated gene products in a manner similar to estrogen, and therefore has been described as possessing both estrogenic and anti-estrogenic properties when consumed as a regular part of the diet (Messina and Loprinza 2001; Lamartiniere 2000). However, when compared to mammalian estrogen, genistein binds less tightly to serum proteins which are dependent upon estrogen activity, making this isoflavone more bioavailable to tissues (Messina and Loprinza 2001; Wu et al 2000). This property accounts for the tissue selective nature of genistein, in which two- to three-fold higher concentrations of this isoflavone are found in breast tissue when compared to blood serum levels (Messina and Loprinza 2001). Moreover, genistein levels in the body appear to increase in a dose-dependent manner, with regular soy consumption resulting in genistein concentrations in the low micromolar level (Messina and Loprinza 2001). In epidemiological studies conducted to date which have examined the role of soy consumption in reducing the risk for breast cancer, researchers have observed up to a 50% reduction in breast cancer incidence, especially among premenopausal women (Messina and Loprinza 2001; Wu et al 2000).

Because genistein functions in a similar manner to mammalian estrogen, one potential explanation for its ability to reduce breast cancer incidence may lie in genistein's capacity to serve as an estrogen agonist, due to its capability to downregulate estrogen receptors and therefore reduce overall estrogenic responses within the body (Messina and Loprinza 2001). As a result, regular soy consumption impacts estrogenic responses via several key mechanisms. First of all, isoflavones such as genistein, have been demonstrated to downregulate the activity of enzymes associated with estrogen metabolism. In particular, isoflavones inhibit lipid aromatase production of estrogen from adipose stores by inhibiting the activity of the cytochrome P450 molecule. The cytochrome P450 molecule, in addition to playing an important role in inflammatory responses due to cell stress, also serves as a key enzyme involved in three hydroxylation steps associated with androgen conversion to estradiol (Messina and Loprinza 2001).

In conjunction with its ability to impact estradiol levels by inhibiting the enzymes involved in estrogen production, genistein also reduces breast cancer risk by affecting ovarian function, specifically reducing luteal phase estradiol production by 9% in women with regular soy supplementation (Wu et al 2000). When combined with the capacity of genistein to upregulate the production of sex hormone binding globulin, the main hormone responsible for removing estrogen from circulation in the body, the isoflavone content of soy products aids in reducing overall levels of estrogen within women, thus reducing the incidence of breast cancers which are estrogen-sensitive (Messina and Loprinza 2001).

Aside from its ability to reduce overall estrogen concentrations within the human body, genistein also exerts a protective effect against breast cancer in an age-dependent

manner, with an earlier exposure- or younger age of consuming soy products- associated with beneficial changes in mammary tissue development (Lamartiniere 2000; Messina and Loprinza 2001). Early genistein exposure stimulates mammary cell proliferation during adolescence, resulting in the enhancement of mammary gland maturation at younger ages, in which fewer terminal end buds (TEBs) and more lobules develop (Lamartiniere 2000; Messina and Loprinza 2001). Because lobules are the most differentiated terminal ductal structures, while TEBs are the least differentiated, but most susceptible to carcinogenesis, the suppression of TEB development in the presence of genistein helps to reduce the potential for breast cancer initiation via two mechanisms: (1) early genistein exposure produces an initial increase in endothelial growth factor (EGF) production during adolescence, thus accounting for the enhancement of mammary gland maturation at a period during the lifecycle when the accumulation of DNA and cell mutations is absent; and (2) early genistein exposure also results in the downregulation of the EGF signaling pathway during adulthood, in turn suppressing the chances of mammary cancer development due to the overproliferation of the mammary stem cell compartment (Lamartiniere 2000; Messina and Loprinza 2001).

### Summary

Over thirty different food sources and plant species were examined via odds ratio analysis for their effects upon breast cancer risk. Out of these thirty-three food groups/species, traditional Thai medicinal plants provided the strongest protection against breast cancer, due to their high phytochemical content, including compounds such as glucosinolates, flavonoids, antioxidant vitamins and phytoestrogens. These plant species mediate breast cancer risk via several important mechanisms, including: (1) the activation

of the phase II detoxification system; (2) the neutralization of free radical oxidative damage; (3) the enhancement of cell mediated immunity; and (4) the regulation of hormonal and metabolic function.

An important contribution of this study to our understanding of the dietary mediation of cancer risk is the synergistic effects of combining different foods upon breast cancer etiology. In the previous chapter, an analysis of individual vitamins for their effects upon cancer risk provided no statistically significant results. This lack of a strong correlation between vitamin intake and breast cancer risk has been replicated in numerous studies. However, as demonstrated by the odds ratio analysis of specific traditional medicinal plants containing high levels of these micronutrients, many of the fruits, vegetables and spices commonly consumed in Thailand produced a significant reduction in cancer risk.

From the perspective of nutritional ecology, this observation substantiates the notion that diverse food sources must be consumed in order to obtain the maximal health benefits derived from micronutrients. Rather than acting in isolation, phytochemicals such as flavonoids and antioxidants work synergistically in order to produce the biological effects associated with cancer prevention, as well as with other hormonal and metabolic functions beneficial to human health. Moreover, while an analysis of the demographic and anthropometric variables for their effects upon breast cancer risk produced inconsistent results, the intake of Thai medicinal plants provided the most consistent predictor of cancer risk in this study.

As a cultural adaptation, the nutritional use of Thai medicinal plants is mediated in large part by the environment, which provides these plant species with their

characteristic phytochemical content and which further enables the Thai population to make use of these species throughout the year. From an historical perspective, Thai medicinal plants have constituted an integral component of Thai cuisine for over seven hundred years, with a reduced dependence upon these food sources occurring only within the past fifteen to twenty years. This combination of culture, environment and history has produced biological adaptations which protect Thai women who maintain this traditional dietary pattern from many of the diseases of affluence found in Western society.

## **Chapter 8**

### **Hypothesis Testing**

#### Introduction

This chapter will present a brief literature review covering the traditional Thai diet and the „Paleolithic“ diet within a Southeast Asian perspective in order to compare and contrast the macro- and micronutrient content of these two dietary regimes. To reconstruct the macro- and micronutrient profiles of the Paleolithic and traditional Thai diets, a metadata analysis was conducted using data and articles published from a broad timespan covering the 1950s to 2000s. For the „Paleolithic“ diet, a metadata analysis was conducted on the research of Cordain (2000; 2002) and Eaton (1985; 1992; 1996; 2007), two researchers who have worked extensively to reconstruct the nutrient profiles of hunter-gatherers. The articles of Eaton and Cordain reconstruct the Paleolithic diet by reanalyzing the hunter-gatherer cultures contained in Murdock’s Ethnographic Atlas, using the micronutrient data from contemporary plant and animal species to determine the average nutrient values associated with hunting and foraging populations.

For the traditional Thai diet, nutrition surveys conducted in the Kingdom of Thailand during the 1950s by the Cornell University Thai Project and the United States Interdepartmental Committee on Nutrition for National Defense (ICNND) were utilized within a metadata analysis in order to develop the nutrient distribution for the traditional Thai diet. The Cornell and ICNND studies represent the only comprehensive dietary surveys conducted in Thailand prior to the introduction of the agricultural technologies

associated with the Green Revolution and to the spread of Western dietary influences into the Thai population. The nutrient profiles derived from this metadata analysis was statistically compared to the macro- and micronutrient profiles of the Thai case and control subjects analyzed for this study using Chi square and ANOVA methods.

### The Paleolithic Diet

As non-communicable chronic diseases such as high blood pressure, diabetes mellitus and cancer have increased in frequency over the past fifty years, epidemiologists and anthropologists have noted that these „diseases of affluence“ seem to arise due to a mismatch between the dietary practices of contemporary human populations and the dietary practices to which the human body is adapted. In particular, researchers have noted the distinct differences in the frequency of these diseases between Westernized nations practicing high fat, low fiber diets and non-Western societies which have maintained their traditional eating patterns. For a majority of developing populations which continue to utilize traditional dietary practices, rates of NCCDs are significantly lower, due in large part to their consumption of fresh, minimally processed foods with high fiber and low fat contents. These characteristics are typical of the „Paleolithic Diet,“ a term which encompasses the dietary practices utilized by early human populations prior to the advent of agriculture.

The Paleolithic Diet represents an average macro- and micronutrient profile which anthropologists believe best reflects the dietary conditions under which the human genome evolved. This basic nutritional pattern would have been practiced by the first anatomically modern humans (AMH) approximately 200-100kya, when early *Homo sapiens* essentially identical in morphology to contemporary humans first exist in the

archaeological record (Eaton 2007). Since the advent of AMH in East Africa, the collective human genome has changed only minimally during the past 100,000 years, with the Late Paleolithic (35-15kya) potentially representing the latest time interval when the collective human genome would have been subjected to the basic nutritional parameters for which it had originally been selected (Eaton 1992). Although patterns of subsistence have changed during the intervening period, most notably with the Agricultural Revolution 10kya and the Industrial Revolution two hundred years ago, genetic adaptations have been unable to keep pace with the rapid changes in human living and eating conditions (Eaton 1992). Therefore, humans are essentially adapted to a Paleolithic, or Stone Age, diet.

Because researchers contend that the Paleolithic Diet constitutes a pattern of subsistence best designed to maximize human health, both anthropologists and epidemiologists have attempted to reconstruct the specific ratio of carbohydrates, fats and protein provided by the typical hunter-gather diet, in order to make recommendations for a contemporary subsistence pattern intended to produce optimal human health. Over the past two decades, S. Boyd Eaton and Loren Cordain have published a series of articles which address the reconstruction of the basic Paleolithic Diet using the data contained in Murdock's Ethnographic Atlas and the nutrient data for contemporary plant and animal species which would have been utilized by hunter-gatherers. In order to develop usable statistics for comparison with the traditional Thai diet and the nutrient intake of the Thai case and control subjects analyzed as part of this study, the statistical values developed by Eaton (1992; 2007) and Cordain (2000; 2002; 2005) were statistically combined as

part of a metadata analysis in order to derive average proportions and values for protein, carbohydrates and fats, as well as for five major vitamins (A, B<sub>1</sub>, B<sub>2</sub>, C and E).

**Characteristics of the Paleolithic Diet.** Although researchers have developed models to describe the basic Paleolithic Diet, foraging human populations would have focused on locally available foods which returned the most amount of energy for the least expenditure of physical activity (Cordain et al 2002; Eaton 2007). Therefore, instead of a universal pre-agricultural diet, the dietary intake of early humans would have varied greatly with latitude based upon the seasonal availability of different floral and faunal species (Eaton 1992). Using the average values derived from the diets of contemporary hunter-gatherer populations around the world, Eaton (1992; 2007) and Cordain (2000; 2002; 2005) have established the characteristics of the basic Paleolithic diet.

Looking at the contribution of the two main subsistence activities, hunting and gathering would have provided roughly equal contributions to the diet of foraging humans, with animal subsistence comprising 59% of daily intake and plant foods providing the remaining 41%. These proportions are based on averages of the extreme values for animal and plant subsistence as determined by environment in influencing species availability (Eaton 2007; Cordain et al 2002). The typical energy intake would have been substantially higher than at present due to the physical expenditure associated with hunting and gathering- averaging about 3000 kcal/day for males and 2750 kcal/day for females (Eaton 2007). The average substrate distribution for a typical Paleolithic diet, as based on the macronutrient ranges for contemporary hunter-gatherers, would have consisted of 32% proteins, 37% fats and 31% carbohydrates (Eaton 2007; Eaton 1992; Cordain et al 2002; Cordain 2002).

Because of the significant contribution of hunting to the Paleolithic subsistence strategy, the average protein intake for early humans greatly exceeded the average values (15.5% of total energy) found in Western diets (Cordain et al 2002). However, the meat which comprised the major source of dietary protein for early humans consisted largely of wild game, with fifty percent of animal foods derived from terrestrial animals and the remaining fifty percent from aquatic animals (Cordain 2002; Eaton 1992). Although epidemiologists have attributed the high incidence of obesity and cardiovascular disease in contemporary populations to diets high in animal products, studies of hunter-gatherers demonstrate that meat consumption alone is not responsible for increases in heart disease. Instead, the proportion of saturated to unsaturated fats found in animal products accounts for the majority of NCCDs in Western populations.

