

DIET, PHYSICAL ACTIVITY AND BONE MINERAL
DENSITY OF POST-MENOPAUSAL SAUDI WOMEN
AND DEVELOPMENT OF A CULTURALLY
ACCEPTABLE FOOD PRODUCT

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Submitted to the Faculty of the
Graduate College
Oklahoma State University
in partial fulfillment of
the requirements for
the Degree of
DOCTOR OF PHILOSOPHY
May, 2020

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ACKNOWLEDGEMENTS

First and foremost, praises and sincere gratitude to Allah, the Omnipotent, who provide me with the supports, good health, and power to achieve success in the accomplishment of this dissertation. Through the Ph.D.'s journey, I have acquired a magnificent and immeasurable opportunity to obtain unlimited knowledge and education that will have an impact on my life and career in the future.

My sincere thanks go to the Food Science Department for offering the chance for me to be a member of their community, to learn and gain valuable professional experience. I am extending my profound thanks and immeasurable appreciation to my supervisor whom I owe a magnificent debt for giving me the benefit of her rich knowledge, perceptive advice, and precious support. My profound gratitude to my committee members Dr. William McGlynn for his cooperation with me throughout my graduate study and Dr. Ranjith Ramanathan for his assistance in guiding me on the right path to complete the dissertation. I would like to thank Dr. Tim Bowser for his collaboration and precious support.

Special appreciation goes to my loved parents, my father, Dr. Salem Alharbi, and my mother, Mrs. Salmah Alhazmi, for giving me the support and surround me with their love. Nothing can express my appreciation for their perseverance and efforts to help me make my dream reality. I want to express my thanks for my brothers Dr. Mohmmmed,

Raid, and Yaser, for making me believe in myself and my sisters Ashwag and Rawan for their persistence prayers and encouragement throughout my Ph.D.

From my heart bottom, I owe my loving thanks to my husband Rakan for honoring my life by his pleasing existence and adding a lovely measure of his spirit to my presence. My children Badr, Kenan, and Ryan, who made my life brighter and making me smile even in hard times.

Great appreciation to the Hospital of King AbdulAziz University for the great collaboration and easing any obstacles that might be experiencing during my collecting data. My appreciation and thanks go to the technical director of the radiology department Mr. Bandr Almutairi for his help and provision of the professional equipment needed throughout the collecting data.

Date of Degree: May, 2020

Title of Study: DIET, PHYSICAL ACTIVITY AND BONE MINERAL DENSITY OF POST-MENOPAUSAL SAUDI WOMEN AND DEVELOPMENT OF A CULTURALLY ACCEPTABLE FOOD PRODUCT

Major Field: FOOD SCIENCE

Abstract:

This study seeks to determine whether higher dietary calcium and vitamin D intakes and increased physical activity are associated with improved bone mineral density (BMD) in Saudi post-menopausal females. A total of 150 women participated in the study. Physical activity questionnaires and three-day food records were used to assess participants' daily routine activities and dietary habits. The levels of physical activity were also tracked over three days using a Letsfit watch, which counted each participant's daily steps. BMD was evaluated using a DXA scan. The findings of the study revealed high osteopenia and osteoporosis prevalence among the participants, estimated at 43.3% and 23.3%, respectively. The study also showed low sunlight exposure and low dietary intake of vitamin D in the participants. There was a positive association between BMD and the number of previous pregnancies and a negative association with the number of years after menopause. There was an observed negative association between daily vitamin D consumption and BMD. However, the result showed no associations among calcium intake, physical activity and BMD.

For centuries, cow's milk was used as a source for calcium and vitamins. However, some people have lactose intolerance or an allergy to cow milk, and they seek alternative products to avoid these health problems. The second part of this study aimed to develop a gluten, dairy, and soy-free product that would be culturally acceptable in the Middle East. The product developed was entirely plant-based, with the primary ingredients being almond (25%), dates (5%), and figs (5%). Laboratory analysis was performed of the developed almond milk product and an existing commercial product (Silk almond milk), evaluating their different compositional properties. The commercial almond milk displayed higher levels of moisture, calcium, vitamin D, ash, and pH. Levels of potassium, carbohydrates, fat, sugar, and protein were higher in the developed product. A comparison between the two products was made using sensory evaluation, with the descriptive sensory evaluation showing that consumer acceptance for the developed product was higher than for the existing commercial product. However, fortification of the developed almond milk product with calcium is recommended.

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PART ONE

**DIET, PHYSICAL ACTIVITY AND BONE MINERAL DENSITY OF
POST-MENOPAUSAL SAUDI WOMEN**

CHAPTER I

INTRODUCTION

1.1 Background

The post-menopausal phase represents a significant change in the life of every woman, marking the end of her reproductive life. Several biological, physical, and psychological changes occur at the onset of menopause. One of these fundamental changes is a decrease in the density of bone mineral (BMD). Low BMD is described as a skeletal status characterized by decreased BMD, leading to increasing weakness. It makes individuals more susceptible to bone fractures. Decreased BMD, when less severe, can be classified as osteopenia. Osteoporosis and osteopenia are indications of structural deterioration resulting in increased bone fragility (Alquaiz et al., 2014).

Osteoporosis alters musculoskeletal function, weakening bones, and increasing the risk of fracture (Veldurthy et al., 2016). Evidence shows that the incidence of fractures related to osteoporosis is very high in both the Middle East and Africa. The population of both regions is aging, with approaching 25% over the age of 50 in 2020. This is forecast to rise to 40% by 2050. This is likely to mean that expected rates of osteoporosis associated fractures quadruple in several countries (Vijayakumar & Büsselberg, 2016).

The imbalance between the activities of osteoblasts (bone formation cells) and osteoclasts (bone resorption cells) contribute to the high risk of fracture, at least for some fragility fractures. It is also caused by a low level of estrogen production due to aging or removal of the ovaries. Estrogen promotes the activity of osteoblasts: the cells that produce bone (Alkhenizan et al., 2017). The mechanism by which low estrogen causes bone loss is complex since estrogen has fundamental effects on bone metabolism through the immune system, oxidative stress, and directly through bone cells. Other causes include alcoholism, kidney disease, and the use of medication that alters calcium metabolism. Such medication includes chemotherapy for cancer, proton pump inhibitors, and glucocorticosteroids. Osteoporosis can be present for many years without any signs or symptoms prior to the assessment of bone mineral density.

Osteoporosis causes severe deterioration and suffering in life quality for those affected. The illness presents itself in various ways, the most dangerous of which are life-threatening fractures following falls or other trauma. The most common include fractures of the neck of the femur and spinal or vertebral fractures, all of which can cause disability (Alkhenizan et al., 2017). Spinal fractures are a significant cause of disability among this group due to the severe, radiating pain caused by the stimulated pain-related nerve endings at the vertebra where band-like fractures occur. Fractures of the hip or femur may cause impaired physical movement resulting in the inability to perform everyday activities and domestic tasks, including cooking and walking. These results suggest that low BMD is a significant risk element for fractures and quality of life (Schuit et al., 2004).

1.2 Prevalence of Osteoporosis

Low BMD has become a very significant worldwide public health issue. According to International Osteoporosis Foundation statistics (NOF), more than two hundred million adults aged 50 and over have been diagnosed with osteoporosis. In the USA, nearly ten million people suffer from osteoporosis, of which eight million are women (Wright et al., 2014). In the Arab world, the statistical picture varies due to the high prevalence of osteoporosis and osteopenia. For example, the prevalence rate among Kuwaiti women aged fifty and over has been reported at 9.9% for osteoporosis and 26.8% for osteopenia (Mahboub, Al-Muammar & Elareefy, 2014). The same paper reports that Saudi females aged fifty and over had elevated rates compared to Kuwaiti females. 40% had lumbar spine osteoporosis and 31% lumbar spine osteopenia. These differences can be attributed to the cultural and religious believes of most Saudi Arabian women, who do not appear in public without covering the whole body, whereas Kuwaiti women have more freedom (Urkevich, 2014).

An essential element of further research should be the development of a better understanding of the underlying risk factors and how to create an effective response in the form of an evidence-based intervention plan.

Current research shows that post-menopausal women, after a duration of five to seven years, might experience approximately 3-5% bone loss per year (Mahboub, Al-Muammar, & Elareefy 2014). This bone loss might be attributed to a lack of estrogen and not to the aging process itself. For Saudi women, a robust association between low level of

literacy, most housewife's lifestyles, and osteoporosis was found. It is suspected that not enough attention is paid to critical nutritional matters by those with these characteristics. For effective intervention and prevention, it is essential to identify osteoporosis risk at as early a stage as possible. Assessing the impact of intake of calcium and vitamin D and levels of physical activity on BMD in Saudi female adults, according to DXA measurements, is one crucial way to identify deficiencies and provide data for intervention plans.

1.3 Risk Factors

Inadequate dietary calcium and vitamin D intakes are major factors that can result in BMD and osteoporosis. Both vitamin D and calcium are vital to bone mass development. Calcium is the most important mineral involved in bone remodeling and maintaining adequate bone mass, and a primary vitamin D function enhances the absorption of calcium from the intestine. The function of vitamin D in its active form, which is 1,25 dihydroxyvitamin D₃, is to stimulate the active trans-cellular pathway of calcium ion absorption (Christakos, Dhawan, Porta, Mady & Seth, 2011). The active trans-cellular pathway of absorption of calcium from the intestines involves calcium influx and occurs by the translocation and basolateral extrusion of calcium ions by the calcium ion channels located on the intestinal membrane. Therefore, inadequate intake of foods high in vitamin D creates a calcium ion imbalance in the body, which leads to dysregulation of 1,25 dihydroxyvitamin D₃ functioning in the absorption of calcium (Veldurthy et al., 2016).

Poor bone mass retention due to the lack of physical activity and exercise among aging women can also contribute to low BMD (Alkhenizan et al., 2017). A majority of the

affected women complain of symptoms such as aches in their joints and muscles while walking or performing physical activities (Alateeq, Almutairi & Al-Dughaiter, 2015). A reduction in physical activity due to the aging process weakens musculoskeletal function, increasing the risk of bone fractures.

Parathyroid gland disorders can also contribute significantly to low BMD and osteoporosis. This health condition results from the excessive production of parathyroid hormone, which increases serum calcium ion levels through resorption of bone tissue, leading to the transfer of calcium from bones to the blood (Alkhenizan et al., 2017). The resorption of bones caused by excessive parathyroid hormone contributes to low BMD and the development of osteoporosis.

Research on a possible link between low physical activity levels and deficiency in dietary calcium consumption in Saudi women, leading to osteoporosis, is limited. The North Africa and Middle East National Consensus Group on Osteoporosis and the US National Osteoporosis Foundation have both stated that lack of physical activity, previous personal fracture history, menopause, smoking, small frame, alcohol consumption, family history of fractures and bone diseases, being on corticosteroid treatment, and having rheumatoid arthritis are the chief risk factors for low BMD (Alquaiz et al., 2014). Mahboub, Al-Muammar and Elareefy's (2014) study of women's health in Saudi Arabia identified several main risk factors. They included age, physical activity, low dietary consumption of calcium, and low serum level of 25- (OH) vitamin D (Mahboub, Al-Muammar & Elareefy 2014).

One recent study in the UAE showed a strong association between the BMD of osteoporosis patients and the intake of calcium, amount of physical activity, and age at the onset of menopause. In addition, a negative correlation was found with caffeine and nicotine intake. Compared to the comparison group that did not have osteoporosis, the finding showed that osteoporosis patients had a lower BMI, lower consumption of calcium, and shorter sun exposure time than those without osteoporosis (Ibrahim, Nabil & Ghaleb, 2019).

1.4 BMD Measurement

BMD examination can be done using dual-energy x-ray absorptiometry (DXA) to measure the mass of mineral per volume of bone. This is widely considered to be the most effective approach used by clinicians. The evaluation of BMD in the management of bone health in females provides an overview of mineral concentration in major bones such as the femur and spine. Although it has limitations, as a non-invasive technique, BMD measurement has been acknowledged as a useful and valuable diagnostic instrument. Gains in BMD are a direct reflection of overall bone strength, though the bone size, shape, architecture, and turnover also play a role.

Based on World Health Organization (WHO) definitions, a measurement of BMD that is 2.5 standard deviations or more below that of the reference population is considered to be osteoporosis (Salamat et al., 2008). In addition, DXA results suggest a general overview of the nutritional status as well as the ability of the body's metabolism to maintain mineral homeostasis in the bones. The results from the DXA measurement of BMD can be used to propose the quantity of supplementary calcium and vitamin D needed in the diet.

1.5 Statement of the Research Problem

Research studies of Saudi women at the onset of menopause have shown that they experience serious problems, including losing bone at a rate of nearly 3% a year. This is imputed to the loss of estrogen, although the mechanisms are inconspicuous (Mahboub, Al-Muammar & Elareefy, 2014). The osteoporosis rate is high in Arab nations (Maalouf et al., 2007). The impact includes reduced mobility and high medication costs, with negative economic consequences. The prevalence of osteoporotic BMD of the lumbar spine and femoral neck of post-menopausal Kuwaiti females has been reported as 20.2% and 12.5 %, respectively. In the same sample population, osteopenia BMD of the lumbar spine and femoral neck was detected in 35.4% and 42.8 %, respectively, indicating that taken together, more than half of the post-menopausal women are affected (Al-Shoumer & Nair, 2012).

One study in Saudi Arabia stratified the spread of osteoporosis and osteopenia by age in decades in post-menopausal Saudi women. Among women aged 50-59, 42.3% were normal, 33.4% had osteopenia, and 24.3% had osteoporosis. Among those aged 60-69, 11% were normal, 27% had osteopenia, and 62% had osteoporosis. The prevalence was even higher among those aged 70-79 years, with only 4.6% in that group normal. 21.5% had osteopenia, and 73.8% had osteoporosis (El-Desouki, 2003). Given the high diversity in the Saudi community, a trial was conducted to assess the osteoporosis prevalence using the arithmetical model of the WHO plan for osteoporosis detection. The estimated prevalence

among women in Saudi Arabia aged group 50-70 years was approximately 23% (Greer, Ahmed, Rifai & Sandridge, 2008).

Saudi Arabia is one of the countries most affected by low BMD and osteoporosis. Pre-menopausal Saudi females have a high prevalence of osteopenia, reported at 24.8%, which is a public health issue necessitating early intervention designed to prevent osteopenia from developing into osteoporosis with age (Arrabal-Polo et al., 2013). Elevated low bone mineral density prevalence rates amongst Saudi women is a matter of serious concern, leading to reduced productivity and increased healthcare costs, and so affecting both the community and the economy. It is important to explore alternative strategies in order to identify evidence-based interventions that could potentially reverse this trend.

Even though safe therapies are available for the management of low BMD, leading to a reduction in fractures, most individuals do not show early symptoms and thus remain undiagnosed and untreated. The majority of those affected in Saudi Arabia have had fractures at one point in their lives but are neither identified nor treated adequately. International organizations have frequently recommended screening for the purposes of making an early diagnosis of low bone mineral density. However, until 2005, Saudi Arabia did not have screening criteria specifying who should be screened, which modality to use, and what age to screen (Beaudoin & Blum, 2005; Zeidan et al., 2016). Screening can provide the necessary background for future researchers to make informed decisions and implement evidence-based interventions for Saudi women, which consider an important health policy issue.

1.6 Goals and Specific Aims

GOAL: To assess the prevalence of osteopenia and osteoporosis in a sample of post-menopausal Saudi women.

GOAL: To determine whether dietary intake of calcium and vitamin D is associated with improved bone mineral density in post-menopausal Saudi women.

SPECIFIC AIM 1: To determine the bone mineral density of post-menopausal women that is associated with a population that eats a healthy diet, compared to a population with an inadequate dietary intake of calcium and vitamin D, to determine whether a difference exists between the two. The sample will include these groups by virtue of the cross-sectional community-based nature of recruiting volunteer participants. The inclusion and exclusion criteria are discussed below in the methodology section.

GOAL: To determine whether levels of physical activity are associated with bone mineral density in post-menopausal Saudi women.

SPECIFIC AIM 1: To determine the bone mineral density of a population with a high level of intense physical activity and compare that with a population with a lower-level of physical activity to determine whether there is a difference, using dual-energy x-ray absorptiometry.

1.7 Purpose of the Study

The recent body of research suggests that physical activity combined with sufficient consumption of dietary calcium and vitamin D could be a useful intervention strategy for low BMD. While there are substantial studies on the impacts of calcium on BMD, more work to

examine the influence of physical activity that results in either limited or increased sunlight exposure is needed. The mechanism of interaction and correlation between physical activity and adequate diet has not been thoroughly investigated and hence remains unclear (Kamalanathan et al., 2014). An understanding of how a combination of exercise and adequate intake of dietary calcium and vitamin D can result in the prevention of osteoporosis offers the potential to lead to more effective intervention options. This project tests the hypothesis that adequate consumption of calcium and vitamin D, coupled with sufficient exercise and sunlight exposure, can help to increase BMD in Saudi women.