An important distinction between the wild game consumed by early humans and the commercial meat utilized by humans today lies in the ratio of structural to separable fats found in meat carcasses. While the structural fat located in cellular and intracellular membranous structures predominantly consists of polyunsaturated fats, separable, or storage, fat found in the intramuscular deposits of commercial meat contains mostly saturated fats (Eaton 2007; Cordain 2002). Saturated fats represent the primary class of dietary lipids responsible for hypercholesterolemia and atherosclerosis, and thus constitute the main factor contributing to cardiovascular disease in Western nations. Since wild game consists largely of structural fats, the animal products used by Paleolithic hunter-gatherers would have contained only 25% of the fat content of contemporary commercial meat (Eaton 2007; Eaton 1992). Moreover, the structural fats

found in wild game would have provided roughly three times the amount of polyunsaturated fat as commercial meat (Eaton 1992).

In addition to eating higher amounts of animal protein than contemporary populations, early humans also consumed high levels of dietary lipids, with fats comprising between 36 and 43% of total energy intake (Cordain et al 2002). While this proportion falls within the range of current Western intake, these values exceed the recommended values as determined by nutritionists. Nonetheless, foraging cultures utilizing this dietary pattern exhibit none of the detrimental health effects associated with a high fat diet. This absence of the signs and symptoms of cardiovascular disease is due to the pattern of dietary lipid intake among ancestral humans, in which fats were obtained as an integral component of whole foods rather than as additives (Eaton 2007).

These observations suggest that the absolute amount of dietary lipids is less important in reducing the risk for cardiovascular disease than the relative concentrations of specific dietary fatty acids (Cordain 2002). Unlike the contemporary Western diet, in which processed foods and commercial meats provide substantially higher amounts of saturated and trans-fatty acids, the wild game and minimally processed plant foods typical of hunter-gatherers consisted predominantly of mono- and polyunsaturated fats (Cordain 2002; Eaton et al 1996). Saturated fats contribute to CVD by increasing serum levels of LDL cholesterol, which remain sequestered within the body and accumulate within arterial walls, resulting in the growth of atherosclerotic plaque. By contrast, mono- and polyunsaturated fats, including the n3-omega lipids, reduce the risk for CVD by removing LDL cholesterol from the body and by increasing serum HDL levels. HDL cholesterol exhibits a protective effect through its anti-arrhythmic, anti-thrombotic and

anti-atherosclerotic properties. Based on this evidence, nutritionists recommend that a healthy diet should provide a polyunsaturated to saturated fat ratio (P:S) of 1.0, with saturated fats comprising less than 10% of total energy intake (Eaton 1992; Eaton et al 1996; Cordain 2002).

While the composition of wild game meat contributed to low saturated fat intake and favorable P:S ratios in hunter-gatherers, the inclusion of minimally processed carbohydrates likewise provided healthy levels of dietary lipids and phytochemicals to the Paleolithic diet. Ancestral hunter-gatherers ate quantitatively less carbohydrates than contemporary humans; however, the amount of fruit and vegetable consumption is nearly double the quantity found in the typical Western diet (Eaton 2007). Among the potentially edible plant species available to ancestral humans, those most exclusively consumed include dicotyledenous fruits, shoots, buds, leaves and roots rather than the cereal grains which characterize the contemporary human diet (Eaton 2007).

The dependence upon cereal grains, and therefore a limited carbohydrate base, by humans today contrasts in several important ways with the more broad-spectrum subsistence pattern of hunter-gatherers: (1) the fruits and vegetables available to foragers grew naturally and without cultivation, and were generally consumed within hours of being gathered; (2) although wild plant foods contain only one-third the calories of contemporary vegetable plant foods, the nutrient content of wild plant foods is especially high; (3) a millennia of agricultural practices have altered the fruits and vegetables consumed in Western nations by reducing their phytochemical content; by contrast, the phytochemicals found in wild, minimally processed fruits and vegetables are both higher in quantity and quality, producing more beneficial health consequences than the nutrients

contained in cereal grains; and (4) on average, the wild plant foods consumed by foragers would have provided substantially more vitamins and minerals than their cultivated counterparts, providing ancestral humans with nearly 1.5 to 5 times the RDA levels of micronutrients each day (Eaton 2007; Eaton 1992; Eaton et al 1996).

In addition to providing high levels of micronutrients, wild plant foods also contribute higher levels of dietary fiber than the contemporary Western diet. The modern refined carbohydrate diet provides only minimal amounts of fiber, whereas the Paleolithic fiber intake generally exceeded 100 g/day (Eaton 2007). Perhaps just as importantly, the contemporary Western diet contributes a disproportionately high level of insoluble fiber; by contrast, the Paleolithic diet provides a more balanced ratio of insoluble to soluble fiber (Eaton 2007). While insoluble fiber plays an important role in absorbing excessive dietary lipids and toxins within the colon, soluble fiber is especially vital for maintaining consistent blood glucose levels due to the slow digestion and low glycaemic/insulin responses produced by high soluble fiber diets (Cordain et al 2002; Eaton 2007).

In summary, the Paleolithic diet can be summarized by the following characteristics: (1) the roughly equal contribution of plant and animal foods to the diet; (2) the use of wild game meat containing high levels of polyunsaturated fats; (3) the inclusion of wild plant foods which provide high levels of phytochemicals and dietary fiber into the diet; (4) favorable concentrations of mono- and polyunsaturated fats in relation to saturated fats; and (5) the seasonal use of varied and numerous uncultivated animal and plant products.

## The Paleolithic Diet within a Southeast Asian Perspective: Moving Towards The Traditional Thai Diet

Southeast Asia presents some unique challenges when evaluating a basic Paleolithic Diet due to the distinct ecological dynamics created by the predominance of tropical rainforests in the region. This environment differs substantially from the Mesoamerican, African and Near Eastern ecosystems upon which archaeologists have based their reconstructions of the ancestral human diet. For example, in contrast with some hunter-gatherer populations located in Mesoamerica and Africa, Southeast Asian tropical rainforests provide local populations with a potentially replenishable supply of diverse plant and animal resources which historically have been supplemented by plant species managed via subsistence horticulture. This reliance in Southeast Asia upon a dual subsistence strategy combining foraged forest products along with horticulturally produced plant matter is due in large part to the environmental constraints imposed upon the region due to post-glacial changes in sea levels.

Between 12000-5000BP, Southeast Asia experienced climatic changes in which sea levels and temperatures were higher than present. This resulted in habitation sites located in upland karst formations or sub-montane areas near small streams and coastal areas (Gorman 1971; Bailey 1989). As demonstrated by the archaeological record in Southeast Asia, hunter-gatherers have historically utilized a combination of wild plant species, such as taro, *Pandanus* and *Canarium*, and aquatic food sources from riverine and marine environments including fish and shellfish (Gosden 1995). Beginning at approximately 10000BP, as determined by archaeological evidence from various sites in Southeast Asia, such as *Khok Phanom Di* in Thailand, hunter-gatherers began altering the tropical forest environment in a manner consistent with horticulture (Kealhofer 2003).

The archaeological sequence in Thailand provides evidence of ongoing forest burning and disturbances at a frequency consistent with the management of open patches related to plant cultivation (Kealhofer 2003). These sites contain phytolithic evidence of cucumbers, peppers, bottle gourds, water chestnuts, broadbeans and peas associated with horticulture, in addition to faunal evidence of the exploitation of freshwater aquatic fauna (snails, fish and crustaceans) and some terrestrial fauna (small herbivorous animals) (Gorman 1971; Shoocongdej 2000; Kealhofer 2003).

As suggested by this evidence, hunter-gatherer populations in Thailand began to selectively cultivate crops while still maintaining a foraging-hunting tradition, possibly to ensure a steady supply of subsistence materials during the short dry season (Higham et al 1992; Kealhofer and Piperno 1994). Among the early potential domesticates, yams, beans, almonds, taro and water chestnuts were utilized initially as wild plant species native to stream environments; later, these species were brought into the forest environment as an early cultivate (Kealhofer 2003). Approximately 7000-6000BP, an important shift occurred in the subsistence strategies of early Thai populations with the addition of rice cultivation as a significant contributor to the traditional diet (Kealhofer and Piperno 1994). At archaeological sites such as *Khok Phanom Di*, *Nong Nor* and *Ban Na Di*, evidence for early rice cultivation includes the remains of beetles commonly associated with stored grains, and pottery vessels containing rice husk and grain impressions (Higham et al 1992; Maloney et al 1989).

Based on the archaeological evidence, even prior to the advent of agriculture, the typical diet of foraging-hunting populations in Thailand relied upon a combination of freshwater and marine fish and shellfish acquired from local streams in conjunction with

gathered wild plant foods and cultivated plant species. Perhaps the single most unique feature of the early human diet in Southeast Asia, when examined from the perspective of the basic Paleolithic Diet discussed above, is the inclusion of early cultivates as an integral part of the diet. This practice reflects a dietary significance of carbohydrates to early Thai populations which predates the origins of agriculture in the region, suggesting that the basic Paleolithic diet, as described by Eaton and Cordain, in which animal protein plays a substantially greater role in the hunter-gatherer nutrient profile, may fail to accurately reflect the dietary practices of early human populations in Thailand and the broader Southeast Asian region.

#### The Traditional Thai Diet

Even after the introduction of agriculture, Thai populations continued to rely heavily upon a combination of rice, fish, vegetables and fruits as the main subsistence base. From the information in King Ramkhamhaeng's stone inscription from the early 13<sup>th</sup> century, rice and fish clearly represented the major ingredients of Thai cuisine- "In the water there are fish, in the field there is rice" (Kosulwat 2002; Yasmeen 2006). In addition to rice and fish, Thais also made use of hundreds of varieties of fruits and vegetables which are available seasonally throughout the year (Yasmeen 2006; Domett 2001). Commonly consumed fruits in Thailand include bananas, coconuts, pineapple, mango, tamarind, papaya and lotus seeds, which provide the main sources of vitamins A, B<sub>1</sub>, B<sub>2</sub>, C and E to the diet (Domett 2001). Vegetables such as swamp cabbage, beans, cucumbers, cauliflower, and tubers are often gathered from home gardens or picked fresh from along the edges of canals and creeks which dominate the contemporary Thai landscape (Domett 2001). These typical Thai plant foods are supplemented with

uncooked leafy green vegetables or herbs, such as mint, green onions, coriander, bamboo shoots, lemongrass, galangal, ginger and basil, which represent basic ingredients in most Thai dishes (Kosulwat 2002; Domett 2001).

As indicated by the extensive list of plant foods above, carbohydrates derived from rice, fruits and vegetables constitute the greatest proportion of the traditional Thai diet, representing approximately 84% of total energy intake (Kosulwat 2002; Hauck 1954). Even when taking into account the complete range of carbohydrate intake among contemporary hunter-gatherer populations, which Cordain et al (2002) estimate to be 22-40% of total energy intake, the proportion of calories derived from carbohydrates in Thailand exceeds most worldwide values. However, several important characteristics define carbohydrate intake in Thailand: (1) the use of seasonally available fresh fruits and vegetables; (2) the incorporation of raw or minimally processed plant foods into the diet; (3) the consumption of plant foods containing high levels of phytochemicals and soluble fiber; and (4) the daily purchase of fresh foods from neighborhood markets (Kosulwat 2002; Yasmeen 2006). This contrasts with the types of carbohydrates consumed in the typical Western diet, which are highly refined, stripping the foods of their nutritional quality.

Fish provides the greatest proportion of animal protein in the typical Thai diet, due to the abundance and availability of fish throughout Thailand (Domett 2001; Yasmeen 2006). Aside from fish, animal meat is generally consumed in small quantities, and usually consists of animal products such as eggs, pork and chicken which are prepared in a number of ways for consumption, including roasting, boiling, salting and fermenting (Domett 2001). Unlike the basic Paleolithic Diet, in which protein represents

approximately one-third of total energy intake, protein content in the traditional Thai diet only constitutes 12% of daily caloric intake (Hauck 1954; ICNND 1960). The reduced importance of animal protein in the traditional Thai diet is reflected in the distribution of food products sold at neighborhood floating or land-based markets, where fresh vegetables and fruits represent nearly 53% of the goods sold, while animal protein constitutes only 25% of the remaining foods (Yasmeeen 2006).