This is a cross-sectional, community-based study that was achieved at the Hospital of King Abdulaziz University, Jeddah, Saudi Arabia. It was conducted from July to October 2019. The study was conducted after King Abdulaziz University's Ethics and Research Committees approval and the Institutional Review Board of Oklahoma State University

CHAPTER II

REVIEW OF THE LITERATURE

2.1 Calcium Intake and Osteoporosis

Bone mineral acquisition can be enhanced through an adequate dietary intake of calcium, coupled with effective physical activity (Pongchaiyakul et al., 2008). Research conducted by Pongchaiyaku et al. (2008) showed that dietary calcium and calcium supplementation have a positive impact on bone mineral density in children and post-menopausal women. A research conducted by Al Quaiz et al. (2014) among post-menopausal women in Riyadh showed that over 60% had an inadequate intake of calcium while the population as a whole had an intake of lower vitamin D and calcium than the recommended daily allowance (RDA) values in Canada and USA (Alquaiz et al., 2014).

These findings confirm that adequate consumption of dietary vitamin D and calcium are very important to the development of strong bones and, thus, to the prevention of osteoporosis. This would favor the practice of supplementing these nutrients among Saudi women as an important public health priority.

Another study conducted in Saudi Arabia showed that women with low levels of education were unlikely to consume diluted yogurt, known as laban, which is an effective calcium source (Mahboub, Al-Muammar & Elareefy, 2014). Laban, a fermented milk product, is generally an important part of the Saudi diet and has widespread popularity and availability. There is very little lactose in this cultured milk product, meaning that even people with lactose intolerance are able to drink it.

Diet has strong links with lifestyle, as addressed in several previous research studies. These studies show that an ideal dietary intake of calcium has a beneficial protective impact. Calcium and vitamin D are fundamental nutrients that improve bone mass, as well as minimizing the loss of bone in post-menopausal females (Carey et al., 2007). Despite the fact that there are other nutrients (macro and micro) integrated into developing and maintaining bone, dietary calcium is known to be the major nutrient that influences bone mass to a greater extent than any other. The adoption of westernized lifestyles in developing countries leads to decreased levels of physical exercise, physical activity, and reduced sunlight exposure. This is also likely a contributing factor to BMD loss and higher rates of osteoporosis, and the same trend is likely to be seen in Saudi women.

A research study conducted by Pongchaiyaku et al. (2008) investigated the relationship between dietary calcium, bone mineral density, and biochemical bone turnover markers among 255 Thai women from rural areas. The first part of their study involved the monitoring of the women's usual dietary intake of calcium through a

quantitative food-frequency questionnaire and three-day food records. The investigators then used DXA to measure bone mineral density. Markers of bone turnover events, inclusively serum osteocalcin, serum total alkaline phosphatase, and type 1 collagen C-telopeptide, were found in half of the women studied. The findings of this research indicate that the participants consumed less dietary calcium per day than recommended, with a mean intake of 265 mg per day. Only three percent of the participants consumed more than 800 milligrams of calcium per day (Pongchaiyakul et al., 2008). The study also found that when age and body mass index were controlled for, those participants who consumed high amounts of dietary calcium had significant high bone mineral density at all measured bone locations.

Consequently, increased bone turnover markers were witnessed among those with the lowest calcium intake. The researchers, therefore, recommended a modification of eating patterns in order to increase the consumption of locally available food, which is rich in calcium, to prevent osteoporosis among the test population (Niafar et al., 2009). A recent study of educated and working pre- and post-menopausal women in Saudi Arabia found a strong correlation between bone mineral density and the number of dairy products consumed per day. It also found a strong correlation between BMD and exposure to sunlight with a more extended period of fertility (Cecily, 2018).

Food options and general dietary behavior are influenced by nutritional knowledge. In order to make bone-healthy food choices, people must be aware of which foods have a sufficient amount of vital calcium. Lack of nutritional education has been cited as one of the major contributing factors to issues of nutrition and malnutrition

among Saudi women. Many Saudi women lack knowledge of recommended calcium intake. They may also not be aware of the negative consequences of diseases associated with inadequate levels of dietary calcium. They may mistakenly perceive high calcium foods, such as dairy products, as fattening. Enhancing fact-based nutritional knowledge is, therefore, a very important step towards reducing osteoporosis prevalence among women in Saudi Arabia (Al-Shoumer & Nair, 2012). Knowledge of and attitudes towards osteoporosis influence osteoporosis-related preventive behaviors. It is hoped that this study will help raise awareness among the participant and their families and may generate further ideas on how to aid the dissemination of nutritional knowledge.

2.2 Vitamin D Intake & Osteoporosis

The incidence of hypovitaminosis (deficiency in vitamin D) increases with age. Lack of intake of vitamin D, coupled with insufficient sunlight exposure, can further compromise the body's ability to maintain bone growth (Al-Mogbel, 2012). The Middle East region, as a whole, consistently ranks as having among the highest vitamin D deficiency rates in the world, which Saudi Arabia is among those countries in which levels of vitamin D deficiency (defined as <50 nmol/l) have been reported to be greater than 35%. (Alkhenizan et al., 2017). The Saudi Diabetes and Endocrine Association has indicated that 30%-50% of women over the age of 40 develop osteoporosis. Their assertion is supported by multiple studies, including a large-scale study conducted in the Jeddah area of patients over age 40. This study found that 18% of participants had osteoporosis, and 40% had osteopenia. Only 7% reported taking regular exercise (Oommen & Alzahrani, 2014).

In another pilot study, Alissa et al. (2011) recruited 122 post-menopausal women from Jeddah's Center of Excellence for Osteoporosis Research to evaluate the impact of nutritional intake on bone mass. After administering lifestyle questionnaires and analyzing dietary intake, bone mineral density, and anthropometric measures were recorded. The findings conclude that the majority of the sample population had a serum vitamin D level of less than 50 nmol/L. Consequently, the study showed that there was a high risk of osteoporosis among the wider population of post-menopausal Saudi women due to low BMD as a result of low intake of vitamin D. By correlating vitamin D intake with the consumption of fatty acids, fiber, cholesterol, and total fat, the findings demonstrated the key role of low intake of vitamin D in the development of osteoporosis and the need for Saudi women to supplement this nutrient (Alissa, Qadi, Alhujaili, Alshehri & Ferns, 2011).

Similar results have been documented by Oommen and Al-Zahrani, who carried out a pilot study among 100 Saudi women aged 40 years and over (Oommen & Alzahrani, 2014). After data collection and analysis, the researchers found that 82% of the women participating in the study could be diagnosed with a deficiency in vitamin D (defined as less than 50 nmol/L). Despite Saudi Arabia having abundant sunlight, only 21% of the sample had nutritionally useful levels of exposure to sunlight. The findings coincide with Al-Mogbel's observation of 100% hypovitaminosis D prevalence among a group of 465 Saudi females aged 19 to 40 and otherwise healthy. Low bone mineral density was recorded among 58% of the women studied. Only 5% were found to have diets high in vitamin D, meaning that the high prevalence of low mineral density and

osteoporosis was linked to a larger vitamin D deficiency at $<25\text{nmol/L}$ (Oommen & Alzahrani, 2014 & Al-Mogbel, 2012).

Almost 20 previous studies have examined levels of serum 25-OH vitamin D in Saudi Arabian adults. Although there is much variability in the prevalence reported in each study, vitamin D deficiency is consistently shown to be high in the studies, ranging from between 41% and 100% in Saudi women, and from 32.5% to 92.6% in Saudi men (Bokhari & Albaik, 2019). A systematic review of the prevalence of vitamin D deficiency (lower than 50 nmol/L) in Saudi Arabia examined all studies carried out for a period of six years between 2011 and 2016 inclusive. This review concluded that the prevalence of vitamin D shortage across different groups (including pregnant and lactating women, newborns, infants, children, and adults) is 81.0%. This is similar to the prevalence seen in neighboring Gulf countries (Al-Daghri, 2018).

Osteoporosis among post-menopausal Saudi women is a major problem, exacerbated by a lack of awareness of predisposing factors, and the issue has been overlooked for a considerable length of time. Saleh Sedrani et al., (1990) revealed extreme levels of deficiency of 25-hydroxyvitamin D among Saudi adults and children, helping to bring this issue to attention as a public health concern (El-Hajj Fuleihan, Adib & Nauroy, 2011).

2.3 Physical Activity and Osteoporosis

Physical activity and regular exercise play a significant role in slowing down the progression of osteoporosis and preserving the structure of trabecular bones. These bones are present at the end of long bones and in the pelvic bones, ribs, and vertebrae (Blake & Fogelman, 2007). Earlier research has shown that exercise can be used strategically to reduce rates of osteoporosis, and other prophylactic interventions have also been studied extensively. The attainment of high bone mass is, to a great extent, related to genetic factors. To a lesser extent, it is influenced by environmental and conditional factors, including exercise. While the subject remains controversial, some studies indicate that physical activity has a substantially valuable effect on osteoporosis prevention. One view is that while physical activity may play a major part in the development of bone mass density, it is also critical in reducing bone mass loss (Al-Shoumer & Nair, 2012). Others state the opposite. Therefore, it is important to evaluate the combined impacts of calcium, vitamin D, and physical activity on BMD in order to develop an effective intervention strategy.

Until now, many factors that could potentially reduce the adverse effects of decreased bone mineral density have not been widely considered by Saudi women. Exercise is one of the most effective ways to increase BMD gradually and maintain it over time.

Alahmed and Lobelo (2018) highlight the benefits of physical activity in one Saudi Arabian primary care center and examine the significance of physical exercise in the promotion of musculoskeletal health and metabolism. They studied the influence of perceptions of physical activity and carried out a cost-benefit analysis. The study concluded that in post-menopausal Saudi women, physical exercise promoted musculoskeletal health by reducing the risk of hip fractures, increasing skeletal muscle mass, and intrinsic neuromuscular stimulation. This led to increased coordination of movement and a lower risk of falls (Alahmed & Lobelo, 2018).

The World Health Organization (WHO) has warned that increasing rates of osteoporosis are likely to be seen among Saudi women in the future. They suggest that weight-bearing exercises should be promoted as a way of slowing down the process of bone mineral loss among Saudi women (Habibzadeh, 2010). The WHO notes that increased exercise has a positive effect on increasing BMD in the lumbar spine and hip region in post-menopausal women. The same research also suggests that a combination of increased exercise and dietary calcium in younger women helps to significantly reduce later life fractures (Habibzadeh, 2010).

Preferences for different types of physical activity vary according to age group, and care should be taken to avoid excessive strenuous load-bearing exercises, especially for those who are already osteoporotic. The extent and degree of any exercise should be adapted to an individual's physical ability, age, and skeletal condition. While children and young adults are encouraged to engage in sporting activities, older women of 50 and above are encouraged to engage in approximately 30-50 minutes of brisk walking at a

suggested pace of 1-5 kilometers per hour (Al-Shoumer & Nair, 2012). This level of exercise is not only sufficient to positively affect BMD but also to reduce total body weight and to improve health more generally.

Moreira et al. (2014) conducted a review of research related to the relationship between physical exercise and osteoporosis in post-menopausal women, studying a variety of different types of exercise. The review considered the available literature up to February 2014. They found that a combined physical activity program, including resistance, impact, and aerobic exercise, enhanced bone mineral density by inducing changes in bone metabolism (Moreira et al., 2014). The review also found that osteogenic exercises resulted in an increase in BMD and contributed to the development of muscle strength and body balance. Improvements in physical function through increased bone mineral density also resulted in an increase in quality of life and a reduction in the incidence of falls and fractures (Moreira et al., 2014).

Weight-bearing on the bones in the course of a day's routine activities has been generally understood to aid bone deposition. Walking is a natural activity for humans and is always recommended to help protect the bone against loss. While previous research suggests a positive increase in BMD with exercise, the extent to which this variable affects BMD has not been established with clarity. The potential for a specific program of exercise to build BMD and reduce osteoporosis has attractive, practical value, but not enough studies have provided the groundwork. Despite the research gap, it has been established that BMD measurements are an important predictor of skeletal injury (Pongchaiyakul et al., 2008). However, not all physical activities have the same impact

on BMD. Weight-bearing exercise increases BMD to a much greater degree than non-weight bearing exercise, which has only a minor impact in comparison. This is because the loss of bone mineral mass often occurs in the limbs and trunk (axial) skeleton (Marques, Mota & Carvalho, 2011). Current research demonstrates that an investigation into the effects of specific physical activities on bone mineral density is needed in order to expand our current knowledge base.

The principle of bone remodeling as a result of mechanical loading is not well understood. Bone is a very dynamic tissue within the body of a living organism, adapting its mass and architecture to the physiological and mechanical environment. During adulthood, bone is constantly being renewed when bone architecture and mass are maintained through bone remodeling. The process of bone remodeling is believed to involve formation and resorption, a continuous process through which bone cells are removed and bone tissue is replaced. This process can result in osteoporosis, especially when there is an imbalance in the remodeling process. Osteoclast cells are involved in the removal of bone, while osteoblasts are involved in the formation of new bone (Alissa, Alnahdi, Alama, & Ferns 2014). These two cell types form the basic unit that controls bone remodeling and, thus, bone mineral density. Remodeling of bones often occurs at the main endo-cortical, intra-cortical, periosteal, and trabecular surfaces, but the rate is more intense in the trabecular than in any other part (Al-Shoumer & Nair, 2012).

Marques, Mota and Carvalho (2011) carried out a meta-analysis to evaluate the effects of exercise interventions on BMD in older people. They found a statistically significant rise in BMD by a mean value of 0.011g/cm³ for the lumbar spine and

0.016g/cm³ for the femoral neck, based on variable mixed load exercises lasting at least 16 weeks. These results indicate that exercise is essential to the development of increased BMD as a result of the role of exercise in stimulating bone metabolism (Marques, Mota, & Carvalho 2011). These findings lend support to the idea of using a program of resistance and endurance exercise to increase BMD, improve health, and increase the quality of life.

A few principles have been found helpful in increasing the osteogenic impacts of physical activity. We know that dynamic stimulation is better than static mechanical stimulation at inducing an adaptive bone response. Mechanical stimulation must exceed a threshold level of intensity to induce osteogenic effects on bone. The frequency and intensity of strain determine the threshold stimulation for bone maintenance. Bone mass is preserved either with a high-intensity mechanical stimulus (less frequently) or with low-intensity mechanical stimuli (more frequently). Abundant calcium and cholecalciferol availability, along with exercise, are required for an adaptive bone response to be seen, especially before puberty and after menopause. For optimum bone health, peak bone mass should be built early in life. In late menopause, calcium and cholecalciferol shortfalls become more damaging as the protective effect of estrogen is lost, leading to impaired calcium absorption and retention (Borer, 2005).

2.4 Dual-Energy X-ray Absorptiometry

Internationally recognized criteria have been set for the detection of osteoporosis during the post-menopausal period, using dual-energy X-ray absorptiometry (DXA) scan T-scores. The International Society for Clinical Densitometry (ISCD) has recommended

the utilization of Z-scores for diagnosis among women of different age groups. Even though an individual would expect Z-scores and T-scores to be extremely alike, particularly in young people, there are very few studies in support of this idea (Carey et al., 2007).

Some research has suggested that a T-score threshold of 2.5 may not be appropriate for all skeletal types, sites, and measurement techniques. This could be an indication of the variance in the prevalence of low BMD when different skeletal sites are measured. Research conducted by Carey et al. found that T-scores as a criterion could not be applied to all measurements of BMD (Carey et al., 2007). Differences in the prevalence of osteoporosis by this measure reflect a number of factors. These include factors age-related bone loss at various locations in the skeleton, variations in the youth and adult reference populations measured by different densitometry equipment, and other technology-related differences. It is, therefore, very important to provide a T-score standard particular to the kind of densitometry assessment performed. This is not only important to obtaining accurate information but also to develop an understanding of the prevalence levels that might necessitate intervention.

DXA scans have numerous clinical benefits when used to measure bone density, including fracture risk prediction, predictions of the effectiveness of anti-fracture treatments and interventions, and the monitoring and evaluation of responses to a given treatment. DXA scans also have consistent reference ranges, offer precise measurements and provide constant hip and lumbar spine DXA scan calibration. All these make them a reliable tool for observing BMD and the identification of at-risk populations (Blake &

Fogelman, 2007). A research study led by Salamat et al. using DXA among women, pre- and post-menopause, established that for both groups, a significant difference was found in women femoral neck and lumbar spine T-scores, with a value of -9.02 and -3.5 respectively at $p < 0.05$ (Salamat et al., 2008). This project confirmed that the difference in the standard deviation of these values could be attributed to a low daily intake of calcium and vitamin D, as well as minimal levels of physical activity.

CHAPTER III

METHODOLOGY

This is a cross-sectional, community-based study that was achieved at the Hospital of King Abdulaziz University, Jeddah, Saudi Arabia. It was conducted from July to October 2019. The study was conducted after King Abdulaziz University's Ethics and Research Committees approval and the Institutional Review Board of Oklahoma State University.