Dietary fats comprise the remaining 4% of total energy intake in the traditional Thai diet, a proportion which represents an exceptionally low value when compared to both the basic Paleolithic Diet and to dietary lipid intake in a majority of Western nations (Hauck 1954; ICNND 1960). Although the breakdown of fat intake into specific fatty acid classes, i.e. mono- and polyunsaturated versus saturated lipids, has never been determined for the traditional Thai diet, research by Carroll et al (1986) suggests that typical Thai foods provide high levels of unsaturated fats and low levels of saturated fatty acids. Carroll et al (1986) further estimate that the traditional Thai diet supplies approximately 5 g/day of polyunsaturated fats, 10 g/day of monounsaturated fats, and 10g/day of saturated fats. Based on current RDA standards, nutritionists currently recommend a saturated fat intake of less than 20-25 g/day; therefore, the traditional Thai diet provides less than half of the maximum amount of saturated fats recommended for a healthy diet (Gebhardt and Thomas 2002).

In summary, the traditional Thai diet is based upon rice as the foundation for energy intake, supplemented by high levels of fresh fruits and vegetables which are available year round on a seasonal basis. Animal protein complements the high carbohydrate basis of the Thai diet, made up largely of fish and shellfish with smaller

amounts of pork and chicken. Finally, dietary fats represent the smallest proportion of total energy intake in the traditional Thai diet, providing high amounts of healthy unsaturated fats and low levels of harmful saturated fatty acids. Although the traditional Thai diet is exceptionally high in carbohydrates, the plant foods consumed in Thailand represent fresh, minimally processed fruits and vegetables which contain high levels of phytochemicals and dietary fiber.

### A Comparative Analysis of the Paleolithic and Traditional Thai Diets

**Hypothesis 1: The traditional Thai diet will provide Thai women with a ratio of micronutrients and substrates comparable to the Paleolithic dietary pattern.**

**Method.** In order to statistically analyze the Paleolithic and traditional Thai diets for similarities and differences in their macro- and micronutrient contents, a metadata analysis was conducted to ascertain the distribution of substrates, dietary lipids and five major vitamins. For the basic Paleolithic diet, research by S. Boyd Eaton, which analyzed 329 hunter-gatherer populations, and articles by Loren Cordain, which examined 229 hunter-gatherer groups, were used in order to develop rough estimates for a basic early human dietary pattern. For the traditional Thai diet, nutritional surveys by Hauck (1954) conducted on 69 Thai villagers and by the United States Interdepartmental Committee on Nutrition for National Defense (1960) covering 513 Thai civilians were collated in an effort to determine the typical nutrient distribution in Thailand. One major limitation to the Thai metadata analysis is the absence of information regarding the specific breakdown of dietary lipids into saturated versus unsaturated fats, and the lack of knowledge concerning vitamin E intake. Therefore a comparison of the Paleolithic and traditional Thai diets was limited to substrate intake and four of the five vitamins of interest analyzed in this study.

**Table 8.1 Comparison of Macro- and Micro-nutrient Content between the Paleolithic Diet and the Traditional Thai Diet.**

	Paleolithic Diet	Traditional Thai Diet	$\chi^2$	<i>p</i> -value
Protein (% energy)	32%	12%	45.23	<0.0000
Carbohydrate (% energy)	31%	84%	215.95	<0.0000
Fat (% energy)	37%	4%	155.02	<0.0000
Saturated fat (% total fat)	23.7%	----	----	----
Monounsaturated fat (% total fat)	42%	----	----	----
Polyunsaturated fat (% total fat)	34.3%	----	----	----
P:S ratio	1.4	----	----	----
Vitamin A (IU)	2240±338.61	1724.52±1391	550.78	<0.0000
Vitamin B <sub>1</sub> (mg)	3.07±0.29	0.56±0.15	192.00	<0.0000
Vitamin B <sub>2</sub> (mg)	5.01±0.2	0.37±0.11	158.08	<0.0000
Vitamin C (mg)	439±54.67	30.48±20.68	408.25	<0.0000
Vitamin E (IU)	28±4.44	----	----	----

This study originally hypothesized that the traditional Thai diet derived a majority of its health benefits by providing micronutrients and substrates in a proportion similar to the basic Paleolithic diet. Using the ANOVA method in order to test for significant differences among the sample means and ratios of both dietary patterns, analysis of the resulting metadata produced Chi square and *p*-values which were utilized in order to determine whether statistically significant differences were observed between the nutrient profiles of the Paleolithic and traditional Thai diets.

H<sub>0</sub>: Statistically significant differences exist between the nutrient profiles of the Paleolithic and traditional Thai diets.

H<sub>A</sub>: No statistically significant differences exist between the nutrient profiles of the Paleolithic and traditional Thai diets.

**Results.** ANOVA testing of the macronutrient distribution in the Paleolithic and traditional Thai diets reveals statistically significant differences in the mean ratio values

for proteins, carbohydrates and dietary fats. As indicated by the previous description of the traditional Thai diet, the proportion of substrate intake in Thailand is characterized by more than a two-fold increase in carbohydrate intake (84%) when compared to the basic Paleolithic diet (31%) (Table 8.1). Although carbohydrate consumption statistically differs in quantity between Thais and typical hunter-gatherers, several qualitative similarities exist, including the use of fresh, minimally processed fruits and vegetables with high micronutrient and fiber content. By contrast, both protein and fat intake are significantly lower in Thailand (12% and 4% respectively) than in the Paleolithic diet (32% and 37% respectively). However, as with the qualitative features associated with Thai carbohydrate intake, the consumption of animal protein and dietary fats in Thailand also mirrors several key traits of the hunter-gatherer diet. For example, the animal protein consumed in Thailand is relatively healthy, providing higher levels of mono- and polyunsaturated fats than saturated fatty acids. This is due in large part to the preferential use of fish and shellfish over commercial meats like pork, beef and chicken. Moreover, the overall dietary lipid profile of the traditional Thai diet reflects a P:S ratio similar to the basic Paleolithic diet, with healthy mono- and polyunsaturated fats predominating over saturated lipids as a result of the inclusion of fresh plant foods into the Thai diet.

Just as the average macronutrient ratios differed between the Paleolithic and traditional Thai diets, the mean micronutrient values, as measured in g/day for vitamins A, B<sub>1</sub>, B<sub>2</sub> and C, also exhibit statistically significant differences when using the ANOVA test. For each of the vitamins examined as part of this study, the traditional Thai diet supplied less than 20-70% of the average daily values found in the basic Paleolithic diet. Even more importantly, for three of the vitamins included in this analysis- thiamin (B<sub>1</sub>),

riboflavin (B<sub>2</sub>) and ascorbic acid (C)- the mean values for the traditional Thai diet fall below current RDA standards by nearly 50%. Only vitamin A intake in the traditional Thai diet exceeds current recommended values by nearly two-fold. However, an important factor to consider when examining nutrient intake in the traditional Thai diet within this framework is the sample population used to estimate vitamin consumption for this study. Because the samples collected by Hauck (1954) and the ICNND (1960) consisted largely of rice farmers in rural areas of Thailand with lower than average socioeconomic characteristics, the mean values for vitamin intake may not reflect the true pattern of micronutrient consumption by early Thai populations. This observation may be especially true, when considering that both past and contemporary Thais have utilized a broad range of seasonally available fruits and vegetables as part of the diet.

**Conclusions.** Based on ANOVA testing of the macro- and micronutrient profiles of the traditional Thai and Paleolithic diets, the alternative hypothesis is rejected and the null hypothesis is accepted. In other words, the traditional Thai diet exhibits statistically significant differences in the pattern of substrate and vitamin intake, with early Thai populations eating higher amounts of carbohydrates and lower quantities of proteins and dietary fats when compared to the basic Paleolithic diet. Moreover, vitamin intake in the traditional Thai diet falls significantly short of the mean values found in the basic hunter-gatherer model. Although this statistical analysis revealed important quantitative differences between the traditional Thai and Paleolithic diets, the significantly lower incidence of non-communicable chronic diseases among Thais prior to the globalization and Westernization of Thai dietary preferences suggests that the amount of each

macronutrient consumed is less important to overall human health than the quality of foods consumed.

An Analysis of the Case and Control Dietary Intakes within the Perspectives of the Paleolithic and Traditional Thai Diets

**Hypothesis 2: The ratio of micronutrient and substrate intake in Thai women without breast cancer will more closely reflect the Paleolithic dietary pattern than Thai women with breast cancer.**

**Method.** In order to test for similarities and differences between the Paleolithic diet and nutrient intake of the Thai cases and controls examined as part of this study, ANOVA testing was conducted in order to derive Chi square and *p*-values to determine statistically significant trends in the data. The same average ratios and values derived from the metadata analysis of the macro- and micronutrient profiles of hunter-gatherers developed by Eaton and Cordain were utilized for the basic Paleolithic diet. Average ratios for substrate and fat intake, and mean values for vitamin intake for Thai cases and controls were developed using the data collected as part of the food frequency questionnaire used in this study.

H<sub>0</sub>: Statistically significant differences exist between the Paleolithic diet and the nutrient intake of Thai controls.

H<sub>A</sub>: No statistically significant differences exist between the Paleolithic diet and the nutrient intake of Thai controls.

**Results.** ANOVA testing of the macronutrient ratios reveals that statistically significant differences exist between the basic Paleolithic diet and the substrate intake of both Thai controls and cases. For both Thai controls and cases, carbohydrate intake represents approximately two-thirds of total daily energy intake, at 66% and 63.6% respectively (Table 8.2). By contrast, carbohydrates only account for one-third (or 31%)

of the typical hunter-gatherer diet. As with the traditional Thai diet, however, both Thai controls and cases make use of a wide variety of fresh fruits and vegetables containing high levels of phytochemicals and dietary fiber as main constituents of their diets.

**Table 8.2 Comparison of Macro- and Micro-nutrient Content between Paleolithic Diet and Nutrient Intake of Thai Cases and Controls.**

	Paleolithic Diet	Thai Cases (Average)	$\chi^2$	<i>p</i> -value	Thai Controls (Average)	$\chi^2$	<i>p</i> -value
Protein (% energy)	32%	20.4%	2.68	0.01	19.3%	4.58	0.03
Carbohydrate (% energy)	31%	63.6%	19.03	0.00001	66%	29.72	0.00001
Fat (% energy)	37%	16.0%	8.30	0.003	14.6%	13.57	0.0002
Saturated fat (% total fat)	23.7%	70.5%	41.83	<0.0000	29.1%	0.90	0.34
Monounsaturated fat (% total fat)	42%	17%	11.13	0.0008	43%	0.02	0.87
Polyunsaturated fat (% total fat)	34.3%	12.5%	9.38	0.002	27.9%	1.08	0.29
P:S ratio	1.4	0.18	----	----	0.96	----	----
Vitamin A (IU)	2240±338.61	7105±4430	702.06	<0.0000	9319±5943	888.99	<0.0000
Vitamin B <sub>1</sub> (mg)	3.07±0.29	52.3±26.8	1583.5	<0.0000	121.1±60.7	2013.8	<0.0000
Vitamin B <sub>2</sub> (mg)	5.01±0.2	1.9±0.8	224.4	<0.0000	5.4±4.5	1000.1	0.19
Vitamin C (mg)	439±54.67	329.7±134	89.32	<0.0000	346.1±147	135.68	<0.0000
Vitamin E (IU)	28±4.44	8.4±2.8	14.19	0.0001	15.3±6.4	17.06	0.00003

Moreover, protein and fat intake for both control and case subjects is significantly lower than the corresponding values for the Paleolithic diet. For example, while proteins represent approximately one-third (32%) of the total energy intake for most hunter-gatherers, the Thai controls and cases only derive 20% of their daily caloric needs from protein. In much the same manner, Thai controls and cases utilize roughly half of the energy (14.6% and 16% respectively) obtained from dietary lipids as ancestral human populations (37%).

However, when examining the proportion of dietary lipids derived from monounsaturated, polyunsaturated and saturated fats, the healthy Thai controls, unlike the

breast cancer cases, display statistical similarities with the Paleolithic diet. Specifically, monounsaturated fats comprise the largest proportion of total fat intake, at 43% for the controls and 42% for hunter-gatherers, followed by polyunsaturated fats, which represent 27.9% of total fat intake for the controls and 34.3% for ancestral humans. Saturated fats provide less than one-third of total fat intake for both Thai controls (29.1%) and hunter-gatherers (23.7%). Perhaps even more importantly, when examining fat intake utilizing the P:S ratio, Thai controls possess a near ideal value at 0.96, which demonstrates the predominant use of polyunsaturated fats among healthy Thai women. By contrast, the P:S ratio for the Thai breast cancer cases (0.18) reflects a significantly greater dependence upon saturated fats as part of the diet. When examining the proportion of dietary fats derived from specific lipid classes, Thai cases obtain 70.5% of their total fat intake from unhealthy saturated fats, with mono- and polyunsaturated fats comprising the remaining 29.5%.

Finally, comparisons of micronutrient intake between the basic Paleolithic diet and the Thai controls and cases likewise reveal statistically significant differences among all three groups. The only exception is the similarity in vitamin B<sub>2</sub> intake among healthy Thai women and ancestral human populations, at roughly 5 mg/day. For vitamins A and B<sub>1</sub>, both Thai controls and cases exceed the daily intake of hunter-gatherers by three- to fifteen-fold, while for vitamins C and E, Thais consume approximately 75% of the daily intake of early humans. Despite these overall differences in micronutrient intake, the daily values of vitamin intake for Thai controls and cases still exceed current RDA recommendations by nearly two- to five-fold.

**Conclusion.** Based on ANOVA testing of the macro- and micronutrient profiles of the Thai controls and cases, the alternate hypothesis is rejected and the null hypothesis is accepted based on the overall statistical differences in substrate and vitamin intake between the Thai subjects and the basic Paleolithic diet. Both Thai controls and cases possess similar substrate ratios and vitamin intake averages which lack statistically significant similarities with the dietary practices of ancestral human populations. However, the typical diet of healthy Thai controls possesses one important characteristic which contrasts with the diet of breast cancer cases- a more beneficial dietary lipid profile. Instead of deriving a majority of their fat intake from the use of saturated fatty acids, Thai controls obtain over two-thirds of their dietary lipids from unsaturated fats.

In addition to contributing to cardiovascular disease through atherosclerosis and the accumulation of fatty acids, saturated fats may contribute to mammary carcinogenesis via several key mechanisms. First of all, saturated fats may increase cancer risk by initiating the expression of genes which promote inflammatory responses, one potential promoter of carcinogenesis, and by inhibiting apoptosis, the body's natural method for eliminating precancerous and malignant cells from the body (Lof et al 2007). In addition, increases in saturated fat possess a weak correlation with elevations in the level of circulating estradiol, an endogenous hormone responsible for the proliferation of mammary endothelial cells in women with estrogen-receptor positive breast cancer (Cho et al 2003). Finally, saturated fats derived specifically from animal products are associated with heterocyclic amines, *N*-nitroso compounds and polycyclic aromatic hydrocarbons produced during the cooking process, organic byproducts which have a

strong positive association with the induction of mammary tumorigenesis (Cho et al 2003; Lof et al 2007).

Although earlier odds ratio analyses of dietary lipid intake have revealed no statistically significant trends in terms of an elevation or reduction in breast cancer risk, the data suggests that overall increases in fat intake may contribute to a higher risk for breast cancer. In this study, though saturated fats, when examined in a separate odds ratio analysis did not contribute to breast cancer risk, the significantly higher levels of saturated fat intake among the Thai breast cancer cases may still contribute to mammary carcinogenesis via the mechanisms described above. The limited sample size of the breast cancer subjects in this study may have contributed to the lack of a statistically significant effect of saturated fats upon breast cancer risk.

**Hypothesis 3: Thai women without breast cancer will have a greater adherence to the traditional Thai diet and the dietary utilization of Thai medicinal plants than Thai women with breast cancer.**

**Method.** In order to test for similarities and differences between the traditional Thai diet and nutrient intake of the Thai controls and cases examined as part of this study, ANOVA testing was conducted in order to derive Chi square and *p*-values which were used to determine statistically significant trends in the data. The same average ratios and values derived from the metadata analysis of the macro- and micronutrient profiles of the Thai samples collected by Hauck (1954) and the ICNND (1960) were utilized for the traditional Thai diet. Average ratios for substrate and fat intake, and mean values for vitamin intake for Thai cases and controls were developed using the data collected as part of the food frequency questionnaire used in this study. Because data on saturated,

monounsaturated, and polyunsaturated fats, as well as for vitamin E, were not available for the traditional Thai diet, these variables were excluded from this analysis.

Beyond an examination of the nutrient profiles, the case and control subjects were also compared to the traditional Thai diet based on the contribution of each food group (i.e. fruits, vegetables, seafood, etc.) to overall nutrient intake. Both Hauck (1954) and the ICNND survey (1960) provide similar data detailing the proportion of total energy intake derived from each food group and were used for the purpose of this analysis. For the Thai cases and controls, the energy derived from each food group was tabulated and percentages were derived based on the average total daily energy intake.

$H_0$ : Statistically significant differences exist between the traditional Thai diet and the nutrient intake of Thai controls.

$H_A$ : No statistically significant differences exist between the traditional Thai diet and the nutrient intake of Thai controls.

**Results.** ANOVA testing of the macronutrient ratios and average vitamin intake reveals that statistically significant differences exist between the traditional Thai diet and the nutritional intake of both Thai controls and cases. As with the traditional Thai diet, carbohydrates provide the greatest amount of calories to the diet for both the controls and cases (66% and 63.6% respectively) (Table 8.3). However, when examining the food groups which contribute to carbohydrate intake, rice comprises the majority of carbohydrate energy intake (66% of total energy), supplemented with smaller amounts of fruits (9.6% of total energy) and vegetables (3.4% of total energy) (Table 8.4). By contrast, for the Thai controls and cases, rice represents only half of the average carbohydrate intake (36.5% and 32.1% of total energy respectively), with fruits (21.9%

and 19.1% of total energy) and vegetables (9.3% and 9.5% of total energy) comprising a greater proportion of energy derived from carbohydrates

When comparing protein intake among the controls and cases with the traditional Thai diet, energy derived from proteins is significantly higher among the Thai subjects (19.3% and 20.4% respectively) recruited for this study than in the traditional Thai diet (12%). In spite of this difference in the contribution of proteins to total energy intake, the controls and cases still derive nearly half of their protein intake from fish products (9.4% and 8.5% of total energy respectively), in much the same manner as the traditional Thai diet (6.2% of total energy). However, unlike the traditional Thai diet (3.8%), animal products derived from commercial beef, chicken and pork provide a greater contribution to total energy intake for the controls and cases (9.4% and 8.5% of total energy respectively).

Fat intake among the Thai controls and cases (14.6% and 16% respectively) also significantly exceeds the proportion of energy derived from fats in the traditional Thai diet (4%). This increase is due in large part to the greater contribution of commercial animal products to the overall diet of the Thai subjects recruited for this study. In addition, the use of discretionary oils and dairy products has increased among contemporary Thais by nearly two-fold when compared to the traditional Thai diet.

The average vitamin intake has also increased significantly in the contemporary Thai diet. While the average daily intake of vitamins in the traditional Thai diet often fell below the recommended daily values for several of the major micronutrients, vitamin intake among the Thai controls and cases not only exceeds the traditional Thai diet, but also current RDA values. Higher levels of vitamin intake among contemporary Thais

relate directly to improvements in the utilization of fruits and vegetables, which have increased by two-fold among the cases and controls when compared to the traditional Thai diet. Even more importantly, the use of fresh, minimally processed plant foods provides higher levels of phytochemicals and micronutrients to the contemporary Thai diet.

**Table 8.3 Comparison of Macro- and Micro-nutrient Content between the Traditional Thai Diet and Nutrient Intake of Thai Cases and Controls.**

	Traditional Thai Diet	Thai Cases (Average)	$\chi^2$	<i>p</i> -value	Thai Controls (Average)	$\chi^2$	<i>p</i> -value
Energy Intake (kcal/day)	1847±203	1421±419	72.37	<0.0000	2062±802	443.63	0.001
Protein (% energy)	12%	20.4%	2.99	0.08	19.3%	3.27	0.07
Carbohydrate (% energy)	84%	63.6%	13.43	0.0003	66%	15.02	0.0001
Fat (% energy)	4%	16.0%	14.31	0.0001	14.6%	15.53	<0.0000
Saturated fat (% total fat)	----	70.5%	----	----	29.1%	----	----
Monounsaturated fat (% total fat)	----	17%	----	----	43%	----	----
Polyunsaturated fat (% total fat)	----	12.5%	----	----	27.9%	----	----
P:S ratio	----	0.18	----	----	0.96	----	----
Vitamin A (IU)	1725±1391	7105±4430	227.49	0.01	9319±5943	502.68	<0.0000
Vitamin B <sub>1</sub> (mg)	0.56±0.15	52.3±26.8	4416.1	0.01	121.1±60.7	5555.8	<0.0000
Vitamin B <sub>2</sub> (mg)	0.37±0.11	1.9±0.8	829.25	0.01	5.4±4.5	731.08	<0.0000
Vitamin C (mg)	30.48±20.7	329.7±134	726.82	<0.0000	346.1±147	957.50	<0.0000
Vitamin E (IU)	----	8.4±2.8	----	----	15.3±6.4	----	----

**Conclusion.** Based on ANOVA testing of the macro- and micronutrient profiles of the Thai controls and cases, the alternate hypothesis is rejected and the null hypothesis is accepted based on the overall statistical differences in substrate and vitamin intake between the Thai subjects and the traditional Thai diet. Both Thai controls and cases derive a similar proportion of their diet from carbohydrates, proteins and fats. While carbohydrates provide the most energy to total daily intake, for the traditional Thai diet

rice represented the major carbohydrate, while for the Thai controls and cases, fruits and vegetables provide as much energy as rice. Even though fish products still comprise the majority of protein intake, energy derived from proteins has increased significantly among contemporary Thai women. In addition, the contemporary Thai diet incorporates a greater use of discretionary fats when compared to the traditional Thai diet, due to an increased reliance upon commercial meats and dairy products.

**Table 8.4 Comparison of Distribution of Food Groups between the Traditional Thai Diet and the Daily Dietary Intake of Thai Cases and Controls by Percent of Total Daily Caloric Intake.**

	Traditional Thai Diet (%)	Thai Cases (%)	$\chi^2$	<i>p</i> -value	Thai Controls (%)	$\chi^2$	<i>p</i> -value
Rice	66%	32.1%	23.19	<0.0000	36.5%	25.54	<0.0000
Vegetables	3.4%	9.5%	4.67	0.03	9.3%	6.09	0.01
Sugar	3.9%	0.8%	1.28	0.28	0.5%	2.36	0.12
Fruits	9.6%	19.1%	4.55	0.03	21.9%	10.58	0.001
Fish	6.2%	8.5%	0.41	0.52	9.4%	1.15	0.28
Other animal products	3.8%	11.3%	6.26	0.01	10.6%	7.24	0.007
Dairy products	1.8%	6.9%	5.64	0.02	4.6%	2.59	0.10
Oils	5.3%	11.8%	3.61	0.05	7.2%	0.48	0.49

These changes to the contemporary Thai diet reflect in part the impact of globalization upon the available food choices for Thai women, with a greater variety of food products providing Thais with the ability to incorporate more options into their daily diet. In some cases, these options include foods previously unavailable to a majority of Thais in the past, including commercial meats and dairy products. Although these new options create statistically significant deviations from the traditional Thai diet for both the control and case subjects, several important qualities contribute to the overall health

benefits of the contemporary Thai diet. For example, Thais still derive important phytochemicals and dietary fiber from the utilization of fresh fruits and vegetables as part of their diet. Moreover, a greater reliance upon fish products as a main source of dietary protein provides a source of healthy unsaturated fats into the diet. This continuation of qualitative aspects of the traditional Thai diet provides contemporary Thais with important health benefits often missing from the typical Western diet.

**Hypothesis 4: Thai women without breast cancer will have a higher intake of Thai medicinal plants as part of their diet than Thai women with breast cancer.**

**Method.** In order to determine whether healthy Thai women have a greater mean daily intake of Thai traditional medicinal plants than Thai women with breast cancer, ANOVA testing was conducted on sixteen different plant species regularly consumed as part of the traditional Thai diet. These medicinal plants include fruits, vegetables and spices which are commonly used to homeopathically treat a variety of illnesses and conditions in Thailand. The average daily intake (g/day) and standard deviations were calculated for the control and case subjects using data from the food frequency questionnaire developed for this study. Although the raw data shows that control subjects consumed a higher quantity of each medicinal plant, as measured by g/day via the food frequency questionnaire, *Chi-square* and *p-values* were derived from the data in order to test for statistically significant trends beyond the observed differences in intake.