3.1 Data Collection and Confidentiality

A snowball sampling technique was utilized for this study, in which research subjects were used to help recruit additional volunteer participants from among their acquaintances, friends, and wider social circles. Recruitment efforts made by community leaders were especially effective. The snowball sampling technique, also called chain sampling, chain referral sampling, or referral sampling, is a non-probability sampling technique. Despite its limitations in making inferences from population-level data, this technique was used, as it is easy to use (via WhatsApp application) and is cost-effective

(Johnson, 2014). This study required an estimated 120-130 women aged 45-65 years who had reached the age of menopause, giving an eventual sample size of over 100. The total number of participants was 150 post-menopausal Saudi women. The study was performed over a period of eight weeks, with a maximum of 20 participants invited to participate in each week. The baseline study was divided into four days. On day one, Letsfit watches were distributed. On days one, two, and three, participants completed a questionnaire giving details of their physical activity and food intake. On day four, DXA scanning was carried out and the questionnaires and Letsfit watches were returned.

A printed invitation with a summary description of the study was distributed to aid in snowball recruitment, with eligible subjects being invited to the hospital. They were given a full explanation of the research study and provided with a consent form. The explanation included a period of training on dietary record-keeping and the use of the Letsfit watch, with time for questions.

Women who were willing to participate signed the consent form and filled out a questionnaire that covered socio-demographics, health, dietary intake, and physical activity. At the initial session, the questions were first explained to the participants by a specially trained assistant who was fluent in Arabic, using a standard script (presented in the appendix). The assistant translated medical terms into layman's language for convenience and better understanding. If the subjects were not comfortable completing the questionnaires alone, they were given individual assistance to do so. The questionnaires, whether filled out with the help of an assistant or not, were reviewed to ensure the participants had understood and had not skipped any questions. This helped to avoid variability and errors.

The questionnaire was designed to obtain information on socio- demographic variables such as age, marital status, educational level, and occupation. It also asked about sun exposure (if the participant was exposed to the sun at all, how many times a week and approximately for how long). It asked questions about medical illnesses, smoking, and personal and family history of fractures (particularly if the participant's mother suffered from osteoporosis, and if so, how her posture was in general and whether she was able to stand straight). Finally, there were questions on obstetric and gynecological history (including the age of menarche, number of pregnancies and children, history of amenorrhea or oligomenorrhea, the number of years post-menopause and previous use of contraceptives). Patients were asked to take home a questionnaire about physical activity and a record food intake over three days and brought this back to the researcher on the final day, along with their Letsfit watches.

Maintaining the privacy and confidentiality of the participants was an essential consideration in this study. Full confidentiality of the s information was maintained throughout, with an explanation of informed consent given. Private rooms were provided for participants to complete the questionnaire and codes were used. All health personnel were required to comply with non-disclosure of participants' personal information. Years post-menopause were calculated by deducting each participants' current age from their age at menopause. Calculating the length of time after menopause is vital to determining the effect of the menopause on the progression of osteoporosis and in testing whether age influences physical activity and dietary intake of calcium and vitamin D.

Stationary vertical height board and balance-beam scale were used to measure participants' height (in cm) and body weight (in kg). For each participant, a calculation

was made for the body mass index using the equation ($BMI = \text{weight (kg)} / [\text{height (m)}]^2$). Participants were then put into four categories, based on their body mass index. WHO international BMI classifications were used: category 1 (underweight) < 18.5 kg/m²; category 2 (normal weight) 18.5 to < 24.9 kg/m²; category 3 (overweight) 25 to < 29.9 kg/m²; and category 4 (obese) ≥ 30.5 kg/m². On the final day of the study, three days after they had completed the questionnaire, selected subjects underwent a DXA scan performed using the same instrument, under the supervision of a single trained DXA operator. This allowed comparability in results and the maintenance of a single standard level of assessment.

3.2 Inclusion and Exclusion Criteria

Post-menopausal Saudi women resident in Jeddah City, aged between 45 and 65, were eligible for this study. Based on self-reports, the participants in this study were screened to exclude women using estrogen supplements or drugs that affect bone metabolisms, such as bisphosphonates, calcitonin, corticosteroids, ketoconazole, rifampicin, phenytoin, and valproic acid. Women with primary hyperparathyroidism, kidney, skeletal, or any other debilitating diseases such as malignancy, liver pathology, chronic renal failure, and cardiovascular disorders likewise were excluded.

3.3 Dietary Measurements

The three-day food intake records that the participants completed were used to evaluate the consumption of calcium and vitamin D-containing foods, including dairy products, and assess the daily calcium intake of each participant. Participants reported their estimated food and beverage consumption during a three-day period. Thorough

instructions were made available to participants to ensure accurate dietary information was obtained. Those participants taking nutritional supplements were asked to record them and provide the investigator with labels of the supplements. After collecting the three-day food intake records, the data was analyzed using the ESHA's Food Processor Nutrition Analysis software. The software provided individual mean vitamin D and daily calcium intakes and was compared with Recommended Daily Allowance (RDA) guidelines, specific to each age level.

3.4 Physical Activity Measurements

The physical activity of all participants was assessed by collecting information about their daily routine activities via the physical activity questionnaire (Appendix 2). Data on levels of physical activity collected through the questionnaires were summarized numerically using metabolic equivalent minutes (MET). The MET was calculated in accordance with the type, frequency, and duration of each exercise for all participants. Levels of physical activity levels were given one of three classifications: low physical activity with MET-min/week less than 500; moderate activity with 500-1000 MET-min/week; and high levels of activity with more than 1000 MET-min/week (Ainsworth et al., 2011).

For more accurate and consistent assessments, Letsfit watches were used to monitor and record physical activity. A Letsfit watch is a physical activity tracker that uses a three-axis accelerometer to analyze motion and acceleration data and provide full information about the frequency, distance traveled, calories burned, and sleep duration of the person who wears it. In the study, the subjects were asked to wear the Letsfit watch for the duration of three days, the period in between filling of the questionnaire and the

time of the DXA scan. Their physical activities were assessed and recorded. The data from each Letsfit watch was noted down with the participant's name, code number, and information. Then the data was correlated with BMD.

3.5 Laboratory Analysis

The participants were asked to visit the biochemical laboratory at King Abdul-Aziz University Hospital in Jeddah in the morning to have their fasting blood samples drawn for analysis. To determine serum levels, all samples from all participants were drawn at the same time of the day to measure serum calcium, serum phosphorus, serum albumin, serum 25-(OH) vitamin D, and alkaline phosphatase levels ALP concentrations (IU/L). Serum calcium and phosphorus were determined by spectrophotometric analysis, while serum 25-(OH) vitamin D was measured using radioimmunoassay after extraction from the sample.

3.6 Measurement of Bone Mineral Density

Bone mineral density (BMD) was evaluated using a dual-energy x-ray absorptiometry (DXA) scan. Quality monitoring operations for the machine were conducted in accordance with the manufacturer's instructions. This was done each morning before assessments of DXA were conducted in order to guarantee precise results. The automatic area of interest was utilized in all proceedings, and BMD was calculated at the femoral neck and lumbar spine (mainly lumbar vertebrae L1 to L4). However, manual adjustments were made on the spine where needed, such as in participants with acute scoliosis. Standard positioning was employed for anterior-posterior scans of the femur, neck, and spine. BMD was expressed as g/cm². Both the T

and Z-scores were obtained. T-score values were considered in the analysis according to the World Health Organization (WHO) recommendations that define criteria for classifying subjects as with normal BMD, osteopenia and osteoporosis. A T-score between -1 and -2.5 indicates osteopenia and a T-score of <-2.5 indicates osteoporosis.

3.7 Statistical Analysis

Statistical analyses were achieved using version 23 of IBM SPSS software for Windows. Univariate analysis for continuous variables was carried out as descriptive statistics showing the minimum, maximum, median 25th percentile, 75th percentile, mean, and standard deviation (SD). The univariate analysis of categorical variables reported each variable's frequency and its corresponding percentage. Bivariate analysis was carried out to test associations between different numeric variables and the value of the BMD T-score using Pearson's correlation. The independent t-test was utilized to compare the value of the BMD T-score across groups. Statistical significance was set at $P<.05$ throughout the analysis.

CHAPTER IV

FINDINGS

4.1 Characteristics of the Participants

A total of 150 Saudi post-menopausal women from Jeddah region participated in this study. The characteristics of those participants are summarized in tables 1 and 2 as categorical and continuous variables.

About 85% (n = 128) of participants were married. In addition to the 28% of women who didn't complete high school, nearly half (47%, n=70) of women stopped their education after high school. The four categories for body mass index (BMI) are: Underweight <18.5, Normal weight = 18.5–<25.0, Overweight = 25–<30.0, and Obese at ≥ 30 . Overweight and obesity were high among participants. Based on the BMI the highest number of women (48.7%, n= 73) were categorized as overweight, while 21.3% (n= 32) were obese. Forty-three women (28.7%) were classified as normal weight based on their BMI. Only twenty-seven women (18%) confirmed frequent exposure to the sun. The only reported medical issues were hysterectomy and diabetes. Hysterectomy was reported by 4 cases while diabetes was reported by 28 participants. Calcium

supplements were taken by 56.7% of the participants, while vitamin D supplements were reported by 53.3%.

Table 1: Demographic, sociocultural and anthropometric characteristics of postmenopausal Saudi women participants

Variables	n	%	
Age	< 51	10	6.7
	51 to <60	109	72.7
	≥60	31	20.7
Marital Status	Single	8	5.3
	Married	128	85.3
	Widowed	9	6.0
	Divorced	5	3.3
Educational Level	Less than high school	42	28.0
	High school complete	70	47.0
	More than high school	37	24.7
Body Mass Index BMI (kg/m ²)	Underweight (< 18.5)	2	1.3
	Normal weight (18.5 – < 25.0)	43	28.7
	Overweight (25 – < 30)	73	48.7
	Obese ≥ 30	32	21.3
Daily outdoor sun exposure	No	123	82
	yes	27	18
History of hysterectomy	No	146	97.3
	Yes	4	2.7
History of diabetes	No	122	81.3
	Yes	28	18.8
Calcium supplements intake	No	65	43.3
	Yes	85	56.7
Vitamin D supplements intake	No	70	46.7
	Yes	80	53.3

Table 2: Demographic and anthropometric data of post-menopausal Saudi women participants

Variables	Mean	S. D.	Median	25 th Pctl	75 th Pctl	Min	Max
Age	57.6	3.6	58	55	60	50	62
Age at menopause (y)	50.2	3.2	50	49	52	38	57
Years since menopause	7.3	4.7	6	4	10	0	21
Number of pregnancies	4.1	4.3	4	2	6	0	10
Weight (Kg)	67.0	12.6	64	60	74	40	153
Height (cm)	156.2	5.8	156	152	160	139	180
BMI (kg/m ²)	27.1	4.1	26.7	23.9	29.3	18.3	39.6

The mean (\pm SD) age of participants was 57.6 (\pm 3.6) years and mean years since menopause was 7.3 (\pm 4.7). The mean number of pregnancies was 4.1(\pm 4.3). Mean BMI was 27.1(\pm 4.1) kg/m², with a range of 18.3 to 39.6.

4.2 Bone Mineral Density (BMD)

The lumbar spine and right femoral neck were measured utilizing Dual-energy X-ray absorptiometry (DXA). According to WHO criteria, the participants were categorized into three groups based on their bone mineral density situation: normal BMD (T-score \geq -1.00), which means healthy bone, low bone mineral density, or osteopenia ($-1.00 >$ T-

score ≥ -2.5) and osteoporosis (T-score < -2.5). The higher the negative value, the more acute the osteoporosis, as shown in Tables 3, 4, and 5.

The mean BMD t score for the spine is -1.45 (SD=1.33) ranging from -4.1 to 2.6 , and the mean's t-score for the right femoral neck is -0.97 (SD= 1.13) ranging from -3.2 to 2.2 . T-score is a comparison of an adult's bone mineral density with that of a healthy 30-year-old.

Table 3: T- score for bone mineral density of the spine and right femur and its distribution in post-menopausal Saudi women participants

	Mean	Standard deviation	Minimum	Maximum
T-score Spine	-1.45	1.33	-4.10	2.60
T-score right Femur	-0.97	1.13	-3.20	2.20

Table 4: T- score for bone mineral density of the spine and its distribution in post-menopausal Saudi women

		T-score (spine)	n	%
BMD	Normal	≥ -1	50	33.3
	Osteopenia	-1 to -2.5	65	43.3
	Osteoporosis	-2.5 & below	35	23.3

The highest number of participants was classified as osteopenia (43.3%) followed by normal (33.3%). About 23.3% of the patients had osteoporosis based on the BMD of their spine.

Table 5: T- score for bone mineral density of the Right Femur and its distribution in post-menopausal Saudi women participants

		T-score (Right Femur)	n	%
BMD	Normal	≥ -1	72	48
	Osteopenia	-1 to -2.5	69	46
	Osteoporosis	-2.5 & below	9	6

Nearly half of the participants had normal right-femur T-score, while 46% of the participants had osteopenia. Only nine participants were categorized as having osteoporosis on the basis of the T-score for their right femur, six of these nine patients also were classified as having osteoporosis based on the T-score of the spine, while three were diagnosed as having osteopenia.

4.3 Laboratory Biomarkers Related to Bone Health

The lab results for calcium, phosphorus, albumin, 25-hydroxy vitamin D, and alkaline phosphatase levels were reported as follows. For serum 25-hydroxy vitamin D 50-120 nmol/L is considered as sufficient for bone health whereas less than 50 nmol/L is classified as insufficient. Among participants, 28.7% had low Vitamin

D (n=43). Only 8% were classified as having a high vitamin D level (n=12). For calcium mmol/L, the level between 2.12-2.52 is taken as a normal category whereas less than or equal to 2.11 is taken as a low category and more than 2.53 is taken as a high category. Most of the participants had normal calcium (n=133, 88.7%). For ALP IU/L, the level of 40-150 is considered as a normal category whereas less than or equal to 39, is classified as low and more than 151 is taken as high category. Almost all of the participants had normal ALP (n=145, 96.7%). For albumin (g/L), the level 40.2-47.6 is taken as a normal category whereas less than or equal to 40.1 is considered as low and more than 47.7 is taken as a high category, which means that most of the percipients had low Alb (n=130, 86.7%). Lastly for P mmol/L. the level between 0.18-1.85 is considered normal whereas less than or equal to 0.17 is taken as low. Almost all the patients had normal P (n=144, 96%).

Table 6: Laboratory data related to osteoporosis for post-menopausal Saudi women participants

		n	%
25(OH) D nmol/L	Low	43	28.7
	Normal	95	63.3
	High	12	8.0
Ca mmol/L	Low	13	7.3
	Normal	133	88.7
	High	4	4.0
ALP IU/L	Low	2	1.3
	Normal	145	96.7
	High	3	2.0
Alb g/L	Low	130	86.7
	Normal	20	13.3
	High	0	0.0
P mmol/L	Low	6	4.0
	Normal	144	96.0
	High	0	0.0

Table 7: Laboratory data for post-menopausal Saudi women participants

	Mean	Std. Deviation	Median	25 th Pctl	75 th Pctl	Minimum	Maximum	% Normal
25(OH) D nmol/L	71.5	34.4	62.7	47.9	89.1	11.1	230.7	63.3%
Ca mmol/L	2.3	0.1	2.3	2.2	2.4	1.9	2.8	88.7%
ALP IU/L	80.1	25.3	77	64	94.3	37	183	96.7%
Alb g/L	36.7	3.9	36.8	34.1	39	23.2	47.0	13.3%
P mmol/L	1.1	0.2	1.1	1	1.2	0.4	1.4	96.0%

Serum 25-OH vitamin was not normally distributed. The median (IQR) was 62.7 (48.0, 89.0)

4.4 Physical Activity Estimates

The mean MET-min/week ranged from a minimum of 115 to a maximum of 664. The median value for MET-min/week was 386, with a mean of 375.7 (± 138.1). A high proportion of the participants (78.6%, n=118) were categorized as having low activity with MET-min/ week less than or equal to 500, while the rest (21.4%, n=32) were considered to have moderate activity with MET-min/ week of 500-1000.

The mean daily Letsfit watch steps for the 3 days measured were calculated. The mean daily steps were 3295 (± 1375) steps. There is a strong positive correlation between physical activity measured as MET-min/week and Letsfit watch steps. The correlation coefficient was $r=0.45$ and $p\text{-value} < 0.001$.

Table 8: Physical activity estimates for post-menopausal Saudi women participants

	Mean	Std. Deviation	Median	25 th Pctl	75 th Pctl	Minimum	Maximum
Physical activity (MET-min/ week)	376	138	386	255	489	115	664
Letsfit watch steps	3295	1375	2863	2347	3829	1761	7291

Table 9: Physical activity categorized by post-menopausal Saudi women participants based on MET-min/week

		n	%
Physical activity (MET-min/ week)	Low	118	78.6
	Moderate	32	21.4
	High	0	0

4.5 Dietary Intake of Calcium and Vitamin D

Calcium intake was compared to the Estimated Average Requirement (EAR) for calcium of 800 mg, which is defined as the estimated need of the person at the 50th percentile. The mean daily calcium intake of the 3 days was calculated from ESHA's Food Processor nutrition analysis software. The mean calcium intake was 927 (\pm 123) mg. Comparing the daily calcium intake to the Estimated Average Requirement (EAR) of 800 mg showed that 126 participants (84%) had calcium intake

compliant with that recommendations but only 2 % of the women met the Recommended Daily Intake (RDI for calcium of 1200 mg).