H<sub>0</sub>: No statistically significant differences exist between the Thai controls and cases in the intake of Thai traditional medicinal plants.

H<sub>A</sub>: Statistically significant differences exist between the Thai controls and cases in the intake of Thai traditional medicinal plants.

**Table 8.5 Comparison of Thai Traditional Medicinal Plant Intake among Cases and Controls.**

Item	Cases Mean $\pm$ SD (g/day)	Controls Mean $\pm$ SD (g/day)	Chi-square	<i>p</i> -value
Bitter melon	11.92 $\pm$ 12.51	14.67 $\pm$ 15.63	2.88	0.08
TumLeung	19.06 $\pm$ 16.56	24.62 $\pm$ 18.50	0.72	0.39
Smooth Loofah	5.18 $\pm$ 8.09	14.10 $\pm$ 16.52	26.15	0.0004
Angled Loofah	10.35 $\pm$ 12.16	17.64 $\pm$ 18.10	8.85	0.002
Pomegranate	3.73 $\pm$ 9.37	3.85 $\pm$ 6.92	5.71	0.01
Mangosteen	18.29 $\pm$ 23.10	27.45 $\pm$ 26.06	0.86	0.35
Krathon	5.70 $\pm$ 10.49	8.67 $\pm$ 12.30	1.49	0.22
Mango	26.66 $\pm$ 27.52	41.20 $\pm$ 32.93	1.88	0.16
Galangal	1.49 $\pm$ 1.47	1.47 $\pm$ 1.60	0.42	0.51
Ginger	1.88 $\pm$ 2.03	3.14 $\pm$ 3.12	10.24	0.001
Turmeric	0.38 $\pm$ 0.77	0.45 $\pm$ 0.75	0.04	0.83
Krachai	0.84 $\pm$ 0.98	0.85 $\pm$ 1.09	0.67	0.41
Sacred Basil	1.69 $\pm$ 1.32	2.32 $\pm$ 1.67	3.2	0.07
Sweet Basil	1.51 $\pm$ 1.38	1.85 $\pm$ 1.65	1.87	0.17
Cumin	0.19 $\pm$ 0.47	0.31 $\pm$ 0.63	4.9	0.02
Lemon Grass	3.78 $\pm$ 2.95	4.39 $\pm$ 3.73	3.19	0.07

**Results.** Based on ANOVA testing of the average daily intake of Thai traditional medicinal plants, five of the sixteen plant species analyzed as part of this study were consumed in statistically significant greater quantities by the healthy Thai controls when compared to the breast cancer cases. With the exception of pomegranate ( $\chi^2= 5.71, p = 0.01$ ), which demonstrated only a weak overall trend in decreasing cancer risk based on odds ratio analysis, smooth loofah ( $\chi^2= 26.15, p = 0.0004$ ), angled loofah ( $\chi^2= 8.85, p = 0.002$ ), ginger ( $\chi^2= 10.24, p = 0.001$ ) and cumin ( $\chi^2= 4.9, p = 0.02$ ) were all associated with a significant reduction in breast cancer risk based upon odds ratio analysis per quintile of intake (Table 8.5). Moreover, three of these five medicinal plants- angled loofah (OR = 0.17,  $p = 0.0000001$ ), ginger (OR = 0.11,  $p = 0.0000001$ ) and cumin (OR = 0.19,  $p = 0.0000001$ ), were among the consumed species with the strongest protective effect against breast cancer. Five additional plant species- bitter melon ( $\chi^2= 2.88, p = 0.08$ ), mango ( $\chi^2= 1.88, p = 0.16$ ), sacred basil ( $\chi^2= 3.2, p = 0.07$ ), sweet basil ( $\chi^2= 1.87, p =$

= 0.17) and lemon grass ( $\chi^2 = 3.19, p = 0.07$ ), were also consumed in greater quantities by the healthy Thai controls; however, the ANOVA testing revealed only a weak statistical trend in terms of significance. With the exception of sweet basil, which possessed a statistically non-significant effect in reducing breast cancer risk, each of these medicinal plants- bitter melon (OR = 0.03,  $p = 0.0000001$ ), mango (OR = 0.15,  $p = 0.0000001$ ), sacred basil (OR = 0.14,  $p = 0.02$ ) and lemongrass (OR = 0.28,  $p = 0.0000001$ ), were also among the consumed foods with the strongest protective effect against breast cancer. Although the raw data for the remaining medicinal plants (tumleung, mangosteen, krathon, galangal, turmeric and krachai) suggest a higher intake among the healthy Thai controls, ANOVA testing did not reveal any statistically significant trends for these species.

**Conclusion.** Although five of the examined plant species (bitter melon, mango, sacred basil, sweet basil and lemongrass) were all associated with only a weak statistical trend towards greater consumption among the Thai controls, when combined with pomegranate, smooth loofah, angled loofah, ginger and cumin, the overall tendency indicates a greater consumption of Thai traditional medicinal plants among the Thai controls. Therefore, the null hypothesis is rejected, and the alternative hypothesis is accepted based upon the higher intake of medicinal plants among the healthy Thai women. The previous ANOVA tests failed to demonstrate any significant similarities between the dietary practices of the Thai controls with either of the Paleolithic or traditional Thai diets. As a result, the statistical trend towards a greater intake of medicinal plants among the Thai controls suggests that the dietary use of medicinal plants

may account for the protective effect against breast cancer found among healthy Thai women.

### Summary

The control and case subjects recruited for this study exhibit striking similarities in their dietary habits due to comparable macronutrient intake ratios and average micronutrient values. Therefore, when analyzing the nutrient profiles of the controls and cases against the Paleolithic and traditional Thai diets, neither the healthy Thai women nor breast cancer cases possess food preferences which reflect either the ancestral human diet or the early Thai diet. In a comparison of the Paleolithic and traditional Thai diets, the macro- and micronutrient profiles for each modality suggest two extremes, with a greater reliance upon animal products at one end, and a dependence upon plant foods at the other. Based upon this observation, the dietary values for the control and case subjects reflect an intermediate zone between the Paleolithic and traditional Thai diets, though still with decidedly Thai attributes. For example, although the inclusion of commercial meat and dairy products, as well as the use of discretionary fats, suggests the influence of globalization and Westernization upon the available food choices in Thailand, a dependence upon carbohydrates still predominates the contemporary Thai diet. Moreover, the continued use of fresh, minimally processed plant foods contributes high levels of phytochemicals and vitamins at amounts which exceed both the values found in the traditional Thai diet and current RDA recommendations.

In spite of the similarities between the nutritional profiles of the control and case subjects recruited for this study, two important differences indicate the potential role for diet in mediating the risk for breast cancer. First of all, as demonstrated by a statistical

comparison with the Paleolithic diet, the Thai controls possess dietary lipid ratios which both mirror the ancestral human diet and which reflect current recommendations for a healthy diet. More specifically, Thai controls derive a majority of their fat energy from unsaturated fats, whereas the breast cancer cases consumed mostly saturated fats as part of their diet. Second, the statistical analyses reveal that Thai controls consume higher levels of Thai traditional medicinal plants as part of their regular diet. As discussed in chapter seven, Thai traditional medicinal plants contain high levels of anticarcinogenic phytochemicals which provide a strong protective effect against breast cancer based on levels of intake. These two features suggest that dietary quality, rather than strict quantitative approaches to nutrition, may provide a better approach towards understanding population differences in disease expression.

## **Chapter 9**

### **Discussion and Conclusions**

#### Introduction

This study addresses the rising incidence of breast cancer in developing populations within the perspectives of biological anthropology and nutritional ecology. Rather than focusing upon traditional epidemiological explanations for population differences in breast cancer incidence, such as anthropometry and specific dietary measures, this research approaches breast cancer etiology in Thailand by examining the dietary use of traditional medicinal plants. The nutritional use of Thai medicinal plants represents a unique cultural adaptation practiced by Southeast Asians with the potential to modify human biology and cancer risk. In particular, the mediation of cancer risk involves the introduction of potent anticarcinogens via plant species containing phytochemicals which are the byproducts of natural plant defenses necessary for survival in a tropical environment.

In order to examine the role of Thai medicinal plant use in the etiology of breast cancer in Thailand, a case control study was conducted using 129 subjects recruited from Bangkok, Thailand, where breast cancer incidence has increased significantly over the past fifteen to twenty years. Anthropometric and demographic data were collected in order to control for the known effects of body composition and reproductive history upon breast cancer risk. In addition, a food frequency questionnaire was administered to subjects in order to collect data on dietary intake and the nutritional use of Thai medicinal

plants. This chapter will review the major findings arising from this data, including a review of the demographic, anthropometric and nutritional data. In addition, these findings will be integrated and discussed within the concept of nutritional ecology, followed by dietary recommendations for cancer prevention and future research considerations.

### Summary of Findings

**Demographic and Anthropometric Data.** The data on reproductive history reflects lifestyle changes in fertility related to the demographic transition, in which women have fewer children at a later age. In this study, these characteristics were associated with the Thai breast cancer cases, which had a later average age at first pregnancy (27.1 years) and fewer number of live births (2.03 children). Moreover, fewer parous cases practiced breastfeeding when compared to the healthy control subjects (88.6% versus 97.9% respectively). As expected, a delay in first pregnancy among the Thai cases was associated with a 15% increase in breast cancer risk (OR = 1.15,  $p = 0.002$ ).

Typically, a delay in first pregnancy after the age of 25 is associated with up to a five-fold increase in breast cancer risk. This risk is due in large part to the reduced capacity of the mammary tissue to maintain the high degree of ductal complexity, characterized by immune-protective hormonal and stem cell changes, acquired during pregnancy (Russo et al 2007; Schneider and Bocker 2006). In the absence of these maturational changes to the breast tissue, women who become pregnant after 25 years of age retain a relatively immature breast structure which predisposes these individuals to

increases in epithelial and stem cell turnover and a heightened response to serum estrogen levels, both risk factors for breast cancer (Lee et al 2003; Britt et al 2007).

The Thai breast cancer cases recruited for this study tend to have higher levels of both income and education, two variables which also possessed a weak positive correlation with an increased age at first pregnancy ( $r^2 = 0.096$  and  $r^2 = 0.117$ ). Therefore, those aspects of the demographic transition present among the breast cancer subjects are related in part to socioeconomic status, with middle- to high-income Thai women characterized by having fewer children at a later age and by practicing breastfeeding at lower rates when compared to the controls.

While a reduction in parity and a delayed age at first pregnancy were associated with an increased risk for breast cancer, one of the unexpected findings in this study was the association of a later age at menarche among the Thai cases (14.7 years) with higher incidence rates of breast cancer. A delayed age at menarche is typically related to decreases in breast cancer risk due to a reduction in lifetime exposure to serum estrogen and to a lengthened period of pubertal skeletal growth characterized by lower levels of growth hormones (Baer et al 2006; DeStavola et al 2004). Because growth hormones are capable of stimulating the rapid proliferation of stem cells, and therefore contribute to the potential accumulation of mutations in the breast stem cell compartment, a later age at menarche reduces breast cancer risk by limiting the degree of stem cell proliferation in adolescent breast tissue (Beaber et al 2008; Onland-Moret et al 2005).

Although skeletal growth is typically decelerated among females with a delayed age at menarche, adult height is ultimately increased, due to a longer period of skeletal maturation. However, the breast cancer cases in this study diverge from the expected

pattern, instead characterized by a shorter average height (155.22 cm) when compared to the healthy controls (158.53 cm). Moreover, rather than increasing breast cancer incidence, greater adult stature among the Thai controls is associated with an 8% reduction in cancer risk (OR = 0.92,  $p = 0.0041$ ). This unexpected pattern, in which a later age at menarche and shorter adult stature contribute to breast cancer risk, suggests a divergent pattern in the timing of menarche and the attainment of adult stature for Thai women. In Thailand, a later age at menarche appears to be associated with a long period of skeletal maturation associated with a slow grow rate, which increases breast cancer risk by exposing the mammary stem cell compartment to greater exposure to endogenous estrogen and growth factors. A later age at menarche and shorter adult height among the Thai breast cancer cases suggests higher levels of growth hormones which act in conjunction with additional lifestyle factors, including parity and age at first pregnancy, to produce increases in breast cancer risk.

While age at menarche and adult height represent variables which diverge from the expected association with breast cancer risk, the use of waist and hip circumference and the waist-to-hip ratio as measures of central obesity reflect the predicted correlation between abdominal fat and cancer risk in Thai women. Unlike body mass index, which only provides an indicator of weight status, the waist-to-hip ratio has been demonstrated to accurately reflect the degree of central fat storage based on numerous epidemiological studies (Okobia et al 2006; Ziegler 1997; Harvie 2003). Among the Thai subjects recruited for this study, basic measures of body composition, including weight and BMI, failed to produce any statistically significant correlations with breast cancer risk. Indeed, the Thai breast cancer cases and controls possess relatively similar BMI values (23.02

and 21.89 kg/m<sup>2</sup>) that fall within the range of normal weight status according to CDC standards.

However, when examining patterns of fat distribution, the Thai cases were characterized by higher levels of abdominal fat storage, as indicated by mean waist and hip circumference values. Increases in waist and hip circumference produced a 10% increase in breast cancer risk as determined by odds ratio analysis (OR = 1.10,  $p = 0.00001$ ; OR = 1.09,  $p = 0.0002$ ). When using waist and hip circumference to calculate the waist-to-hip ratio, the Thai breast cancer cases were also distinguished by having greater WHR values than the controls. At WHR values greater than 0.85, considered to be the threshold for unhealthy levels of abdominal fat storage, the risk for breast cancer increases six-fold, reflecting the expected pattern between central obesity and breast cancer risk (OR = 6.13,  $p = 0.002$ ). In Western nations, breast cancer risk based on WHR tends to follow an age-related pattern, with higher WHR values producing a protective effect in premenopausal women, and an increased risk in postmenopausal women. Although a similar analysis based on age-status in this study did not produce any statistically significant results, the overall trends suggest an opposite effect in Thai women, with higher WHR values increasing breast cancer risk to a greater degree among pre-menopausal, rather than post-menopausal, women (OR = 2.92,  $p = 0.33$ ; OR = 1.71,  $p = 0.24$ ).

Central fat distribution increases breast cancer risk by producing endocrinological and immunological changes associated with the metabolic syndrome. In particular, abdominal fat increases endogenous estrogen production via the conversion of androgens in adipose tissue, while concurrently reducing the production of sex-hormone binding

globulin, the main hormone responsible for removing estradiol from the blood stream (Ballard-Barbash 1994). Moreover, abdominal fat increases the production of the 16 $\alpha$ -OHE variant of estradiol, a metabolic product of estrogen synthesis which initiates breast stem cell differentiation and the process of carcinogenesis (Telang et al 1992; Gupta et al 1998). In addition, central adiposity contributes to immunological changes which increase breast cancer risk, including decreases in cell-mediated immunity (NK cells, leukocytes and T helper cells), increases in inflammatory mediators associated with tumor production (prostaglandins, C-reactive proteins) and increases in reactive oxygen species (Rose 2004; Harvie 2003; Sauter and Scott 2008).

Overall, the demographic and anthropometric data both confirm and deviate from the expected risk patterns for breast cancer. While certain reproductive variables, including a later age at first pregnancy, a fewer number of live births and a lower frequency of breastfeeding characterize the Thai breast cancer cases and increase breast cancer risk as expected, an older age at menarche among the healthy controls presents an unexpected finding which protects against cancer. By contrast, measures of central fat storage produce predictable associations with breast cancer risk. Both waist and hip circumference, as well the waist-to-hip ratio, produce significant increases in breast cancer risk in the Thai breast cancer cases associated with hormonal and immunological changes related to abdominal fat storage. When combined, these variables suggest that Westernization over the past fifteen to twenty years has influenced breast cancer rates in Thailand via the demographic transition and lifestyle changes which have contributed to decreases in fertility and increases in central adiposity. In turn, these modifications to

traditional reproductive patterns and the increase in central adiposity in Thailand provide one key to understanding the recent increase in breast cancer among Thai women.

**Nutritional Data and Hypothesis Testing.** Just as the reproductive history and anthropometric data reveal the importance of Westernization upon breast cancer etiology in Thailand, the nutritional data underscores how the globalization of food choices within the framework of a traditional diet can have profound influences upon both biology and disease expression. The data acquired from the food frequency questionnaire reveals overall similarities between the Thai cases and controls in the proportion of calories derived from proteins, carbohydrates and fats. Even though the healthy controls consumed significantly more calories per day ( $2062 \pm 802$  kcal/day) than the breast cancer cases ( $1421 \pm 419$ ), for both groups of subjects, carbohydrates comprise approximately two-thirds of total energy intake, while proteins and fats each represent one-third of daily caloric intake. The striking similarities between the cases and controls suggest that both groups consume relatively similar diets. However, the odds ratio analyses conducted on both the broader macro- and micronutrient values, as well as on specific dietary variables and Thai medicinal plants indicate that important differences exist in the diets of the Thai cases and controls recruited for this study.

When examining carbohydrate intake among the Thai subjects (~60% of energy/day), the levels of consumption exceed the ratio recommended by the basic Paleolithic diet (31%), while at the same time falling below those values considered normal for the traditional Thai diet (84%). In spite of these differences in carbohydrate intake, an important qualitative feature of the Paleolithic and traditional Thai diets is the reliance upon fresh, seasonally available and minimally processed fruits and vegetables.

These days, refined carbohydrates are regularly consumed in Thailand due to globalization; however, among the healthy Thai controls, refined carbohydrates contribute a smaller proportion to overall energy intake, while unrefined carbohydrates predominate. Using odds ratio analysis to analyze the impact of consuming complex carbohydrates upon breast cancer risk, the data reveal that cancer risk decreases by 49% as unrefined carbohydrate intake increases (OR = 0.51,  $p = 0.00001$ ). Therefore, one important distinguishing characteristic of the healthy control diet is a greater reliance upon complex carbohydrates provided by fresh fruit and vegetable intake.

An important component of complex carbohydrate intake in Thailand is the incorporation of medicinal plants into the regular diet. Thai medicinal plants include fruits like mangosteen and krathon, vegetables such as bitter melon and ivy gourd, and spices like lemongrass and galangal which are commonly utilized within Thai cuisine. When comparing the case and control subjects for their intake of Thai medicinal plants, the healthy Thai controls consume higher gross quantities, as determined by both the raw data and by ANOVA testing, of the sixteen medicinal plants examined in this study. Although over forty-five individual foods were analyzed for their effects upon cancer risk, fifteen out of the nineteen items which produced a statistically significant reduction in breast cancer risk consisted of Thai medicinal plants.

The protective effect provided by Thai medicinal plants ranges from a 29% to a 97% reduction in cancer risk. Among the important mechanisms contributing to this protective effect includes the presence of high levels of anticarcinogenic phytochemicals found in Thai medicinal plants. These compounds include carotenoids, phenolics, alkaloids and organosulfurs which protect against tumor initiation via several key

methods, including: (1) the induction of phase II detoxifying enzymes; (2) free radical scavenging activity; (3) the inhibition of DNA mutagenesis; (4) the inhibition of cell proliferation; and (5) the induction of cell cycle arrest and apoptosis (Acamovic and Brooker 2005; Dillard and German 2000; Lampe 2003).

Within the context of this study, Thai medicinal plants constitute an important component of carbohydrate intake among the healthy Thai controls. As a cultural adaptation unique to Southeast Asian populations, the dietary use of Thai medicinal plants facilitates biological responses which promote immune function while simultaneously inhibiting both tumor initiation and promotion via environmentally and endogenously produced toxins and carcinogens. Therefore, aside from the importance of unrefined carbohydrates as a defining characteristic of the traditional Thai diet, the nutritional use of Thai medicinal plants represents another key component which characterizes traditional dietary practices in Thailand.

As suggested by the data in this study, one important consequence of the globalization of food choices is the reduced dietary reliance upon medicinal plants as a cultural adaptation among the Thai breast cancer cases. As Thai medicinal plants are increasingly replaced by refined carbohydrates in the Thai diet, the protective effect against breast cancer provided by a reliance upon carbohydrates is negated by the presence of processed sugars and cereal grains which are devoid of nutritional value. Based upon this relationship between carbohydrate alternatives and breast cancer risk, the nutritional use of Thai medicinal plants represents another significant qualitative feature which distinguishes the dietary preferences of the healthy control subjects from the Thai breast cancer cases.

Similar to carbohydrate intake, the proportion of energy derived from proteins and fats among the cases and controls also lie intermediary to the values characteristic of the Paleolithic and traditional Thai diets. Protein represents approximately 20% of the daily caloric intake of the cases and controls in this study, an increase from 12% of the total energy intake typical of the traditional Thai diet. Seafood represents the conventional source of protein in the traditional Thai diet, and among the cases and controls, the proportion of calories derived from fish and shellfish is roughly similar to the traditional Thai diet. Therefore, the remaining protein intake is derived from the consumption of commercial meat products, once again highlighting the effects of globalization upon food choices in Thailand.

This influence of Westernization is not only evident in the food choices contributing to protein energy intake, but also in the use of dairy products and discretionary fats, which have increased significantly over the past fifty years. While the traditional Thai diet derives only 1.8% of daily energy intake from dairy products and 5.3% from discretionary fats, the consumption of these specific food groups has increased almost two-fold among contemporary Thai women. However, as with carbohydrate intake, the breast cancer cases and controls have different patterns of utilizing dairy products and dietary fats. For the Thai cases, dairy products and discretionary fat intake (6.9% and 11.8% respectively) represent a significantly higher proportion of daily energy intake when compared to the controls (4.6% and 7.2%). Furthermore, when examining the types of dietary lipids contributing to total energy intake, the breast cancer subjects are characterized by the utilization of significantly greater amounts of saturated fats (70.5% of total fat intake) when compared to the controls (29.1% of total fat intake). As

the ratio of polyunsaturated to saturated fats (P:S) reveals, the controls (P:S = 0.96) rely on significantly higher levels of healthy unsaturated fats, while the cases (P:S = 0.18) utilize considerably lower quantities.

Based on the P:S ratio, the controls consume a healthy combination of dietary fats, while the cases rely on saturated fats at levels above the daily recommended allowance due in large part to a greater reliance upon commercial meats, dairy products and discretionary oils. Among individuals deriving high levels of total energy intake from saturated fats, breast cancer risk is elevated via several mechanisms, including: (1) the initiation of genes which promote inflammatory responses; (2) the inhibition of apoptosis and cell cycle arrest; (3) the increased production of estradiol; and 4) the association with heterocyclic amines, *N*-nitroso compounds and polycyclic aromatic hydrocarbons which induce carcinogenesis (Cho et al 2003; Lof et al 2007).

As demonstrated by the statistical analyses of these dietary data, the process of globalization produces changes in food options which encourage developing populations like Thailand to adopt new food resources within the framework of a traditional diet. For the Thai breast cancer cases recruited for this study, the process of globalization involves the loss of one traditional component- the nutritional use of medicinal plants, and the replacement of distinctive dietary features, i.e. a reliance upon complex carbohydrates and low levels of protein and fats, with foods typical of a more Westernized diet. These non-traditional foods include commercial meats like beef, pork and chicken, as well as discretionary fats and dairy products such as milk, cheese and yogurt which would not have been available to Thais fifty to sixty years ago. Only within the past two decades, as global corporations have intensely marketed the „nutritional value“ of consuming

mass-produced foods, have Thais started utilizing non-traditional foods as part of their diet (Yasmeen 2006; Kosulwat 2002).

The use of these non-traditional foods among the Thai breast cancer cases reflects the burgeoning middle-class within Thai society, in which sufficient disposable income is available for utilization upon convenience foods that are high in refined carbohydrates and saturated fats (Yasmeen 2006; Kosulwat 2002). Beginning twenty years ago, as income levels in Thailand increased due to rapid modernization, incidence rates of overweight/obesity and the diseases of affluence arising from Western-style diets concomitantly increased in frequency (MOPH 2005; Kosulwat 2002). Even more important, diseases such as breast cancer now represent the most significant causes of mortality among Thai women, reflecting the temporal shift towards globalized food choices (MOPH 2005). Therefore, while globalization has contributed in part to both the demographic transition and the higher rates of overweight/obesity among the Thai breast cancer cases, another key component of Westernization is the rapid loss of traditional dietary patterns which have enabled Thais to represent one of the healthiest populations in the world (Kosulwat 2002). Among the case subjects in this study, globalization in particular can be defined as a reduced reliance upon medicinal plants which constitute not only a majority of carbohydrate intake in the traditional Thai diet, but also the main source of natural cancer-fighting compounds.