The mean daily vitamin D intake for the 3 days was calculated from the dietary record using ESHA’s Food Processor nutrition analysis software. Vitamin D intake had a mean of 104 IU (± 40). This mean was obtained from food intake in addition to the instructed amount of supplement taken by the participants due to vitamin d deficiency. The current RDA for vitamin D is 600 IU. However, the previous recommendation for vitamin D intake IU was used to define adequate vitamin D intake because all the participants were below the current RDA. Comparing the daily Vitamin D intake to the previous recommendation of 200 IU showed that 98% of participants had intakes below 200 IU per day.

Table 10: Summary of calcium and vitamin D intake of post-menopausal Saudi women participants

	Mean	Std. Deviation	Median	25 th Pctl	75th Pctl	Minimum	Maximum
Daily Ca intake (mg)	927	123	923.6	832.5	1013.8	632	1245
Daily Vit D intake (IU)	104	40	92.8	79.5	121.5	34	287

Table 11: Calcium and vitamin D intake and its categories for post-menopausal Saudi women participants

		N	%
Daily Ca intake	Intake meeting EAR	126	84%
	<EAR	24	16%
Daily Vit D intake	Intake \geq 200 IU	3	2%
	< 200 IU	147	98%

4.6 Associations between T- scores for BMD and other variables

Table 12: Correlation of the spine T-score with other variables

Variables	Pearson's correlation coefficient	P-value
Age	-.091	.270
BMI	.124	.131
Years since menopause	-.171*	.036
No of pregnancies	.191*	.019
VitD nmol/L	-.108	.186
Ca mmol/L	.006	.945
ALP IU/L	.042	.611
Alb g/L	-.081	.323
P mmol/L	.163*	.047
Average Ca intake	-.033	.689
Average vitamin D intake	-.201*	.014
Average Letsfit watch steps	.071	.386
Physical activity (MET-min/ week)	0.12	0.14

* Correlation is significant at the 0.05 level

The number of pregnancies and P level in blood were positively correlated with the spine T-score. The more pregnancies the woman had, the higher the spine T-score for the BMD. Years since menopause and daily vitamin D intake were negatively correlated with the spine T-score. The higher the number of years after menopause, the lower the spine T-score of BMD. The same is observed with vitamin D intake, a higher daily vitamin D intake was associated with low spine T-score. This relationship is not likely to be causal. It may be explained if participants with low BMD were instructed to take more vitamin D as a sort of some kind of advice.

Table 13: Correlation of the right femur T-score with other variables

Variables	Pearson's Correlation coefficient	P-value
Age	-.125	.127
BMI	.006	.942
Years since menopause	-.205*	.012
No of pregnancies	.121	.140
VitD nmol/L	-.107	.194
Cal mmol/L	-.111	.175
ALP IU/L	.043	.598
Alb g/L	-.128	.120
P mmol/L	.076	.352
Average Ca intake	.021	.800
Average vitamin D intake	-.142	.083
Average Letsfit watch steps	.055	.503
Physical activity (MET-min/ week)	0.09	.291

* Correlation is significant at the 0.05 level

The only significant correlation found was between years since menopause and the right femur T-score. The correlation was negative, which means the more the years after menopause the lower the BMD and T-score for the right femur.

Statistical analysis was done to check for the association between different factors and spine & right-femur T-scores for which the data was presented in Tables 14,15,16,17,18, and 19.

Table 14: Independent t test comparing the spine T-score and right femur T-score between participants with adequate and inadequate calcium intake

Calcium intake		N	Mean	SD	P value
BMD spine T- score	Inadequate Dietary Ca	24	-1.82	1.07	0.14
	Adequate Dietary Ca	126	-1.38	1.37	
BMD Right Femur T- score	Inadequate Dietary Ca	24	-1.35	1.09	0.70
	Adequate Dietary Ca	126	-0.90	1.12	

Independent t-test comparing the spine T-score and the right femur T-score between participants with adequate and inadequate calcium showed no significant difference.

Table 15: Independent t-test comparing the spine T-score and the right femur T-score between participants based on their median vitamin D intake

Vitamin D intake		N	Mean	Std. Deviation	P-value
Spine T-score	Above median	75	-1.62	1.04	0.12
	Below median	75	-1.29	1.56	
Right Femur T- score	Above median	75	-1.07	0.97	0.28
	Below median	75	-0.87	1.26	

Independent t-test comparing the spine T-score and the right femur T-score between participants by the median of vitamin D intake showed no significant difference.

Table 16: Independent t-test comparing the spine T-score and the right femur T-score between participants based on their median physical activity as MET-min/ week

Physical activity MET-min/ week		N	Mean	Std. Deviation	P-value
Spine T-score	Above median	75	-1.31	1.37	0.21
	Below median	75	-1.59	1.28	
Right Femur T-score	Above median	75	-0.93	1.00	0.66
	Below median	75	-1.01	1.25	

Independent t-test comparing the spine T-score and the right femur T-score between participants of the upper half and lower half of physical activity MET-min/ week showed no significant difference.

Table 17: Independent t test comparing the spine T-score and the right femur T-score between participants of the upper half and lower half of physical activity MET-min/ week

Physical activity MET-min/ week		N	Mean	Std. Deviation	P-value
Spine T-score	Moderate physical activity MET-min/ week	32	-0.99	1.41	0.027
	Low physical activity MET-min/ week	118	-1.58	1.29	
Right Femur T-score	Moderate physical activity MET-min/ week	32	-0.82	1.04	0.397
	Low physical activity MET-min/ week	118	-1.01	1.15	

Independent t-test comparing the spine T-score and the right femur T-score between participants with low and moderate physical activity MET-min/ week showed a significant difference regarding spine T-score but no significant difference regarding the right femur T-score. For the spine T-score, those with low physical activity had lower T-score than those with moderate physical activity.

Table 18: Independent t-test comparing the spine T-score and the right femur T-score between participants based on their median Letsfit steps.

Letsfit watch steps		N	Mean	Std. Deviation	P-value
Spine T- score	Above median	75	-1.40	1.26	0.67
	Below median	75	-1.50	1.41	
Right Femur T- score	Above median	75	-0.90	1.10	0.44
	Below median	75	-1.04	1.15	

Independent t-test comparing the spine T-score and the right femur T-score between participants of the upper half and lower half of average Letsfit steps showed no significant difference.

Table 19: Independent t-test comparing the spine T-score and the right femur T-score between participants of the upper half and lower half of average Letsfit steps

Letsfit watch steps		N	Mean	Std. Deviation	P-value
Spine T- score	>= 5000.00	20	-1.20	1.18	0.36
	< 5000.00	130	-1.49	1.35	
Right Femur T- score	>= 5000.00	20	-0.72	1.09	0.28
	< 5000.00	130	-1.01	1.13	

Independent t-test comparing the spine T-score and the right femur T-score between participants with average Letsfit steps below 5000 and those above or equal to that showed no significant difference.

CHAPTER V

DISCUSSION

5.1 Bone Mineral Density (BMD)

The prevalence of osteopenia and osteoporosis in this study's sample of post-menopausal Saudi women was found to be 43.3% and 23.3%, respectively, based on the spinal T-score. Nearly half of the participants had normal right femoral T-score, while 46% of the participants had osteopenia. Only nine participants were categorized as having osteoporosis on the basis of their right femoral T-score. Of those, six were diagnosed as having osteoporosis based on their spinal T-score, while three were diagnosed as having osteopenia. This prevalence is consistent with expectations based on previous studies of the prevalence of osteoporosis and osteopenia in the region. Due to the high levels of diversity in the Saudi community, there was a trial to assess the osteoporosis prevalence using the mathematical pattern of the World Health Organization plan for osteoporosis detection.

The estimated prevalence among women in Saudi Arabia women aged 50-70 was approximately 23% (Greer, Ahmed, Rifai, & Sandridge, 2008). One other study in Saudi Arabia stratified the prevalence of osteoporosis and osteopenia by patient age in decades among post-menopausal Saudi women. It showed that prevalence was higher in older age groups. Among those aged 50-59, 42.3% were normal, 33.4% had osteopenia, and 24.3% had osteoporosis. In the age group 60-69 years, 11% were normal, 27% had osteopenia, and 62% had osteoporosis. The prevalence was even higher in the group aged 70-79 years, of whom 4.6% were normal, 21.5% had osteopenia, and 73.8% had osteoporosis (El-Desouki, 2003). Saudi Arabia is one of the countries most strongly affected by low bone mineral density and osteoporosis. To compare regionally, the prevalence of osteoporosis and osteopenia in Kuwaiti women aged fifty and over has been reported at 9.9% and 26%, respectively (Mahboub, Al-Muammar, & Elareefy 2014).

The same study reported that Saudi women aged 50 years and over had even higher rates for spinal osteoporosis and osteopenia, at 40% and 31%, respectively. The high prevalence of low BMD among Saudi women indicates a serious public health concern that is not only affecting the community but also the economy in general, due to reduced productivity and the increased costs of medical treatment and care, and hospital admissions resulting from complications of osteoporosis. It is, therefore, important to explore alternative strategies to identify evidence-based interventions that could potentially reverse this trend.

5.2 Risk Factors and BMD

When participants were asked about their exposure to sunlight, only 18% reported outdoor sun exposure. This is considered a very low percentage, especially when considering that Jeddah is a sunny city most of the year. This may be due to the high average temperatures in Jeddah, making sun exposure uncomfortable. Cultural factors also play a role, as even when they do spend time outside, women may be poorly exposed to sunlight, due to wearing clothing that covers the whole body. The percentage of sun exposure in Jeddah among women aged 40 years or more was found to be just 21% in a previous study (Oommen & Alzahrani, 2014). Sun exposure rates were found to be higher in the eastern region. There, the percentage of women gaining regular sunlight exposure (a minimum of twice per week between 9 am and 12 pm for 40 minutes) was estimated at 68% in a study carried out during winter. Winter temperatures in the east range from 18–25 °C during the day (Elsammak, Al-Wosaibi, Al-Howeish & Alsaeed, 2010). This may mean that climactic differences play a significant key role in differences in the amount of sun exposure gained by women in the eastern and western regions of Saudi Arabia.

The results of this research showed a negative correlation between BMD in the spine and the right femur and after menopause. The greater the number of years after menopause, the lower the BMD T-score. This is similar to the findings of Mahboub et al. (2014) that women may suffer up to 5% bone loss per year, five to seven years after menopause (Mahboub, Al-Muammar & Elareefy, 2014). So, BMD is negatively correlated with the number of years after menopause (Modanloujouybari, Heidari,

Hosseini & Javadian, 2018). Loss of bone during this period may be attributed to a deficiency in estrogen and not necessarily to the aging process itself (Raisz, 2005).

It also observed that the number of pregnancies undergone is positively correlated with the spinal T-score. Women who had experienced high numbers of pregnancies had higher spinal BMD T-scores. The effect of parity on BMD in this study is similar to the results of a systematic review of 10 studies. The impact appeared at a specific site in which a significant correlation between parity and bone mineral density of the hip was found. The same correlation was not found with BMD of the spine or femoral neck (Song et al., 2017). Another systematic review and dose-response meta-analysis revealed a linear reduction of hip fractures. This included a reduction of the nonlinear osteoporosis fracture risk among post-menopausal women, associated with the growing parities number of more than five live births (Wang et al., 2016).

A Canadian multicenter Osteoporosis Study (CaMos) concluded that increasing parity showed no relationship with considerable fragility fractures or bone mineral density, after dominating for 20 variables and completion of 15 years of follow up (Cooke-Hubley et al., 2017). Furthermore, a recent systematic review of seven cross-sectional studies of Korean women concluded that the OR for osteoporosis was 1.43 in women after menopause, with high parity compared to those with low parity. Although the result of this study seems to contradict other studies, the stronger evidence is that there is no increase in the risk of osteoporosis with increased parity. Moreover, this last study is limited to Korean women (Lee, Choe, Choi & Lee, 2019).

5.3 Laboratory Biomarkers Related to Bone Health

Analysis of the calcium and vitamin D levels in the blood of the participants found the following results. 28.7% of participants had low vitamin D, and only 8% were considered to have high levels of vitamin D. In relation to calcium levels, the results were better, with only 7.3% showing low blood calcium levels. Low levels of vitamin D were reported in many other studies, with a vitamin D deficiency prevalence in adult Saudi females of 62.7% (Mokdad et al., 2015). Saudi Arabia is among those countries in which levels of vitamin D deficiency (<50 nmol/l) are greater than 35%. The Middle East region consistently ranks among the highest in the world for vitamin D shortage (Alkhenizan et al., 2017). 55% of a sample of Saudi women over 50 were found to have low vitamin D levels (Al-Turki et al., 2008).

In a pilot study, Alissa et al. recruited 122 post-menopausal women from Jeddah's Center of Excellence for Osteoporosis Research and found that the majority of the sample had vitamin D serum levels below 50 nmol/l (Alissa, Qadi, Alhujaili, Alshehri & Ferns, 2011). Identical results documented by Oommen and Al-Zahrani, who conducted a pilot study of 100 Saudi females aged 40 years and over. The researchers conclude that 82% of the participants had vitamin D deficiency (Oommen & Alzahrani, 2014).

The findings coincide with Al-Mogbel's observation of 100% hypovitaminosis D spread among a group of 465 Saudi females aged 19 to 40 (Al-Mogbel, 2012). The status of vitamin D amongst Saudi adults has been examined in almost 20 studies. Although there is much variability in the prevalence reported in each study, levels of vitamin D

deficiency are very high, ranging from 41% to 100% in Saudi women (Bokhari & Albaik, 2019). A systematic review of the prevalence of vitamin D deficiency in Saudi Arabia examined all studies between 2011 and 2016 and concluded that the prevalence amongst various groups of women and girls (including pregnant and lactating women, children, and adults) was 81.0%. This is similar to the prevalence recorded in neighboring Gulf countries (Al-Daghri, 2018). Generally, mean serum levels of 25(OH)D were much lower in Saudi Arabian post-menopausal women than in women from western countries. They were, however, similar to levels amongst other Arab women, suggesting the presence of cultural factors affecting vitamin D levels, such as the duration of sunlight exposure (Dehlawi, 2013).

The positive correlation observed between P level in blood and the spine T-score is consistent with the fact that BMD does not contain calcium only, but it also contains calcium phosphate. Therefore, during bone-building throughout the growth period, a sufficient concentration of serum phosphate (1.5-2.0 mmol/L) is required (Heaney, 2004). Almost 85 % of the phosphorus in the body was found in BMD, where it plays a vital role, similar to that of calcium, in the process of bone repair (Vorland et al., 2018). Moreover, it was found that adequate phosphorous intake was positively correlated with BMD and decreased osteoporosis risk (Cho & Lee, 2015).

Most of the participants had low Alb (n=130, 86.7%), but no association between albumin level and bone mineral density was found. According to the National Health and Nutrition Examination Survey (NHANES) of 15,539 individuals, there is an independent correlation between osteoporosis and hypoalbuminemia. The odds of osteoporosis

occurring with hypoalbuminemia were 5.37-fold when adjusting for confounding factors (Afshinnia & Pennathur, 2016). Prealbumin, usually used with albumin, is an indicator of nutritional status and was found to be correlated with BMD. Prealbumin levels decreased as BMD decreased. In addition, an association was found between Prealbumin and BMD after adjusting for confounding factors using both partial correlation and multiple linear regression (Li et al., 2017).

One study included 21,121 patients showed a gradual reduction in the rate of osteoporosis from 28.0% at ALb level of 3 g/dL or lower to 9.3% at Alb higher than 4 g/dL. This reflects the fact that a lower percentage of osteoporosis is associated with a higher level of albumin (Afshinnia, Wong, Sundaram, Ackermann & Pennathur, 2016). One older study reached a conclusion that there is no significant difference in the mean of Alb levels through the three BMD classifications in either males or females. It found that previous correlations were likely to be due to unsuitable modifications of age-related reductions in Alb levels and the prevalence of extremely weak osteoporosis patients in previous studies (Lunde, Barrett-Connor & Morton, 1998).

5.4 Dietary Intake of Calcium and Vitamin D

Daily intake of calcium was calculated and compared to the Recommended Daily Allowance (RDA) of 800 mg and showed that 84% of the participants had a calcium intake level that was compliant with the recommendations. However, when comparing participants' daily vitamin D intake to the Recommended Daily Allowance (RDA) of 200 IU, it found that 98% of the participants were below the recommended level. This

striking observation of low intake of both calcium and vitamin D is similar to the findings of the Oommen & Alzahrani (2014) study. They observed that only 5% of participants consumed diets rich in vitamin D and concluded that a high prevalence of low BMD and osteoporosis was linked to a high incidence of deficiency of vitamin D (at $<25\text{nmol/L}$) (Oommen & Alzahrani, 2014).

The study found a negative correlation between the estimated daily vitamin D intake and BMD. A high daily vitamin D intake is associated with a low spinal T-score. This relationship is not likely to be causal. It may be attributed to the fact that patients with low BMD were routinely being instructed to take more vitamin D

Cross-sectional research was carried out with 300 post-menopausal Saudi women. No association between calcium and vitamin D dietary intake and bone mass levels at different sites across the body was found. The study also found that dietary calcium and vitamin D intake was meaningfully lower than the recommended standard (Alissa, Alnahdi, Alama & Ferns, 2014). The literature showed a positive correlation between vitamin D intake and BMD, contrary to this study's observations. Rising vitamin D consumption was found to lead to the development of BMD in Korean women aged > 50 years (Yoo, Kim & Ly, 2019). A randomized controlled trial found that consuming milk fortified with calcium and vitamin D daily by healthy women after menopause induced a significant boost in BMD at the neck of the femur (Reyes-Garcia et al., 2018). An increase in bone mineral density at the spine was found in the treatment group of a clinical trial two years after receiving calcium and vitamin D supplements (Bæksgaard, Andersen & Hyldstrup, 1998).

By comparison, a recent systematic review and meta-analysis of the effects of vitamin D supplementation on musculoskeletal health involving 81 randomized controlled trials (n=53 537 participants) showed a contradictory result to that which is generally expected in clinical practice. Bolland, Grey & Avenell (2018) found that supplementation with Vitamin D does not prevent fractures or have a clinically significant impact on BMD. The researcher claimed that no differences between the impacts of low and high vitamin D doses were found. The authors concluded that there is an insignificant rationalization for using vitamin D supplements to preserve or develop musculoskeletal health (Bolland, Grey & Avenell, 2018). One recent study in the UAE found an association between the BMD of osteoporosis participants and calcium consumption. The study also found that osteoporosis patients had a lower BMI, lower intake of calcium, and lower levels of sun exposure compared to the control group (Ibrahim, Nabil & Ghaleb, 2019).

The questionnaire in this study included questions about calcium and vitamin D supplementation. The answers revealed that calcium supplements were taken by 56.7% of the participants and vitamin D supplements by 53.3%. However, these findings were not used due to the lack of sufficient information provided about the dose, quantity, and frequency at which the supplements were taken.

5.5 Physical Activity Estimate

Almost 3/4 of the participants in this study (78.6%) were categorized as having low physical activity with MET-min/week less than or equal to 500. 21.4% of them were considered to have moderate activity levels with a MET-min/week of 500-

1000. We found no correlation between either the participants' history of physical activity (calculated as the number of MET-min/week) or the mean number of steps (based on the Letsfit watch measurements) and BMD calculated as a T-score. This was true in relation to both the spine and the right femur. Comparing the spinal T-score and the right femoral T-score between participants in the upper and lower halves of physical activity (MET) showed no significant difference. Comparing the spinal T-scores and the right femoral T-scores of participants with low and moderate physical activity (MET) did show a significant difference in spinal T-scores, but no significant difference in right femoral T-scores. There was a high standard deviation in the Letsfit watch steps, which could be explained by the fact that some participants were not wearing the watch constantly, leading to unmeasured steps. It might also be that there were some outliers in the data collected.

For spinal T-scores, those with a low level of physical activity had lower T-scores than those with moderate levels of physical activity. Comparing spinal and right femoral T-scores between participants of the upper and lower halves of average Letsfit steps showed no significant difference. Comparing spinal and right femoral T-scores between participants with average Letsfit steps (below 5000) and those above or equal to that showed no significant difference. In reviewing the literature, some studies found an association between physical activity and BMD.

A systematic review found that a combined physical activity program, including resistance, impact, and aerobic exercise enhanced BMD by inducing changes in bone metabolism (Moreira et al., 2014). The review also found that osteogenic exercises

resulted in an increase in BMD and contributed to the development of muscle strength and body balance. Improvements in physical function through increased BMD improved quality of life and reduced the incidence of falls and fractures (Moreira et al., 2014).

Another systematic review based on 43 randomized controlled trials involving 4320 participants showed the presence of the impact of physical activity on BMD in the intervention groups compared with control groups. They concluded that physical activity could help women avoid bone loss after menopause (Howe et al., 2011). In a meta-analysis carried out by Marques, Mota and Carvalho (2011) to estimate the impact of exercise interventions on the BMD of older adults, the researchers established a significant increase in BMD based on variable mixed load exercises lasting at least 16 weeks (Marques, Mota & Carvalho, 2011). Some researchers have found that while physical activity may not play an essential role in the development of bone mass density, it is critical in reducing the loss of bone mass (Al-Shoumer & Nair, 2012).

One study concluded that, in post-menopausal women in Saudi Arabia, physical exercise promoted musculoskeletal health. It was found to decrease the risk of hip fractures and increase skeletal muscle mass and intrinsic neuromuscular stimulation. This resulted in increased coordination of movement and a lower level of risk of falling (Alahmed & Lobelo, 2018). Another study concluded that increased exercise could increase bone mineral density in the lumbar spine and hips in post-menopausal women (Habibzadeh, 2010).

5.6 Negative Results Reached

This research hypothesized that physical activity combined with sufficient dietary intake of vitamin D and calcium could be useful in the prevention and treatment of low BMD. However, our results did not show any association between the presence of osteoporosis or osteopenia and the intake of vitamin D supplements, calcium supplements, or the calculated calcium intake. The average vitamin D intake was negatively correlated with the spinal T-score for BMD. This finding is consistent with the conclusion of a systematic review of risk factors for low bone mineral density in healthy women aged between 40 and 60 (Waugh et al., 2008). That study showed no consistent or sufficient proof of the influence of either calcium consumption or exercise on BMD. That study also demonstrated that evidence of correlations between BMD and other possible causal or co-linked factors (such as smoking, age at menstruation, history of absence of menstruation, family history of osteoporosis, and race) was inconsistent (Waugh et al., 2008). In an extensive systematic review, 19 researchers assessed the correlation between serum 25(OH)D and BMD in post-menopausal women and older men. This review showed that there was reasonably strong proof of an association between serum 25(OH)D concentrations and alterations in bone mineral density in the hips (Cranney et al., 2007). Several studies specified particular serum 25(OH)D low concentrations in which increasing falls, fractures, or bone loss occur (Cranney et al., 2007).

CHAPTER VI

CONCLUSION

After menopause, women experience structural, physical, biological, and physiological changes. Their bone density becomes lower, their skeletomuscular systems become weaker, and their susceptibility to bone fractures increases. These factors together can be classified as either osteopenia or osteoporosis, depending on the severity of bone mineral deterioration. These medical conditions are associated with several risk factors, which can be summarized by (but are not exclusive to): low dietary calcium and vitamin D consumption, low levels of sunlight exposure, hormonal disturbance, and reduced physical exercise. As a result, osteopenia and osteoporosis can cause poor life quality and impaired physical mobility.

The research on this topic in Saudi women at the onset of menopause is limited. Therefore, this study aimed to establish whether higher dietary intake of calcium and vitamin D and higher levels of physical exercise is associated with improved BMD. The study's participants were post-menopausal Saudi women resident in Jeddah City, aged

between 45-65 years. Participants were asked to take home a Letsfit fitness tracker watch, a questionnaire about their physical activity, and a three-day food intake form. They were asked to detail their food intake over three days and returned it along with the questionnaire and watch at the end of the study. The study excluded women who were using estrogen supplements or drugs known to affect bone metabolism.

This study has shown that there is a high prevalence of osteopenia and osteoporosis, a low prevalence of sun exposure, and low dietary intake of vitamin D among post-menopausal Saudi women. There was a positive association between bone mineral density and the number of pregnancies, but a negative association with the years after menopause. A negative association was also observed between the daily intake of vitamin D and BMD. This association cannot be casual but can possibly be explained by a higher vitamin D intake among women who already have low BMD. Some of these women will be taking vitamin D with the intention of strengthening their bones. In addition, no association was found between calcium intake, physical activity and BMD. This may be explained by the limitations of the study design. Based on previous studies, we would recommend awareness campaigns and public health interventions directed toward Saudi post-menopausal women. These should be designed to increase rates of sun exposure, increase levels of physical activity, and improve diet so that it contains adequate amounts of vitamin D.

6.1 Study Limitations

Certain limitations would be expected to impact the generalization of the study's results. This includes the fact that the research was designed as a community-based cross-

sectional study, meaning that associations could not be considered to be authoritative proof of causal relationships. It is also essential to note that the measurement of calcium and vitamin D dietary intake, as well as physical activity at a single time point, might not reflect longstanding effects. Another limitation is that the study was carried out within a single region, Jeddah, and thus cannot be generalized to all Saudi women.

Part of the study was assessed by self-reporting, which may be misleading and contain some degree of bias. This might include social desirability bias in the way that participants recorded their dietary data and physical activity. There was little control in terms of supervision and ensuring the accuracy of the records. Diet and food consumption may be important, with one individual consuming more food than another, thus receiving more nutrients. Some participants may be reluctant to record the actual food that they consume daily, or they may fail to record foods accurately. It is expected that this limitation will have influenced the vitamin D negative correlation, as some participants did not answer questions about doses of vitamin D supplements taken.

Research has shown that quantities vary with respect to individuals, groups, regions, and cultures. Similar quantities recorded by any two participants may not necessarily mean the same thing for each participant. In addition, it is worth noting that dietary habits vary not only from country to country but also from one region to another. The nutritional habits of a specific region may not necessarily reflect the habits of the whole country or a specific age group within a community.

6.2 Study Recommendations

Based on the research study findings and the literature review of the related studies, the following actions are recommended:

- Implementation of awareness campaigns directed towards Saudi post-menopausal women to increase their rates of sun exposure and physical activity.
- Organization of social and sports events that target the participation of Saudi women, especially older women, with the goal of raising their physical activity levels.
- Encouraging post-menopausal women to seek DXA scanning as a screening tool for osteoporosis and osteopenia, as recommended by international guidelines.
- The raising of public awareness of the high prevalence of osteoporosis and osteopenia and the measures that can be taken to reduce the risks.
- Encouraging diets rich in vitamin D.
- Performing more extensive research to assess the relationship between physical activity and BMD in the form of longitudinal cohort studies.

PART TWO

DEVELOPMENT OF A CULTURALLY ACCEPTABLE FOOD PRODUCT

CHAPTER I

INTRODUCTION

1.1 Background

Across the world, milk is considered as one of the most staple, valuable, and well-known food. It has been consumed as food by various age groups for centuries due to its nutritional value. However, milk can be harmful among certain groups of the population. It may cause unhealthy effects like cow milk allergy, lactose intolerance, anemia, and heart disease as a result of some compounds. Because of such issues, concerns about consuming cow's milk has been increasing among both healthy and at-risk people (Kneepkens & Meijer, 2009; Swagerty, Walling, & Klein, 2002). Plant-based milk alternatives may be preferable for many. Depending on its components, non-dairy milk can be a rich source of minerals, vitamins, dietary fiber, antioxidants, non-allergic proteins, and essential fatty acids. Moreover, it lacks cholesterol as well as lactose and saturated fatty acids, so it can be consumed by people trying to control their cholesterol intake as well as those who are lactose intolerant (Kundu, Dhankhar & Sharma, 2018).

One of the most popular non-dairy milk types is almond milk, manufactured from the nuts of almond trees. Almond milk is characterized by its creamy texture and nutty taste and is available in sweetened and unsweetened, and plain and flavored forms (Sethi, Tyagi & Anurag, 2016). Sales of almond milk have grown rapidly in the last decade. According to a new report conducted by Grand View Research, the global almond milk market has reached \$5.8 billion and is projected to hit \$13 billion by 2025 (Starostinetskaya, 2019). Various types of almond milk such as Silk Protein Chocolate Pea & Almond & Cashew Milk, Califia Farms Coconut Almond Milk, Vanilla Almond Milk, Breeze Almond milk Horchata, and Almond Breeze Mexican Hot Chocolate have been produced to meet consumer demand for different flavors and ingredients

1.2 Objective of the Study

The objective of this study was to develop a dairy, gluten, and soy-free milk product that would be culturally acceptable in the Middle East.

Specific food product development objectives were:

- To develop a plant-based milk alternative using almonds, figs, and dates.
- To compare the nutrients in the product developed with a similar commercially-available product
- To compare the sensory characteristics of the developed almond milk product with a similar commercially-available product using a consumer panel made up of Middle Eastern women.

CHAPTER II

REVIEW OF THE LITERATURE

2.1 History of Almond Milk

During ancient times, almond milk was a common ingredient in the food, beverages, and medicines. It was first used in the Middle East in the 13th century, though almonds are also native to India and North Africa. The milk was popular in the medieval period because it could be stored without spoiling for a long time, unlike cow's milk (Shurtleff & Aoyagi, 2018). Islamic people made and used almond milk during the fasting season because it was known to be nutritious and low in calories. In Africa, it is first known to have been used in Egyptian cookery in the 14th century (Shurtleff & Aoyagi, 2018).

During the Middle period, almond milk was also widely used in regions of Europe as a substitution for dairy milk during Lent. In England, almond milk is first mentioned in the literature at the end of the 14th century. During this time, it was often provided to children as it is easily digestible.

By the middle of the 19th century, almond milk had become a common ingredient in Indian cookery books. In California, information about almond milk was first recorded in the 19th century. Contemporary research has supported the value attributed to almond milk as it has been determined to be nutritious (Alozie & Udofia, 2015).

2.2 Almond Milk Consumption and Propagation Stages

Almond milk consumption has been growing in recent years. During the medieval era, production was low and was carried out using traditional grinding methods. There was a lack of adequate information about its production and value, reducing its potential popularity. In recent years, as the nutritional benefits of almond milk have become better understood and as this knowledge has spread, production steadily increased (Shurtleff & Aoyagi, 2018). As technology has evolved, modern mechanized processes have replaced traditional grinding methods. In addition to increasing production speed, contemporary almond milk products are higher in quality than early products made with traditional methods.

Recently, demand for almond milk has increased considerably. Currently, global almond production is between 1 and 1.5 million metric tons and is no longer confined to the Middle East (Foreign Agricultural Service (FAS), 2017). The world's largest almond producer is the USA, with production concentrated in California. Other major almond-producing countries include Italy, Turkey, Australia, Morocco, Spain, Greece, Iran, and Portugal (FAS, 2017).

According to Nielsen (2016), almond milk sales have increased by 250% during the previous five years. Although almond milk represents only 5% of the milk market, it has the largest market share (approximately 60%) of non-dairy milk and is more profitable than other milk substitutes (Dharmasena et al., 2015). Figure 1 shows recent changes in sales figures and the 2015 market value.

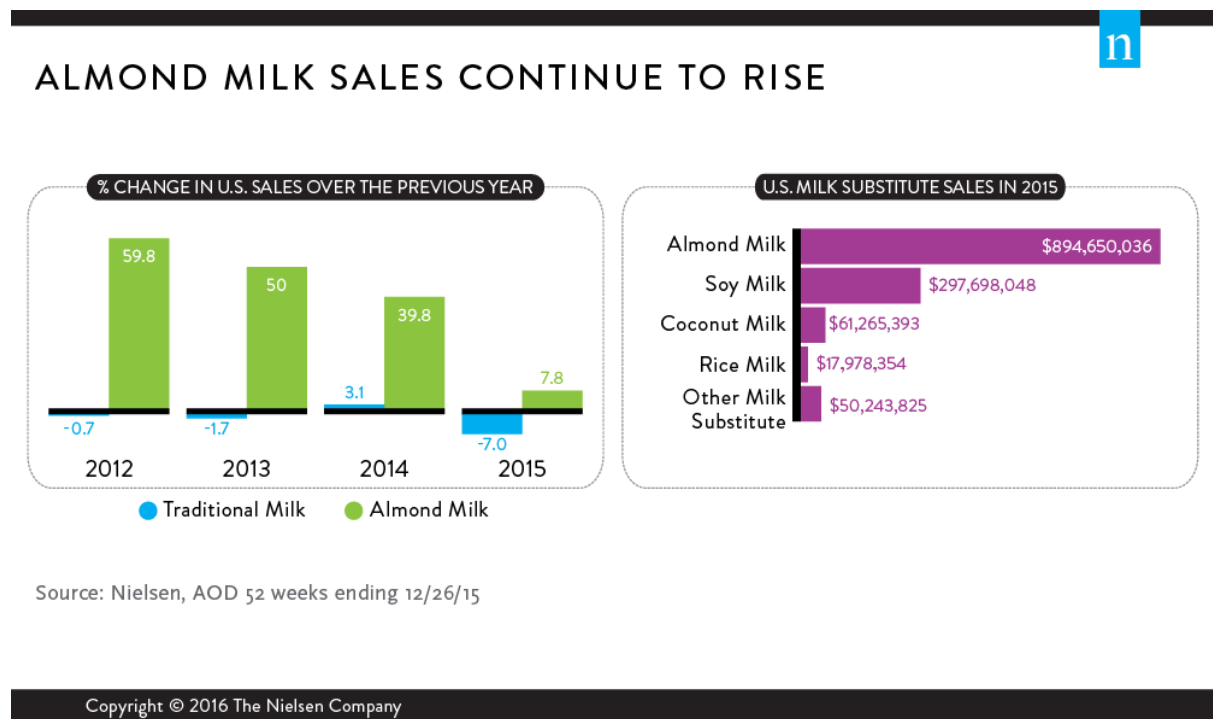


Figure 1. Almond milk sales and 2015 market value (adapted from Nielsen Company, 2015)

2.3 Almond Milk Production Process

There are various methods for producing almond milk. Domestic almond milk production is a simple process. First, almonds are washed in running water. They are then soaked in clean, warm water for around 12 hours (Berger, Bravay & Berger, 2013). The almonds are then removed from the water and rinsed. More water is then added, and the cleaned and soaked almonds are liquefied in a blender, using maximum speed. After

blending, the liquid and pulp are separated using a strainer or cheesecloth as the filter. The resulting filtrate is almond milk.