Epidemiologists have alternatively touted either the basic Paleolithic diet, with a high protein and low carbohydrate intake, or a high carbohydrate, low fat pattern such as the traditional Thai diet as the best nutritional method for achieving optimal human health and reducing the incidence of NCCDs. Eaton and Cordain (1985; 2002; 2007), for

example, have described how a high protein diet characteristic of ancestral humans has the capacity to stabilize cholesterol levels and the symptoms of the metabolic syndrome in the short term. By contrast, other nutritional agencies, including the CDC and the WHO-FAO, contend that high carbohydrate and low fat intake best ameliorates insulin resistance by promoting weight loss.

However, as demonstrated by the dietary preferences of the Thai control subjects recruited for this study, the proportion of energy derived from each macronutrient is less important than the quality of foods consumed on a regular basis. Although the healthy controls derived the same proportion of energy from carbohydrates, proteins and fats as the breast cancer cases, the maintenance of traditional Thai dietary practices, even with the influences of globalization, produces optimal health results. In particular, the continued reliance upon traditional Thai medicinal plants as a main component of carbohydrate intake, rather than a dependence upon refined carbohydrates, represents an important qualitative feature which protects the control subjects from both breast cancer and the metabolic correlates of overweight/obesity.

The odds ratio analyses of medicinal plant intake represented one of the salient findings of this research, as these plant species consistently represented the strongest protective mechanisms against breast cancer. Neither macronutrient nor micronutrient intake provided significant associations with a reduction in cancer risk. Therefore, beyond the macro- and micronutrient ratios which characterize traditional dietary practices in Thailand, the key defining strength of the traditional Thai diet lies not in the quantity of consumed nutrients, but rather in the reliance upon the nutritional use of medicinal plants. This observation, in and of itself, provides some important factors to

consider when attempting to define specific nutritional guidelines for optimal human health, as will be discussed in the next section.

### Framing the Study Results within the Context of Nutritional Ecology: Nutrient Recommendations for Cancer Prevention and Optimal Human Health

As rates of overweight/obesity and the concomitant diseases of affluence have increased in frequency worldwide over the past fifty years, one of the main concerns for public health officials has been to define specific dietary guidelines for promoting optimal human health. This effort has contributed in part to a revived interest in the dietary practices of ancestral humans and hunter-gatherer populations. Because contemporary humans essentially maintain the same genomic sequence as early human populations living 10-100 kya, researchers have contended that the only dietary pattern appropriate for humans is the blueprint provided by our hominid ancestors. In this dietary model, animal protein contributes the greatest proportion of calories to daily energy intake, supplemented by smaller amounts of complex carbohydrates. Due to the considerable amount of calories derived from animal meats, dietary fats likewise contribute a significant amount of calories to the Paleolithic Diet; however, because of the reliance upon lean game animals, these dietary fats are predominantly unsaturated.

As demonstrated by recent clinical studies, a high protein, low carbohydrate diet does indeed confer several important health benefits in the short term, including the stabilization of blood glucose levels and the normalization of serum cholesterol. This amelioration of the metabolic correlates of insulin resistance results from the higher thermic effect for animal protein, which requires more energy for digestion, balanced by the intake of minimally processed carbohydrates. In this combination, carbohydrates negate the acid-producing effects of a high protein diet. For these reasons, researchers

have questioned the validity of recent dietary guidelines which attribute the recent increase in overweight/obesity and the metabolic syndrome to the consumption of red meat. Instead, anthropologists have suggested that returning to our hunter-gatherer roots, by using high levels of lean animal meats complemented by fresh sources of carbohydrates may provide the answer for improving human health in the twenty-first century.

Indeed, the research protocol designed for this study was developed within the constraints of this framework, in the assumption that traditional diets from developing populations acquire their health benefits by best reflecting the dietary practices of our early human ancestors. However, as the data outcomes reveal, both the traditional Thai diet and the nutrient profiles of the study subjects diverge significantly from the values expected for the basic Paleolithic diet in important quantitative ways. In particular, the traditional Thai diet contrasts with the typical hunter-gatherer diet due to its dependence upon high quantities of unrefined carbohydrates and a low reliance upon animal protein. Though the dietary preferences of the Thai controls also lacked a statistical correlation with either of these dietary models, the nutritional profile for the healthy subjects recruited for this study still reflect an essentially traditional Thai diet due to several qualitative features- specifically the use of fresh, minimally processed fruits and vegetables and the derivation of dietary proteins from fish and mollusks.

This lack of concordance between the traditional Thai diet, the basic Paleolithic model, and the nutritional profile of the healthy Thai controls suggests that instead of a single, overarching nutritional approach to optimal human health, several different models exist. Perhaps even more importantly, rather than striving to find the perfect

quantitative recipe for human health via a precise combination of macronutrients, phytochemicals, vitamins and minerals, public health researchers need to utilize a qualitative approach to nutrition which looks at food in terms of its overall quality and health benefits. Within this framework, nutritional guidelines for optimal human health represent conditionally-based recommendations based upon a combination of both individual and broader ecological factors.

This conceptual approach to nutrition and health represents the theoretical praxis of nutritional ecology, which addresses human health via the complex interaction between diet, somatic maintenance, and physical wellbeing within the constraints of the natural environment (Hockett and Haws 2003; Raubenheimer et al 2009). Using this theoretical basis, human health is determined neither by net caloric intake nor by strict macronutrient ratios, but rather by consuming a diverse diet capable of providing the dozens of essential nutrients necessary to achieve optimal bodily function and health conditions in humans (Hockett and Haws 2003; Ulijaszek 2002; Leitzmann 2005). And because the human body rarely relies upon a single nutrient to perform its vital functions, consuming a diversity of foods in order to obtain these synergistically active dietary compounds represents an important qualitative feature of any guideline for a healthy diet. Consequently, the ability to acquire these nutrients in the absence of a global economy is determined in part by the ecological resources available to each population.

In Thailand, the tropical environment restricts the availability of animal protein derived from large game or domesticated animals, but in exchange provides an abundance of freshwater fish from inland estuaries and seasonally available fruits and vegetables derived from both forest and riverine environments. Perhaps even more

importantly, because the Thai tropical environment likewise increases the number of potential pathogens and predators with the capacity to affect the reproductive fitness of these plant species, the fruits and vegetables available to Thais have developed intricate chemical compounds necessary for their survival. These phytochemicals, found in significant concentrations in Thai medicinal plants, play a similar role within the human body, by neutralizing free radical oxidative species and repairing cell damage due to toxins, pathogens and teratogens. Therefore, while the nutritional use of Thai medicinal plants represents a cultural adaptation characteristic of Southeast Asian populations, this practice is also enabled in part by the unique environmental conditions which have shaped plant evolution in the region. In other words, the cultural practice of using medicinal plants within a nutritional context is made possible in part by the tropical environment in which these plant species have evolved.

As suggested by this relationship in Thailand between dietary intake and ecology, the environment shapes not only the physiological adaptations made by the organism in response to its habitat, but also the nutritional resources available to that individual. In this sense, we can talk about „dietary efficiency“ as a necessary adjunct to reproductive fitness when examining the human condition. Within this conceptual framework, the capacity to attain optimal somatic function and health is a product of dietary efficiency, or the ability to obtain the full suite of nutrients necessary to maintain the human body regardless of the net energy return from calories. Dietary efficiency in turn is determined by the types of food resources made available by specific environmental conditions. Therefore, dietary efficiency can be measured in individuals by examining the capacity of organisms to utilize as diverse a complement of food sources as made possible by the

environment in which that individual lives. Consequently, individuals consuming a diet consisting of diverse food sources would possess more dietary efficiency than individuals utilizing only a limited number of food options. In the context of this study, the healthy Thai controls would be classified as having greater dietary efficiency due to their utilization of more diverse food sources- namely traditional medicinal plants, than the breast cancer cases.

As suggested by the nutritional ecology framework and the concept of dietary efficiency, nutritional recommendations for achieving optimal human health should focus more on the qualitative properties of foods rather than the net macro- and micronutrient returns provided by specific foods. Using these principles to develop nutritional guidelines for both cancer prevention and optimal human health, the following recommendations based on the results from this study have been developed using qualitative, rather than quantitative, approaches to dietary efficiency:

- 1) Utilize seasonally available fruit and vegetable species.
- 2) Choose plant species which are locally available.
- 3) Favor minimally processed foods over highly refined food options.
- 4) Consume animal products which are lean or which contain high levels of omega-3 fatty acids, such as fish and shellfish.
- 5) Limit the use of discretionary oils and sugars.
- 6) Obtain essential micronutrients from whole foods rather than from nutritional supplements.
- 7) Prepare meals which contain diverse food sources, rather than fewer restricted options.
- 8) Limit the amount of dietary lipids obtained from saturated fats.

As reflected by these guidelines, dietary efficiency and optimal human health can be achieved by utilizing flexible recommendations which can accommodate different biological needs and environmental situations. Rather than representing a standardized nutritional guideline designed to fit every individual's dietary needs, these

recommendations allow for a certain degree of flexibility necessary to accommodate changing nutritional needs throughout the human lifecycle. Moreover, these recommendations take into consideration the ecological environment, by emphasizing the use of locally available resources in order to ensure that plant and animal food sources maintain the highest levels of micronutrient content possible during the transit between harvest and consumption. In essence, these dietary principles reflect the main characteristics of a diet based on the principals of nutritional ecology which confers the significant health advantages upon individuals practicing these nutritional profiles.

#### Future Research Considerations

The results from this study have provided important quantitative measures for determining the main factors contributing to breast cancer incidence in Thailand. These factors include the demographic transition, which has reduced parity among the breast cancer cases, and the globalization of food choices, which has replaced the nutritional use of Thai medicinal plants with refined carbohydrates and a greater reliance upon dairy products, commercial meats and discretionary fats. In particular, this study highlighted the importance of medicinal plant use as a key cultural component of the traditional Thai diet. Among the healthy Thai controls, Thai medicinal plants were consumed in higher quantities and represented the primary dietary component contributing to a reduction in breast cancer risk.

Because previous epidemiological studies have yielded inconsistent results in terms of the effects of specific macro- and micronutrients upon breast cancer risk, the absence of statistically significant associations in this study (with the exception of unrefined carbohydrates and vitamin B<sub>2</sub>) is not surprising. This lack of statistical

correlation may be due in part to smaller sample sizes and the inability to account for the effects of food preparation techniques upon nutrient availability. In future studies reexamining breast cancer incidence in Thailand, improvements upon this study would include measures of food preparation and a determination of the locations from which subjects purchase their foods, in order to determine the quality of consumed foods.

Aside from establishing the role of Thai medicinal plant use in providing the essential health benefits to the traditional Thai diet, this study also highlights the importance of utilizing qualitative methods for determining dietary recommendations to optimize human health. As demonstrated by the quantitative similarities in the dietary intake of the control and case subjects recruited for this study, food quality, rather than quantity, provides the most salient explanation for the protective effect provided by nutritional intake among the healthy controls. These qualitative features of the dietary intake characteristic of the Thai controls reflect similar elements found in both the Paleolithic and traditional Thai diets. Some of these qualities include the seasonal use of minimally processed fruit and vegetable species, the focus upon healthy sources of animal protein, and reliance upon unsaturated sources of dietary lipids.

As suggested by both the Paleolithic and traditional Thai diets, diverse nutritional options from local sources provide the full suite of essential nutrients required to sustain optimal human health. This complex relationship between human health, nutrition and the environment represents the main conceptual basis of nutritional ecology, which emphasizes the development of dietary principles grounded in food quality rather than food quantity. The concept of dietary efficiency reflects this importance of determining nutritional quality via the micronutrient content instead of the net caloric return provided

by foods. Within this framework, a concept of dietary efficiency can be established as a means for measuring the capacity of individuals to acquire the essential nutrients required to sustain optimal human health and development. From the perspective of biological anthropology, the concept of dietary efficiency provides a means for making nutritional recommendations which take into account not only the individual's needs throughout the human lifecycle, but also the constraints placed by the environment upon available food resources.