Commercial almond milk production involves precise, sophisticated processes. Production begins with the heating of almonds at 80-90°C to form an aqueous dispersion of partially de-oiled almond powder (Berger et al., 2013). The powder is then proportioned at 8% ±1% and then mixed with 0.1% of a stabilizing agent (Sethi et al., 2016), usually a hydrocolloid, and left for some time to allow the compound to solubilize. Next, the mixture is cooled to about 55°C in order to raise its viscosity. The aqueous solution is then taken through the grinding phase for two minutes at a maximum acceleration of 38,000 m/s². Centrifugal clarification is used to remove large particles that can be detected on the roof of the mouth or tongue (Berger et al., 2013). The resulting products undergo Ultra-High Temperature treatment (UHT) by pasteurization at 90° C for 15 seconds. Homogenization during cooling is maintained at 10,000 hPa (Hectopascal Pressure Unit). The product is then packaged and sold for consumption (Sethi et al., 2016).

2.4 Almond Water Activity and Moisture Content

Almond water activity and moisture content influence its shelf-life. Moisture content refers to the total amount of water available in a product (Syamaladevi et al., 2016). The relationship between almond milk products and moisture content is expressed either in terms of 'dryness' or 'wetness.' 'Dryness' refers to the product's water content per unit of mass. 'Wetness' refers to the quantity of water present per unit of mass of a substance.

Controlling moisture content, oxygen, and temperature is vital for the preservation of almond quality and shelf-life stability. Moisture content in almonds is expressed as a percent of the number of grams of water in 100 g of almonds (Capanoglu & Boyacioglu, 2008) and should be maintained at 6% maximum or less (Syamaladevi et al., 2016). Dates are classified as having an intermediate moisture content, which has been defined as a maximum of 0.6 of water activity, and between 20–23% moisture content (Habib & Ibrahim, 2011).

Water activity (a_w) represents the chemically-available (unbound or free) water in a product at a given temperature. Research shows that water activity is strongly associated with food preservation, because low moisture content minimizes chemical and enzymatic reactions, preventing the growth of microorganisms. The level of water activity in low moisture foods such as almonds is influenced by the humidity of the storage environment as well as temperature. Storage temperatures must be kept low as almonds start spoiling at temperatures above 38°C (Syamaladevi et al., 2016). When stored in cool and dry conditions, almonds have water activity levels ranging from a_w 0.3 to 0.6. In general, bacteria and mold do not grow at a water activity level below 0.8 (Almond Board of California, 2014).

Figure 2 shows a diagrammatic summary of the relationship between moisture content and water activity in almonds.

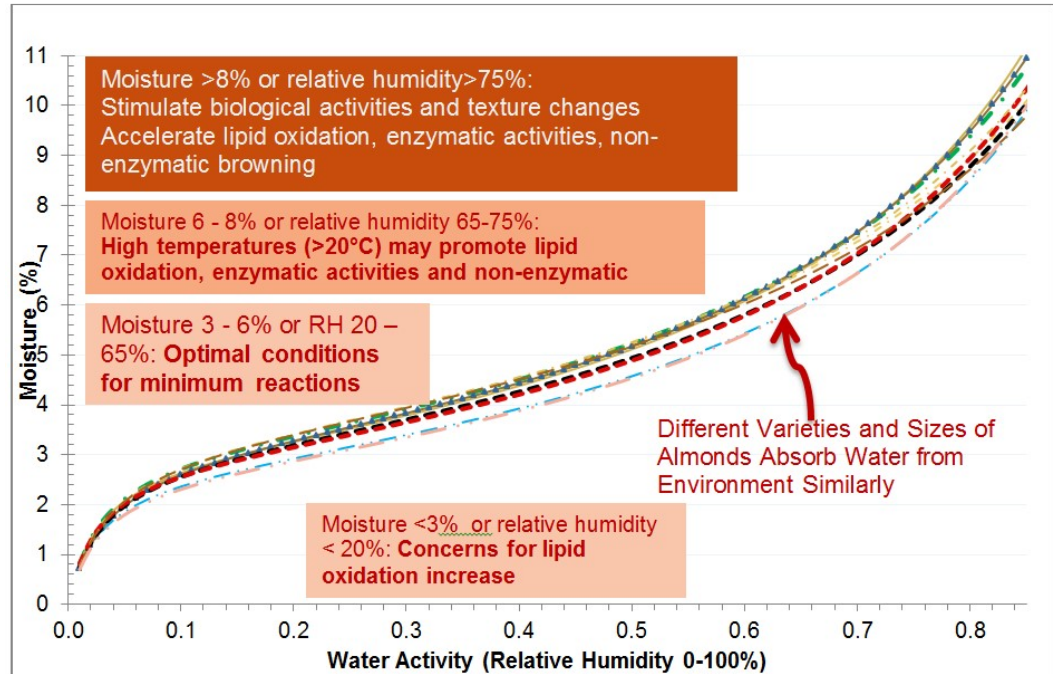


Figure 2. Managing humidity and temperature of almonds to ensure shelf-life (adapted from the Almond Board of California, 2010).

2.5 Almond Milk Shelf Life

'Shelf-life' was described by Fu and Labuza (1993) as “the amount of time a product remains within an acceptable sensory, nutritional, and safety level.” When a food product’s shelf-life is calculated, many relevant factors are considered. These include environmental factors such as storage conditions, oxygen, temperature, humidity, microorganisms, insects, and processing conditions. Other factors that determine or influence shelf life include the ingredients and the composition and nature of the product. The characteristics of almond milk products and the composition of the product (including the amount of unsaturated fats, moisture content, water activity, and roast level) also affect shelf-life.

Packaging also affects shelf-life, with proper packaging offering physical protection against moisture and gas penetration. Further, packaging materials should be free of odors and moisture (Syamaladevi et al., 2016).

The recommended shelf life of refrigerated almond milk is between 7-10 days of opening to ensure safe consumption. A container that appears bloated, or almond milk that appears curdled or slimy, are indications of stale milk that should be discarded (Syamaladevi et al., 2016). To achieve a good shelf-life, proper storage is required. After the ultra-high temperature pasteurization process, almond milk can be put into sterile, shelf-safe packs (Sethi et al., 2016). Almond milk has between 1-3 months' shelf-life if it is unopened and stored under the right conditions.

2.6 Almond Milk Packaging and Labeling

Almond milk is often packaged using recyclable materials such as plastic or glass, or alternatively in hard composite cartons. Composite cartons are often made by laminating three different materials: high-quality paperboard, polyethylene, and aluminum foil (Mexis & Kontominas, 2010). The paperboard supplies the stiffness needed while the polyethylene material is used to provide a tight seal inside and outside the carton. The aluminum foil provides a barrier against light and oxygen, helping to preserve the milk without the need for refrigeration.

Food labeling is a key component in marketing and is legally required in the United States by the Food and Drug Administration (FDA). It discloses whether the product is appropriate for all consumers, or whether it should be avoided by some. It imparts vital information about production and use, including ingredients, handling, and

preparation instructions. All of this information could influence consumer health and the environment. Food products are usually labeled with their key features, such as product name, manufacturer's name and address, ingredients, date of manufacture, shelf life, expiry date, and other key or required information. This could include warning statements about any allergy-causing components or ingredients. In the case of almond milk, this would include almond nuts (“Guidance for Industry: Food Labeling Guide,”2013) .

2.7 Almond Milk Allergies

Despite almond milk’s popularity and noted benefits, it is not without potential adverse side effects. Research shows that it is not a suitable product for those who are allergic to nuts. In allergic people, it can cause diarrhea, nausea, and facial swelling (Al Tamimi, 2016). And almond allergies are not rare: almonds are one of the nuts to which the largest number of people are allergic.

2.8 Almond Milk Product Competitors

Almond milk is not the only plant-based milk on the market. Soymilk was the first commonly produced and sold plant-based milk. It began to be mass-produced to provide high levels of nutrients to those who are hypersensitive to milk protein and/or are lactose intolerant. Isoflavones are the major bioactive component in soy. These are known to contribute to the prevention of osteoporosis, cardiovascular disease, and cancer (Omoni & Aluko, 2005). However, consuming soymilk may cause allergic reactions in some people. This is the major drawback of soymilk products.

Alozie and Udofia (2015) compared almond milk to soymilk. They observed significant variations in mean mineral content and sensory scores across the milk samples tested. Almond milk showed higher levels of crude fiber and ash. Concentrations of potassium, calcium, magnesium, phosphorus, zinc, and iron were significantly higher in almond milk than in soymilk. Sensory evaluation indicated substantial variation across samples, but overall the study concluded that almond milk was preferable to soymilk when considering color, taste, flavor, and overall acceptability (Alozie & Udofia, 2015).

A similar study analyzed almond milk and soymilk samples in different proportions (100% soybean, 100% almond nut, 50% almond/50% soy, 40% almond/60% soy, and 60% almond/40% soy). The study concluded that milk prepared with 100% almond nuts had a higher concentration of nutrients than the other milk (Kundu et al., 2018).

Another popular plant-based milk is coconut milk, used for many years in Asia. It has been hailed for its power to boost the immune system as it contains lauric acid (Sethi et al., 2016). It is also thought to help support heart health. However, consumption of coconut milk is limited, which can be attributed to its higher saturated fatty acid content.

Other popular plant milk products include flax milk, sesame milk, hemp milk, and rice milk (Vanga & Raghavan, 2018). Each of these products has a small share of the market.

2.9 Ingredients of Developed Almond Milk

2.9.1 Figs

Figs, scientifically known as *Ficus carica* L., are native to the Middle East and Asia. They are one of the oldest fruits known to have been consumed by humans. Historical evidence dating back to about 2500 B.C documents the use of figs for their medical and nutritional benefits. Figs were used to cure or treat a diverse range of illnesses, including gastric issues, inflammation, and cancer (Mawa, Husain & Jantan, 2013). Figs were common plants during the time of Jesus, with fig trees being mentioned in both the Holy Quran and Old and New Testament of the Bible. Figs were also used to sweeten different dishes before sugar became available and replaced them (Mawa et al., 2013).

Figs are a nutritious fruit that contains phenolic compounds, minerals, soluble sugars, organic acids, and vitamins A and K (Slatnar, Klancar, Stampar & Veberic, 2011). Common minerals found in figs include magnesium, copper, iron, calcium, potassium, phosphorus, and strontium. They also provide calories and contain proteins, carbohydrates, and fiber. Their high fiber content makes figs suitable for nourishing and toning the intestines, working as a natural laxative (Mawa et al., 2013). As a rich source of dietary fiber, figs can have a positive effect on weight loss and management, helping to induce satiety.

Figs may play a key role in decreasing the risk of developing breast cancer. A study conducted on post-menopausal women diagnosed with breast cancer found a 34-50% reduction in the risk of developing breast cancer for women who consumed fiber-

rich fruits such as figs (Suzuki et al., 2008). A systematic review of the efficiency of *Ficus carica* in boosting bone health, examining all studies from 1946 to 2016, concluded that *Ficus carica* plays a role in the promotion of bone health. The authors suggested that figs could have pharmaceutical use in the future (Idrus et al., 2018).

2.9.2 Almonds

As discussed above, almonds, scientifically known as *Prunus amygdalus*, are believed to have originated from the Middle East, parts of the Indian subcontinent and North Africa. The almond tree has been cultivated for human use for many centuries, partly due to the nutritional and health value of its nuts (Yada, Lapsley & Huang, 2011). Throughout human history, almonds have been valued for their religious, ethnic, and social significance.

Currently, almonds are known for their nutritional value and are known to have multiple health benefits. They are a rich source of vitamin E and other minerals, including iron, magnesium, potassium, and calcium. Further, they are low in sugar and rich in protein. Although almonds contain high levels of fat, it is nearly 66% monounsaturated fat and 26% polyunsaturated fat (United States Department of Agriculture, 2011).

Eating almonds can increase levels of vitamin E in the bloodstream, with almonds containing 34% vitamin E (α -tocopherol). Vitamin E plays a pivotal role and is a strong antioxidant source. It protects the body's cells from harm caused by free radicals and oxidative stress (Rizvi et al., 2014). Almonds can also help reduce blood pressure and increase blood flow, averting the artery-clogging oxidation of cholesterol (Jambazian,

Haddad, Rajaram, Tanzman & Sabaté, 2005). A study has shown that consuming 10g of almonds daily improves HDL-cholesterol levels and reduces triglycerides as well as total cholesterol (including LDL-cholesterol and VLDL-cholesterol) (Jamshed, Sultan, Iqbal & Gilani, 2015). The cardio-protective properties of almonds are due to their micronutrient contents, including polyphenols. These act as antioxidants and are widely believed to be beneficial for a range of health issues, including cardiovascular concerns (Berryman et al., 2011).

Recent studies indicate that including almonds in the diet may help to decrease the risk of diabetes type II. Almond consumption by patients with type II diabetes has been associated with weight reduction and decreased levels of fasting glucose, triglycerides, and LDL-cholesterol (Lovejoy et al., 2002). A similar piece of research found that the nutrients in almonds have beneficial effects on glucose homeostasis and on controlling weight (Kim, Keogh, & Clifton, 2017). Rajaram and Sabaté (2008) noted the following:

“Review of the available data to date suggests that adding nuts to habitual diets of free-living individuals does not cause weight gain. In fact, nuts have a tendency to lower body weight and fat mass. In the context of calorie-restricted diets, adding nuts produces a more lasting and greater magnitude of weight loss among obese subjects while improving insulin sensitivity (p. 579)”.

2.9.3 Dates

Dates, a fruit produced by a flowering palm plant traditionally found in desert areas, are scientifically known as *Phoenix dactylifera*. They are thought to have originated in

the Middle East, particularly Mesopotamia. Fossil evidence shows that dates have been in existence for at least 50 million years, making them the oldest cultivated fruit in the world (Al-Shahib & Marshall, 2013). In the Middle East, dates have been considered an essential food for thousands of years. In pre-historic Egypt, dates were used to make wine (Flowers et al., 2019). During Ramadhan, Muslims have traditionally eaten dates to break their fast because they increase blood sugar and provide energy. In the Roman era, dates were popular and widely cultivated for their nutritional value.

By the late 18th and early 19th centuries, missionaries from Spain were growing date palms in California and near the Mexican border (Russo, 2007). Today, dates are widely cultivated in North Africa, Egypt, South Asia, and the Horn of Africa. Despite this widespread global production, the Middle East produces 2/3 of the world's dates, with production levels highest in Egypt, Iran, Iraq, and Saudi Arabia.

Numerous studies claimed that dates have many health benefits, providing an extensive range of fundamental nutrients. Dates are a rich source of several minerals and vitamins. Dates help provide energy, containing 70–80% carbohydrate in two forms (glucose and fructose). Both of these are easily absorbed directly into the bloodstream (Al-Farsi, Alasalvar, Morris, Baron & Shahidi, 2005). Additionally, dates are a great source of antioxidants, potassium, dietary fiber, magnesium, protein, copper, manganese, iron, vitamin B6, and phenolics, giving them a high nutritional value. Dates' high oleic acid content also makes them useful for people with cardiovascular disease (Al Juhaimi, Özcan & Ghafoor, 2012). Further, over time it has been believed that consuming dates

reduces the need for augmentation of labor during pregnancy, working as a facilitator to the delivery process (Al Bochi, 2018).

2.9.4 Water

Water is a naturally occurring compound with many uses. Water is a crucial component in the production of the proposed almond milk, just as it is in many other fruit and juice processing industries. The production of almond milk requires water as both a cleaning agent as well as a solvent.

2.10 Developed Almond Milk Advantages and Health Benefits

Almond milk has myriad health advantages and health benefits in the human body (Choudhury, Clark & Griffiths, 2014). The almond milk developed for this study is a beverage alternative that is unlikely to cause unintended weight gain. For people participating in weight loss programs, almond milk can be taken as an alternative for dairy milk. Unsweetened, plain almond milk contains less fat and fewer calories than fat-reduced cow's milk. On average, the calorie content of almond milk is 50% less than that of cow's milk. It has zero cholesterol (Choudhury et al., 2014). Fortifying almond milk with other fruits such as figs and dates that contain large amounts of vitamins and minerals makes almond milk an even better choice for human consumption, with greater potential health benefits, but does raise its calories values.

Observational studies have revealed that consistent intake of almond milk can help to reduce heart disease risk, possibly because of its healthy fatty acid content. The major fatty acid in almond oil is oleic acid, which has been found to encourage beneficial

changes in blood lipids (Kamil & Chen, 2012). Almond milk also can help in the development of strong bones. It may lower an individual's risk of arthritis and osteoporosis and may improve the immune system. Platt, Josse, Kendall, Jenkins, and El-Sohemy (2011) assessed the postprandial effects of consumption of 60g of almonds on human osteoclast precursors. The result showed that almond intake reduced osteoclast composition and function, providing proof of the relationship between almond and a decreased risk of developing osteoporosis. Research also shows that 100g of almond are rich in antioxidants and contain 25.1 mg of the flavonoids that are essential in lessening the number of free radicals in the body. However, removing almonds' skin can cause a reduction of approximately 25%-50% of antioxidants, as these are concentrated in the skin (Ros, 2010).

Although a small percentage of figs and dates have been added to the almond milk developed for this study, the milk still contains sugars from these fruits. This could be of concern to many consumers, especially those with diabetes. Veberic, Jakopic, and Stampar (2008) conclude that due to the antioxidant character of the phenolic content of figs, they should be considered to be a healthy ingredient and a good alternative to the consumption of sweets. Further, Tang, Shi & Aleid (2013) suggested that dates potentially play a role in preventing and controlling diabetes. The almond milk developed for this study used figs and dates without any additional sugar.

Lactose intolerance is defined as the lack of ability to break down lactose into galactose and glucose and then absorb them (Labrie et al., 2016). Lomer, Parkes and Sanderson (2008) found that lactose intolerance is affected by various factors, including

ethnic origin. Some Asian countries have the highest percentage of lactase non-persistence, at almost 100%. South American and African countries represent over 50%. Northern Europeans, North Americans, and Australians have low rates of lactose intolerance. Among of the populations of the UK, Finland, and northern France 17% suffer from lactose intolerance. The almond milk developed in this study is lactose-free, making it suitable for people who are lactose intolerant.

The protein gluten is mostly present in rye, wheat, and barley, and it is therefore present in common foods made from these grains, such as bread. Celiac patients need to consume gluten-free foods, as gluten can damage their small intestine, interfering with nutrient absorption (Hosseini et al., 2018). The milk developed in this research is made only of almonds, figs, dates, water and sea salt, all of which are gluten-free.

CHAPTER III

METHODOLOGY

3.1 Description of the Product and Process Flow Diagram

Almonds, dates, figs, and water were among the ingredients used to produce almond milk for this study. The Ikhlas' dates used were purchased from a market located in Jeddah, Saudi Arabia. Great Value Whole Natural Almonds and a Shahia Dried Fig Garland were obtained from a Walmart supermarket (Edmond, OK). All of these fruits were properly stored during the production process. Fruits, pastes and liquids were stored in therefrigerator at 35.6- 46.4 °F (2-8 °C) when not being used.

Table 20 below illustrates the final product formulation.

Table 20: Almond milk formulation

Ingredients	%w/w
Almond	25
Fig	5
Date	5
Water	65
Total	100

The present formulation was created after products developed during several previous research trials were compared with commercially available almond milk and found to be weak in some areas. The product formulation was modified several times before being finalized. The results from the pilot study were all positive, indicating that the present product formula was the best for the market of those tried.

3.2 Production Process

One kilogram of fresh almonds was purchased from the market, with 250 grams used in the formulation of the almond milk. The almonds were measured using a Shimadzu balance scale (ME-204 MS-205DU, ATX-224, Shimadzu, Japan). Hot water (70 °C=158°F) was added to the weighed amount to soften the almond skins. The soaked almonds were cooled and then refrigerated. The overall soaking period was 12 hours, and skins were then removed manually. It is possible to make almond milk without removing the skin, which could be ground finely along with the rest of the almond. This would have improved the nutritional qualities of the product, reduced waste, and might have had a positive effect on the product's texture and color. However, a decision was made to remove the skins, since soaking and removing the skin was shown to reduce phytate contents, which could lessen the bioavailability of some micronutrients (Lin et al., 2017).

Figs and dates were soaked separately in hot water (70 °C=158°F), they were then stored in the fridge after cooling. The total period of the soaking process was 12 hours. The soaked figs then were added to a blender (NutriBullet model, serial number: 86831254, Homeland Housewares, US) and processed for five minutes at a speed of 24,000 rpm until a fig paste was produced. Date seeds were removed by hand, and the

deseeded dates and 50 grams water were added to the blender. The dates were then processed for five minutes at a speed of 24,000 rpm until a date paste was acquired.

The separated almonds, fig paste, and date paste were added to the remaining 500 grams of water in the NutriBullet blender (serial number: 86831254, Homeland Housewares, US), which was then run for five minutes at a speed of 24,000 rpm. The blender was then switched off, and the contents were given 10 minutes to settle. After a homogenous mixture was obtained, the blended material was filtered using Nut Milk Bag's reusable 12" x 10" (30cm x 25cm) cheesecloth bags (BESTOMZ). The filtered milk was heated at 72 °C using a stainless-steel double boiler for 15 seconds, occasionally being stirred by hand (Brown, 2011). It was cooled using an ice bath in a large container and continually stirred by hand until the temperature of the mixture was reduced to 5°C. Milk frothing thermometer (Update International, model number: THFR-17) was used to track the temperature during the posterization process period. The milk was packaged in a glass bottle with a secure lid, and the bottle was stored in a refrigerator at 2-8 °C. A portion of the filtered product was then drawn off for analysis. Figure 3 presents the milk production process as a flow chart.

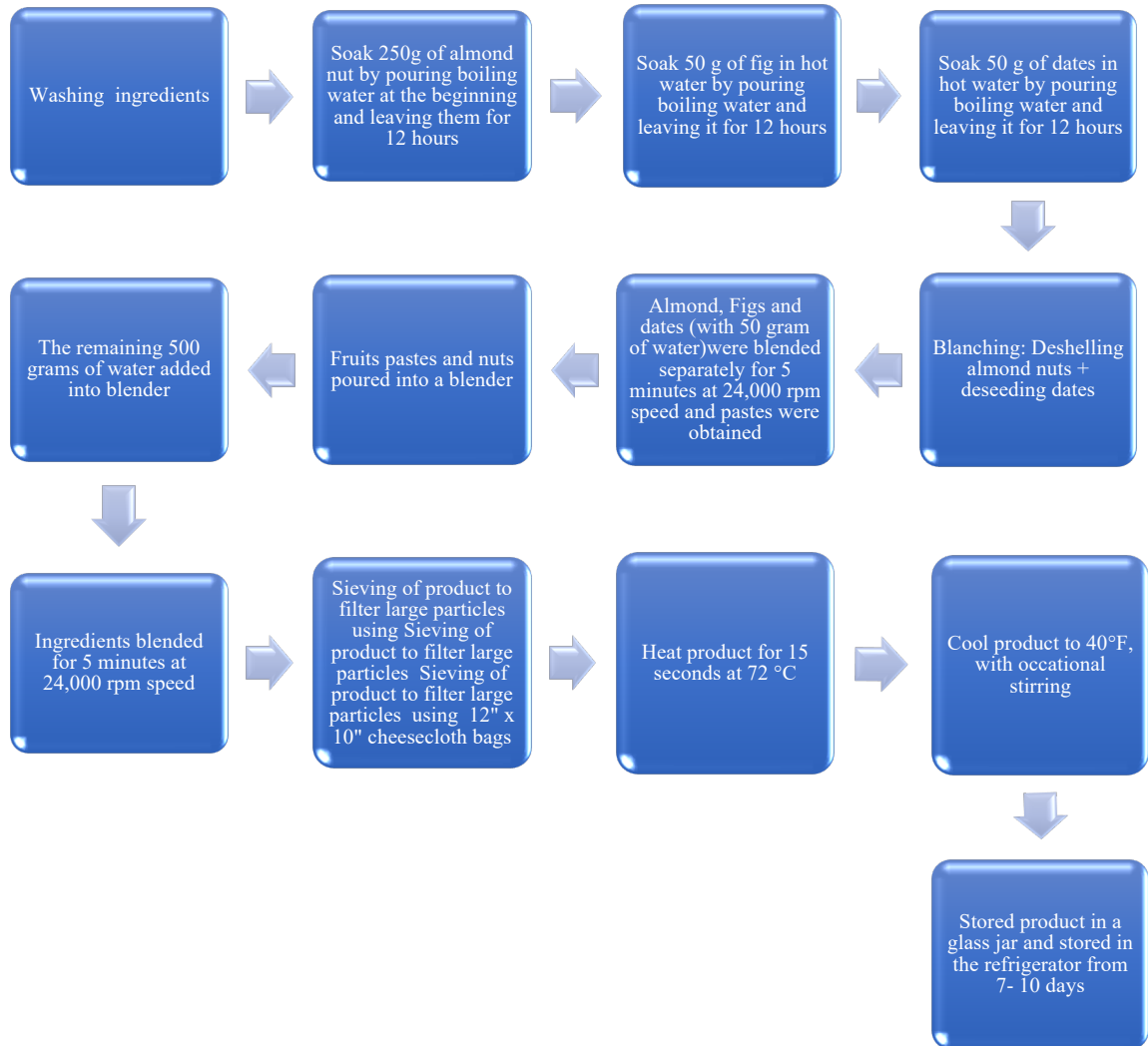


Figure 3. Almond milk production process flow diagram

3.3 Chemical Analysis

3.3.1 Proximate Analysis, Carbohydrate, and Water Activity

Proximate analysis methods were used to examine the moisture, ash, fat, and protein content in six samples (three of which were of the almond milk developed in this study and three of which were of commercial almond milk). In addition, carbohydrates and water activity were analyzed. All of the analyses were conducted in a chemistry lab

located in the Robert M. Kerr Food and Agricultural Products Center (FAPC) at Oklahoma State University, Stillwater, OK by laboratory staff. Moisture content, ash, carbohydrate, and protein tests were performed in triplicate, while fat analysis was performed in duplicate.

Five grams of each sample was placed in the oven at a temperature of $102^{\circ}\text{C} \pm 1$ for 16-18 hours for drying in accordance with the AOAC Official Method 950.46. The moisture percentage was determined from the loss in weight.

$$\% \text{ Moisture (wt/wt)} = [(A - B) / A] \times 100$$

The AOAC Official Method 920.153 was used to test ash content and expressed as a percentage of the initial weight of the product. The crude protein content of the product was determined by drying 0.2 grams of each sample at $102^{\circ}\text{C} \pm 1$ for 16-18 hours using an air oven. The AOCS Ba 4e-93 methodology was used; the general protein conversion factor is 5.18. The formula utilized is $\% \text{ Protein} = \% \text{ N} \times 5.18$.

The AOAC Official Method 991.36. was used to determine crude fat content. Five grams of each sample was dried at $102^{\circ}\text{C} \pm 1$ for 16-18 hours using an air oven. The extraction was performed using ethyl ether and petroleum ether.

Norajit, Gu, and Ryu (2011) described the total carbohydrate calculation as $\text{Carbohydrate content} = 100 - (\text{ash} + \text{fat} + \text{moisture} + \text{protein})$. Water activity was determined using an Aqua Lab Meter at 24.6°C , with the dew point sensor of the meter measuring water activity.

3.3.2 Micronutrients and Dietary Fiber Analyses

Calcium, dietary fiber, potassium and vitamin D2, and D3 content analyses were conducted in the Mérieux NutriSciences Lab. The AOAC 991.43 methodology was adopted for the analysis of both calcium and dietary fiber. The levels of vitamin D2 and D3 in both milk samples were analyzed using the AOAC 2011.11 (UHPLC-MS/MS) method.

3.3.3 Other Chemical Analyses

A refractometer (Model: MASTER-53 α . Cat.No. 2351) was used to analyze the sugar level of the two samples. In a Brix refractometer, the sugar percentage (Degrees Brix ($^{\circ}$ Bx)) was determined as a function of the index of refraction in the solution. The average pH level of these two samples was measured using a Corning 32 pH meter. This process took less than 10 seconds.

3.4 Subjective Perceptions Feedback

Although the triangle test, in which the participants taste three samples of two different products, is commonly used as a sensory test, a paired comparison test was adopted instead. The paired comparison test is an easy acceptance test that minimizes confusion among panelists while identifying the sample that is higher in an attribute. It involves assessing only two samples at a time (Vaclavik, Christian & Christian, 2008). Two cups, one from the almond milk developed in this study and one from the commercially-available almond milk, were used to determine the extent to which consumers liked the product. Feedback was solicited to aid product development and improvements and to assess the product's market potential. A questionnaire was given

using a nine-point hedonic scale in which (9) meant “like extremely,” (8) “like very much,” (7) “like moderately,” (6) “like slightly,” (5) “neither like nor dislike,” (4) “dislike slightly,” (3) “dislike moderately,” (2) “dislike very much,” and (1) “dislike extremely.” The questionnaire also asked about several essential attributes, including color, odor, texture, sweetness, flavor, and the overall acceptance rating of the product.

The almond milk produced in this study and the commercially-available almond milk (Unsweetened Silk Almond Milk (WhiteWave Foods, North America)), were compared. The commercially-available milk is unsweetened, reduced-fat almond milk, selected for comparison because it is the commercial milk most similar to our product. Unsweetened Silk Almond Milk is a processed, unsweetened, gluten, and dairy-free product. It is fortified with vitamins and minerals, added to increase its nutritional value. Ingredients such as natural flavor, locust gum, gellan gum, and ascorbic acid (Figure 4) are added to the product to help avoid bacterial growth, prevent spoilage, protect freshness and increase shelf life.

Nutrition Facts	
Pure Almond Almondmilk, Dark Chocolate - From Silk	
1 container (226g)	
Servings Per Container 1	
Amount Per Serving	
Calories 120	Calories from Fat 25
% Daily Value*	
Total Fat 2.5g	4%
Saturated Fat 0g	0%
Trans Fat 0g	
Polyunsaturated Fat 0.5g	
Monounsaturated Fat 1.5g	
Cholesterol 0mg	0%
Sodium 190mg	8%
Total Carbohydrates 23g	8%
Dietary Fiber 1g	4%
Sugars 21g	
Protein 2g	
Vitamin A	10%
Vitamin C	35%
Calcium	45%
Iron	10%

* Percent Daily Values are based on a 2000 calorie diet. Your Daily Values may be higher or lower depending on your calorie needs.

Figure 4. Nutritional facts of flavored Silk almond milk

3.4.1 Procedure

Fifty women from the Middle East, aged 25-65 years old, were recruited for this study, with help from the Islamic Society of Greater Oklahoma. They posted an announcement of the study on Facebook and distributed flyers in the area surrounding their mosque. The panelists assembled in a specific hall in the Islamic Society of Greater Oklahoma (Masjid An-Nasr). Participation in the sensory analysis was open for three days. The schedule for the first two days was open from 9 am to 5 pm, with the last day by appointment only. Participation took no more than one hour. The project was IRB-approved (IRB application number was AG-19-52).

Consent forms were given to all of those who participated in the study. First, the consent form was read to them. Then, any questions they had on it were answered. The

participants were individually required to sign their informed consent forms before proceeding with their participation in the project. All participants were allowed to keep one of the two signed consent forms for their records.

There were two tasting rounds, during which the participants were divided into two groups (A and B). A copy of the questionnaire was distributed to the two groups, and a paired comparison test was used for the sensory evaluation. A sample of the almond milk developed for this study was presented to group A in the first round, and a sample of commercial almond milk was presented in the second round. Group B was provided with a commercial almond milk sample in the first round, and a sample of the almond milk samples developed in this study in the second round. Approximately 15 ml of milk was placed into a waxed cup for each participant. Unsalted crackers and a bottle of water were also provided to allow participants to separate the taste of each sample. The samples were provided with three-digit codes. It is worth noting that this method followed the ISO 5495:2005 standards for sensory testing.

3.5 Statistical Analysis

A statistical analysis of the differences in sensory evaluation between the product developed for this study and the commercially available product was made using paired sample t-tests. The data from triplicate proximate and mineral analysis samples were analyzed using a Mann-Whitney U test. The ANOVA test is usually used to test the difference in the mean between two groups. In this case, the Mann-Whitney U test (non-parametric test) was used because the data were not normally distributed.

CHAPTER IV

FINDINGS

4.1 Sensory Analysis

The developed product and the commercially available one had many statistically significant differences (Table 21). The highest differences in scores were observed in odor (0.9 (± 1.7)), sweetness (0.9 (± 1.7)), and mouthfeel (0.8 (± 1.5)). The overall acceptance score for the developed product was higher than the commercial one by an average of 0.7 (± 1.3). The decision to report only one number after the decimal in the mean and SD was taken as reporting more than one decimal number holds little clinical value for a hedonic scale.

Table 21: Sensory quality evaluation for the commercial almond milk (control) and the developed almond milk (treatment)

Characteristics	Treatment		Control		Difference		T-score	df	P-value
	Mean	SD	Mean	SD	Differences in two Means	SD			
Color	6.9	1.2	6.4	1.2	0.5	1.3	2.875	49	0.006
Flavor	7.8	1.2	7.2	1.3	0.6	1.5	2.806	49	0.007
Sweetness	8.3	1.1	7.4	1.2	0.9	1.7	3.572	49	0.001
Odour	7.3	1.2	6.4	1.1	0.9	1.7	3.745	49	< 0.001
Mouthfeel	7.4	1.1	6.6	1.1	0.8	1.5	3.848	49	< 0.001
Overall acceptance	8.1	0.9	7.4	1.0	0.7	1.3	4.054	49	< 0.001

Nine-point hedonic scale: ranges between (9) = "like extremely" & (1) = "dislike extremely"

4.2 Chemical Analysis

There was a marginally significant difference between the pH levels of the two products. The median pH for the developed product was 5.68 (Table 22), compared to that of the commercial product, which was 7.41 ($p = .0495$). The sugar test (% Brix) also showed a significant difference ($p = 0.0463$); for the developed product, it was 12.73%, which compared to 4.20% in the commercially available product.