In the future, as researchers continue to address disease prevention from the perspective of nutrition, the use of a nutritional ecological approach emphasizing dietary efficiency over standardized recommendations represents a more salient approach to improving human health. By utilizing this approach, the beneficial aspects of food consumption are emphasized as integral components of human health, thereby making dietary intake a complementary approach to traditional pharmaceutical methods for enhancing the human condition. Rather than focusing upon the negative connotations associated with dietary intake, this emphasis upon foods as contributors to optimal human health provides an important means for understanding population differences in disease expression. As researchers continue to investigate the health consequences of different dietary models practiced throughout the world, the use of the nutritional ecological model and the concept of dietary efficiency will provide scientists with the means for understanding not only the etiology of many non-communicable chronic diseases, but also the qualitative features which provide traditional diets with their main health benefits.

Appendix A  
Enrollment Questionnaire

ส่วนที่ 1: ข้อมูลประชากร

1. คุณเกิดวันที่เท่าไร

\_\_\_\_ / \_\_\_\_ / \_\_\_\_  
(วัน) (เดือน) (ปี)

2. ขณะที่คุณอยู่ที่กรุงเทพมหานครใช่ไหม

\_\_\_\_  
ใช่    ไม่ใช่

3. คุณอยู่ที่กรุงเทพมหานครกี่ปี

\_\_\_\_\_

4. คุณจบการศึกษาระดับอะไร

\_\_\_\_\_

5. คุณทำงานหรือเปล่า

\_\_\_\_  
มี    ไม่มี

5(a). คุณทำงานเกี่ยวกับอะไร

\_\_\_\_\_

6. คุณทำงานเดือนละเท่าไร

\_\_\_\_\_

## ส่วนที่ 2: ข้อมูลทางการแพทย์

7. คุณมีประจำเดือนครั้งแรกอายุเท่าไร  
\_\_\_\_\_ ปี

8. คุณเคยตั้งครรภ์ไหม  
\_\_\_\_\_  เคย  ไม่เคย

8(a). คุณครรภ์ครั้งแรกอายุเท่าไร  
\_\_\_\_\_ ปี

8(b). คุณมีลูกกี่คน  
\_\_\_\_\_ คน

9. คุณเคยให้ลูกทานนมจากเต้าไหม  
\_\_\_\_\_  เคย  ไม่เคย

9(a). คุณให้ลูกทานนมจากเต้านานกี่เดือน  
\_\_\_\_\_ เดือน

10. ประจำเดือนของคุณหมดแล้วหรือยัง  
\_\_\_\_\_  หมด  ยังไม่หมด

10(a). คุณประจำเดือนหมดอายุเท่าไร  
\_\_\_\_\_ ปี

10(b). คุณน้ำหนักเท่าไรตอนประจำเดือนหมด  
\_\_\_\_\_ กิโลกรัม

11. มีใครคนในครอบครัวเป็นมะเร็งเต้านมไหม  
\_\_\_\_\_  มี  ไม่มี

11(a). คนในครอบครัวที่เป็นมะเร็งเต้านมเป็น:

- คุณแม่
- น้อง/พี่สาว
- ป้า/น้าผู้หญิง
- ยาย/ย่า

12. คุณเคยเป็นมะเร็งเต้านมไหม

เคย  ไม่เคย

12(a). คุณเป็นมะเร็งเต้านมมานานเท่าไร

เดือน

13. คุณสุขสบายหรือเปล่า

สุข  ไม่สุข

13(a). คุณสุขสบายวันละกี่ม้วน

- 1 ถึง 5 ม้วน
- 6 ถึง 10 ม้วน
- 10 ถึง 15 ม้วน
- มากกว่า 16 ม้วน

14. ตอนคุณอายุ 18 ปีน้ำหนักของคุณเท่าไร

กิโลกรัม

### ส่วนที่ 3: ข้อมูลโภชนาการ

คุณทานอาหารต่อไปนี้เดือนละกี่ครั้ง

อาหาร	กินกี่ครั้ง					
	ไม่เคย	1 ครั้งต่อสัปดาห์	2-4 ครั้งต่อสัปดาห์	5-6 ครั้งต่อสัปดาห์	ทุกวัน	1-3 ครั้งต่อเดือน
<b><u>ส่วนที่ 1: เนื้อสัตว์</u></b>						
ไก่						
เป็ด						
เนื้อวัว						
เนื้อหมู						
ปลาดุก						
ปลาช่อน						
ปลากะพงแดง						
ปลาทุ						
กุ้ง						
ปู						
ปลาหมึก						

หอย							
หอยนางรม							
หอยแมลงภู่							
<b><u>ส่วนที่ 2: ผัก</u></b>							
บวบเหลี่ยม							
หน่อไม้							
หัวปลี							
ถั่วงอก							
มะระ							
พริก							
ผักคะน้า							
ดอกกุยช่าย							
กุยช่าย							
ผักกาดกวาดตั้ง							
หน่อเลียง							
ผักบุ้ง							
แตงกวา							
หัวไชเท้า							
มะเขือยาว							

หัวหอมต.น							
เห็ดโคน							
เห็ดหูหนู							
ผักกาดขาว							
กระเจี๊ยบ							
ผักกาดดอง							
ฟ.กทองเทศ							
ฟ.กเทศพ.ม							
ฟ.กทองพื้นเมือง							
หอมแดง							
บวบหอม							
ดอกฟ.กทอง							
เผือก							
พริกชี้หนู							
ตำลึง							
แห้ว							
ถั่วพู							
ถั่วฝักยาว							
<b><u>ส่วนที่ 3: ผลไม้</u></b>							

กล้วย							
มะพร้าว							
ทุเรียน							
ฝรั่ง							
ขนุน							
มะนาว							
ลำไย							
มะม่วง							
มะละกอ							
สับปะรด							
ทับทิม							
ส้มโอ							
เงาะ							
มะขาม							
แตงโม							
ชมพู่							
ส้ม							
กระท้อน							

มังกุด							
น้อยหน่า							
ลิ้นจี่							
แก้วมังกร							
<b><u>ส่วนที่ 4: เครื่องเทศ</u></b>							
กระวานขาว							
ผักชี							
ยี่หระ							
ข่า							
กระเทียม							
ขิง							
ตระไคร้							
สะระแหน่							
กะเพรา							
โหระพ้า							
ขมิ้น							
กระชาย							
<b><u>ส่วนที่ 5: น้ำตาลและน้ำมัน</u></b>							
ชีอิ้วดำ							

น้ำตาลทราย							
ซีอิ๊วหวาน							
น้ำปลา							
น้ำมันพืช							
น้ำตาลปีบ							
น้ำมันถั่ว							
กะปิ							
ซีอิ๊วขาว							
น้ำมันหอย							
<b><u>ส่วนที่ 6: ก๋วยเตี๋ยว และข้าว</u></b>							
วุ้นเส้น							
ปะหมี่							
ก๋วยเตี๋ยว							
ขนมจีน							
แป้งข้าวเจ้า							
แป้งข้าวสาลี							
แป้งมัน							
ข้าว							
ข้าวเหนียว							

ข้าวกล้องแดง							
ขนมปังสีข้าว							
ขนมปังแบ่งหมี							
<b><u>ส่วนที่ 7: ถั่วเหลือง</u></b>							
เต้าหู้							
น้ำเต้าหู้							
<b><u>ส่วนที่ 8: เครื่องดื่ม</u></b>							
ชา							
กาแฟ							
น้ำกระเจี๊ยบ							
น้ำมะพร้าวอ่อน							
โซดา							
น้ำผลไม้							
<b><u>ส่วนที่ 9: นมและไข่</u></b>							
นํ้านม							
นมเปรี้ยว							
ไข่							
เนยแข็ง							

**Appendix B**  
**Ethics Committee Approval Mahidol University**



คณะแพทยศาสตร์ โรงพยาบาลรามาธิบดี มหาวิทยาลัยมหิดล  
ถนนพระราม 6 กทม. 10400  
โทร. (662) 354-7275, 201-1296 โทรสาร (662) 354-7233  

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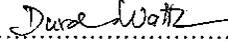
Faculty of Medicine, Ramathibodi Hospital, Mahidol University  
Rama VI Road, Bangkok 10400, Thailand  
Tel. (662) 354-7275, 201-1296 Fax (662) 354-7233

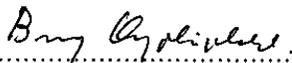
**Documentary Proof of Ethical Clearance Committee on Human Rights  
Related to Researches Involving Human Subjects  
Faculty of Medicine, Ramathibodi Hospital, Mahidol University**

No. MURA2006/333

<b>Title of Project</b>	The Nutritional Use of Thai Medicinal Plants and The Etiology of Breast Cancer in Thai Women
<b>Protocol Number</b>	ID 09 – 49 – 22
<b>Principal Investigator</b>	Miss Joanna Tatomir
<b>Official Address</b>	Department of Anthropology The University of Michigan

*The aforementioned project has been reviewed and approved by Committee on Human Rights Related to Researches Involving Human Subjects, based on the Declaration of Helsinki.*

<b>Signature of Secretary</b>	
<b>Committee on Human Rights Related to Researches Involving Human Subjects</b>	Assoc. Prof. Duangrudee Wattanasirichaigoon, M.D.

<b>Signature of Chairman</b>	
<b>Committee on Human Rights Related to Researches Involving Human Subjects</b>	Prof. Boonsong Ongphiphadhanakul, M.D.

<b>Date of Approval</b>	November 15, 2006
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**Appendix D**  
**University of Michigan Human Subjects IRB Approval**



Health Sciences Institutional Review Board (IRB) • 540 East Liberty Street, Suite 202, Ann Arbor, MI 48104-2210 • phone (734) 936-0933 • fax (734) 998-9171 • irbhsbs@umich.edu

**To:** Ms. Joanna Tatomir

**From:** Charles Kowalski

**Cc:**

Andres Frisancho  
Joanna Tatomir

**Subject:** Initial Study Approval for [HUM00001149]

**SUBMISSION INFORMATION:**

Study Title: The Nutritive Use of Thai Medicinal Plants and the Etiology of Breast Cancer in Thailand

Full Study Title (if applicable):

Study eResearch ID: [HUM00001149](https://eresearch.umich.edu/HUM00001149)

Date of this Notification from IRB: 6/5/2007

Initial IRB Approval Date: 7/9/2006

**Current IRB Approval Period: 7/9/2006 - 7/8/2007**

**Expiration Date:** 7/8/2007

UM Federalwide Assurance (FWA): FWA00004969 expiring on 5/10/2009

OHRP IRB Registration Number(s): IRB00000245

**NOTICE OF IRB APPROVAL AND CONDITIONS:**

The IRB Health Sciences has reviewed and approved the study referenced above. The IRB determined that the proposed research conforms with applicable guidelines, State and federal regulations, and the University of Michigan's Federalwide Assurance (FWA) with the Department of Health and Human Services (HHS). You must conduct this study in accordance with the description and information provided in the approved application and associated documents.

**APPROVAL PERIOD AND EXPIRATION:**

The approval period for this study is listed above. Please note the expiration date. If the approval lapses, you may not conduct work on this study until appropriate approval has been re-established, except as necessary to eliminate apparent immediate hazards to research subjects. Should the latter occur, you must notify the IRB Office as soon as possible.

**IMPORTANT REMINDERS AND ADDITIONAL INFORMATION FOR INVESTIGATORS**

**APPROVED STUDY DOCUMENTS:**

You must use any date-stamped versions of recruitment materials and informed consent documents available in the eResearch workspace (referenced above). Date-stamped materials are available in the "Currently Approved Documents" section on the "Documents" tab.

**RENEWAL/TERMINATION:**

At least two months prior to the expiration date, you should submit a continuing review application either to renew or terminate the study. Failure to allow sufficient time for IRB review may result in a lapse of approval that may also affect any funding associated with the study.

**AMENDMENTS:**

All proposed changes to the study (e.g., personnel, procedures, or documents), must be approved in advance by the IRB through the amendment process, except as necessary to eliminate apparent immediate hazards to research subjects. Should the latter occur, you must notify the IRB Office as soon as possible.

**AEs/ORIOs:**

You must inform the IRB of all unanticipated events, adverse events (AEs), and other reportable information and occurrences (ORIOs). These include but are not limited to events and/or information that may have physical, psychological, social, legal, or economic impact on the research subjects or others.

**SUBMITTING VIA eRESEARCH:**

You can access the online forms for continuing review, amendments, and AEs/ORIOs in the eResearch workspace for this approved study (referenced above).

**MORE INFORMATION:**

You can find additional information about UM's Human Research Protection Program (HRPP) in the Operations Manual and other documents available at: [www.research.umich.edu/hrppp](http://www.research.umich.edu/hrppp).



**Charles Kowalski**  
Chair, IRB Health Sciences

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