The nutritional content showed a significant difference between the two products except for the total dietary fiber, vitamin D3 content, and water activity. Calcium was significantly lower for the developed product at 27.80 mg/100g versus the commercial product at 199.00 mg/100g. However, potassium content was significantly higher for the

developed product (105.00 mg/100g) than that of the commercial product (12.20 mg/100g). Vitamin D₂ was also different; for the developed product. It was < 0.75mcg/100g lower than that of the commercial product (2.03mcg/100g). The total vitamin D content was significantly lower for the developed product (< 0.55mcg/100g) than for the commercial product, which equals to 2.03mcg/100g.

The moisture percentages were different. The one for the developed product (82.94%) was significantly lower than that of the commercial product (94.76%) ($p = .0431$). Ash content percentage also showed a significantly lower value for the developed product (0.54%) than that of the commercial product (0.63%) ($p = .0369$). On the other hand, fat content percentage was a significantly higher value for the developed product (5.73%) than of the commercial product (0.91%) ($p = .0495$). Further, protein content percentage was significantly different, where the developed product (2.02%) contained higher protein concentration product (0.35%) ($p = .0463$). Finally, the carbohydrate percentage was significantly higher for the developed product (8.79%) than that of the commercial product (3.37%) ($p = .0431$).

Table 22: Chemical analysis for the developed almond milk (treatment) and the commercial almond milk (control)

	Treatment	Control	P-value*
	Median	Median	
Average PH	5.68	7.41	0.0495
Sugar test			
%Brix	12.73%	4.20	0.0463
Nutrients			
Calcium mg/100g	27.80	199.00	0.0495
%TDF	<0.45%	<0.45%	
Potassium mg/100g	105.00	12.20	0.0463
Vitamin D ₂ mcg/100g	<0.75 mcg/100g	2.03 mcg/100g	
Vitamin D ₃ mcg/100g	<0.55 mcg/100g	<0.55 mcg/100g	
Total Vitamin D mcg/100g	<0.55 mcg/100g	2.03 mcg/100g	
% Moisture	82.94	94.76	0.0431
% Ash	0.54	0.63	0.0369
% Fat	5.73	0.91	0.0495
% Protein	2.02	0.35	0.0463
% Carbohydrates	8.79	3.37	0.0431
Water Activity (Aw)	0.99	0.996	0.2752

* Mann Whitney U test was used because data is not normally distributed

CHAPTER V

DISCUSSION

5.1 Sensory Panel Analysis

The sensory profile of food is the most significant assessment factor for product quality. It is used to predict consumer reactions to a product and to determine its likeability. A group of 50 Middle Eastern women, aged from 25 to 65 years, served as the sensory panel in this study. After tasting both the almond milk developed for this study and the commercially available almond milk, the panelists ranked both milks' sensory qualities, including color, flavor, sweetness, odor, and mouthfeel (the physical sensation of food or drink in mouth). They did this using a nine-point hedonic scale from 1 to 9.

Statistical analysis compared the sensory qualities of the developed almond milk to the commercially available Silk almond milk. Mean (\pm SD) differences between the ratings of the samples were calculated and compared. Then, the mean difference between the two products for each sensory quality was revealed for its significance. For color, the mean (\pm SD) of the developed product was 6.9 (\pm 1.2), whereas the mean for the

commercial almond milk was 6.4 (± 1.2). This represents a significant difference between the means of the two products (value of 0.5(± 1.3)). The T-score for color was 2.875 ($p = .006$). The mean difference in flavor between the two products was significant, with the T-score for flavor being 2.806. In terms of sweetness, the difference was significantly higher, with p -value = .001. This indicates that the developed almond milk product was considered much sweeter.

For the characteristic of odor, the mean was also comparatively and significantly higher for the developed product ($p < .001$). The analysis of mouthfeel also showed that the women who tested the product gave a significantly lower score to the commercial product than to the developed almond milk. The corresponding T-score was 3.848 ($p < .001$). The higher score given to the developed almond milk may be explained by its higher fat content, which would be expected to produce a better mouthfeel. The overall acceptance rate for the developed almond milk product was significantly higher than for the commercial almond milk, with a mean higher rate of 0.7 (± 1.3).

From the results obtained, and because the P -values for the mean difference were < 0.05 , it can be concluded that both the sweetness and the flavor of the developed almond milk were preferred by participants, compared to the commercially available almond milk. Further, the overall acceptance level of the developed almond milk was high (P -value of the mean difference < 0.001). Twenty-one participants gave the developed almond milk a score of nine, and 19 gave a score of eight. One participant gave a score of five. Four, twenty-four, and four participants gave the scores nine, eight,

and five, respectively for the commercial product. The aim of the study was to develop an almond milk product that is culturally acceptable to women in the Middle East. The sensory evaluation results indicate that the developed almond milk product could become a competitor to commercially available almond milk products.

5.2 Proximate Analysis, Carbohydrate, and Water Activity

The moisture percentage of the commercially available almond milk was calculated as 94.76%. The moisture percentage of the developed almond milk was 82.94%. The p-value of 0.0431 for the difference in moisture content between the two samples indicates a significant difference. The moisture content in the developed almond milk product was similar to Alozie and Udophia's (2015) almond milk. Their milk had a moisture content of 86.11%. Alozie and Udophia stated that the higher moisture content of their milk, in comparison to soymilk, could be attributed to the fact that the primary ingredient of almond milk is water. It could also be attributed to the higher solids content in the tested product.

The percentage of ash in the developed almond milk product was lower than that in the commercial almond milk (0.54% versus 0.63%; $p = 0.0369$). Alozie and Udophia (2015) reported that their ash content obtained from ash was 3.04%, higher than the current study. Another study conducted by Kundu et al. (2018) developed almond milk using three different ratios of almond to water (1:1) (1:2) (1:3). The results showed that the higher the water content in the product, the lower the ash level.

The protein content of the two samples was significantly different. In the developed almond milk, it was 2.02%, and the commercially available almond milk, 0.35% ($p = 0.0463$). This difference can be attributed to the addition of dates and fig to the developed almond milk. The higher protein content that results may be beneficial for health (Chen, Lapsley & Blumberg, 2006).

A substantial amount of fat was reported in the developed almond milk, which had a fat content of 5.73%. The commercial-product had a fat content of 0.91% ($p = .0495$). Almonds contain mono-unsaturated and poly-unsaturated fats, which are considered “good fats.” Good fats help aid digestion and even weight control or reduction as they reduce hunger and promote satiety (Wien et al., 2003). They are also beneficial in keeping levels of harmful cholesterol low. However, calculating the difference in fat calories between the two products revealed that the developed almond milk product had 100 Kcal/cup more than the commercial product (5 grams difference in fat*250-gram cup* 9 Kcal/gram of fats/ 100 grams= 108 Kcal/cup). It should be noted that the human body does not absorb all of the fat in almonds, because some fats are inaccessible to digestive enzymes. Compounds, such as phytic acid form non-absorbable complexes when they bind with minerals (Liu et al., 2017). Additionally, in the developed almond milk product, there were no added chemicals or substances. In the commercially available milk product, ascorbic acid was used to prevent the degradation of unsaturated fatty acids and to inhibit their oxidation (Cami3n, Milagro, Fern3ndez & Mart3nez, 2008).

The carbohydrate content of the developed almond milk was significantly higher than that of the commercially available almond milk, with values of 8.79 % and 3.37%, respectively ($p = .0431$). The additional carbohydrates found in the developed almond milk product can be attributed mostly to the inclusion of dates. One MEDJOOOL date has 18 g of carbohydrate, and 3 DEGLET NOOR dates contain 16 g. One Khalas date (the dates used in the milk) contains 32 g of carbohydrate. (Al Bochi, 2018). Nevertheless, there was no added sugar or chemicals in the developed almond milk, and therefore it can be consumed by those not restricting carbohydrates.

In the developed almond milk, median water activity was found to be 0.99, lower than that of the commercially available almond milk, for which the median was 0.996. This difference is not statistically significant. As both samples were liquid, the resultant values for both products were high.

Water activity is considered an important element of a food product, affecting characteristics such as appearance, texture, spoilage, and taste. To protect the commercial almond milk from spoilage and to improve and preserve its stability, ultra-high-pressure homogenization was performed at 10,000 hPa (Hectopascal Pressure Unit). for the commercial product, knowing that ultra-high-pressure homogenization is suitable for liquid products like almond milk

5.3 Micronutrients and Dietary Fiber Analysis

Because the ingredients of the developed almond milk are rich in dietary fiber, the final product might be expected to have a substantial percentage of fiber. Still, the results

of both products were the same (less than 0.45%). This is because the almond milk production process goes through several stages, including detaching pulp from the nut milk, leading to the removal of most fiber from the eventual product (McIndoo, 2015).

Vitamin D2, the plant source of vitamin D, was expected to be found in the products, but not vitamin D3. This is because vitamin D3 is produced by the skin after sunlight exposure and is not usually found in food. Both vitamins D2 and D3 were found in the developed almond milk, but they were calculated as being less than 0.75 mcg/100g and 0.55 mcg/100g, respectively. The total vitamin D level found in the milk was < 0.55 mcg /100g. The commercially-available almond milk showed a remarkable amount of vitamin D2 (2.03 mcg/100g) and D3 (almost 0 mcg/100g), giving a total vitamin D level of 2.03 mcg/100g. Fortifying the commercially-available product with vitamin D meant that levels of the vitamin were higher than in the developed almond product.

The amount of calcium in the developed almond milk was found to be 27.8 mg/100g. The commercial almond milk had an accumulated total calcium level of 199.0 mg/100g. The developed almond milk had a higher calcium content than that found in a sample of almond milk in another study (16.01 mg/100g; Kundu et al. (2018)). In an additional comparable study, a lower value for calcium also was measured for almond milk (13.10 mg/100 ml) (Yetunde et al., 2015).

The reason for the elevated calcium level in the commercially available almond milk (199.00 mg versus 27.80 mg/100 g; $p = .0495$) is that minerals, including calcium carbonate, which represents a high level of calcium, were added to that milk. The

developed almond milk contained only food ingredients without any additives. The lack of additives in the developed almond milk product means that it can be tolerated by patients prone to kidney stones, or patients with irritations or allergies to additives. Moreover, the body struggles to absorb calcium carbonate, compared to naturally-occurring calcium in food (Vavrusova & Skibsted, 2014). As commercially-available almond milk is fortified with added calcium, the level is dependent on the brand (Katz, 2018).

Proportionately higher amounts of potassium were found in the developed almond milk compared to the commercially available almond milk: 105 mg/100 versus 12.2 mg/100. These differences were significantly significant ($p = .0463$). Both figs and dates are excellent sources of potassium, resulting in high levels being found in the milk (Vora & Vora, 2017).

5.4 Other Chemicals Analysis

The average pH reading obtained for the developed almond product was 5.68. For the commercial product, it was 7.41, a significant difference ($p = .0495$). The developed almond milk was produced with 25% almonds, 5% figs, 5% dates, and 65% water. The pH of figs is around 4.6, and the pH of the dates is around 6.3-6.6 (McGlynn, 1992). Almonds have a pH level of around 6. Because of the low pH values of its individual components, the overall pH level of the developed almond milk was lower than the commercial milk. The presence of calcium carbonate in the commercial product, which is sparingly soluble in water, led its pH to be slightly above 7 (median pH of 7.41).

According to Blamire (2000), most bacterial growth occurs when pH levels are between 6.5 and 7.0, which means the developed almond milk product has a pH level that should inhibit bacterial growth and extend shelf life

The sugar concentration of the developed almond milk was 12.73 grams, while the sugar level of the commercial almond milk was 4.20, a significant difference ($p = 0.0463$) The commercial product was unsweetened almond milk, but the developed almond milk product consisted of 5% figs and 5% dates, both of which contain a high amount of glucose and an even higher amount of fructose (Vora & Vora, 2017). However, these types of sugars are plant-based natural sugars. They are readily absorbed, sweet, and help induce satiety. This means they may reduce the total calorie intake in comparison to fat-rich food (Al-Farsi & Lee, 2008).

5.5 Comparison among Cow's milk, Soymilk, Almond Milk and Coconut Milk

A comparison of the nutrients in a 100-gram portion cow's milk, soymilk, almond milk, and coconut milk was carried out in order to differentiate between cow's milk and plant-based milk. This comparison was made using data on each type of milk from the U.S. A Department of Agriculture (Table 23).

It was found that almond milk can be a good source of calcium if fortified, and a good source of healthy unsaturated fats. However, cow's milk has higher levels of protein, folic acid, vitamin D, carbohydrates, and total saturated fats than almond milk

Table 23: Nutrient comparison among cow’s milk, soymilk, almond milk, and coconut milk

	Cow’s Milk, Whole	Soymilk (unsweetened with added vitamin D and Calcium)	Almond Milk (unsweetene d)	Coconut Milk
Water	88.13 g	93.14 g	96.54 g	94.57 g
Energy	61 kcal	33 kcal	15 kcal	31 kcal
Protein	3.15 g	2.86 g	0.4 g	0.21 g
Total lipid (fat)	3.25 g	1.61 g	0.96 g	2.08 g
Carbohydrate	4.8 g	1.74 g	1.31 g	2.92 g
Calcium	113 mg	124 mg*	184mg*	188 mg*
Vitamin C, total	-	-	-	-
Folate, total	5 µg	-	1 µg	-
Vitamin E, added	-	-	6.33 mg	
Vitamin D (D2 + D3),	51 IU*	49 IU*	41 IU*	1 µg
Total saturated fatty acids	1.865 g	0.206 g	0.08 g	2.083 g
Monounsaturated Fatty acids	0.812 g	0.392 g	0.59 g	-
polyunsaturated fatty acid	0.195 g	1.009 g	0.24 g	-

* Fortified

CHAPTER VI

CONCLUSION

The definition of milk is restricted to dairy products. However, non-dairy beverages, made from plant extracts, do exist as an alternative to animal milk. These beverages are not called “milk” in many countries, including European countries. There are a number of different types of milk and milk alternatives, including soymilk, coconut milk, rice milk, and almond milk, each with its own unique benefits. Among those kinds of milk, it is almond milk that enjoys the highest consumer demand.

Almond milk is a good source of calcium and minerals. It has become a popular alternative to dairy milk and is enriched or fortified with Vitamin D and calcium. Its major benefit is that it is suitable for those who are intolerant of lactose or have an allergy to milk. Also, almond milk, as with cow’s milk, is gluten-free so that it can be safely consumed by individuals with celiac disease. Further, it is unsweetened, and therefore does not raise blood sugar levels.

Almond milk products that are commercially-available at present contain added chemicals, which may be of some concern. The almond milk developed in this study is a vegan, gluten-free, dairy-free milk. It contains only four ingredients, all nutritious and healthy: 25% almonds, 5% figs, 5% dates, and sea salt (in addition to water).

The developed almond milk product was compared with a popular commercial almond milk product: “Silk Almond Unsweetened Milk.” In most of the analyses, the developed almond milk product was found to be better than the commercial milk product, based on the significant difference in mean values. The flavor of the commercial milk product is unsweetened, but the developed almond milk product tasted very sweet and was therefore preferred by most of the women who participated in the study. The milk contains natural sugars from figs and dates. However, precautions should be taken with diabetic patients who are usually restricted from consuming sugar.

To increase its nutritional value and flavor, the commercial product has added chemicals, some of which can be harmful to people with certain conditions. In the developed almond milk product, only plant-based natural fruits were used. In order to reduce bacterial growth, increase shelf life, and protect freshness, ascorbic acid is used in the commercially-available product. Ascorbic acid is primarily Vitamin C, which may prevent the healthy unsaturated fats present in almonds from being oxidized. These unsaturated fats are not harmful to humans, but rather they help to reduce high blood cholesterol levels. In addition, the pH level of the developed almond milk was low, which helps to reduce bacterial growth and increase shelf life without additives. More shelf-life

testing, such as microbial testing, sensory evaluation, chemical testing, and storage conditions, is required to determine product quality on the shelf.

In commercially-available milk products, calcium carbonate and vitamin D2 often are added to increase vitamin D and calcium levels. Though calcium carbonate is a source of calcium, it might not be tolerated by specific patient groups, including those prone to kidney stones. Moreover, calcium carbonate is not absorbed well by the human body compared to the forms of calcium more commonly found in foods. On the other hand, no chemicals were added to the developed almond milk product to increase its nutritional value. For this reason, the developed almond milk product contains only a small amount of calcium. Since drinking enough of the developed almond milk product to obtain the recommended daily amount of calcium (approximately 1000 mg calcium) would lead to a high-calorie intake, calcium fortification is highly recommended.

This study sought to compare the developed almond milk product and the selected commercially-available product through a sensory panel evaluation. While the laboratory analysis evaluates the compositional properties of these two products, the sensory panel study helps examine consumers' perceptions of differences between products. In conclusion, although there were no taste tests to compare the developed almond milk product and cow's milk, customers with lactose intolerance or an allergy to milk will definitely accept the developed almond milk product.

6.1 Study Recommendations

- Since calcium content in the developed almond milk product is lower than in the commercial product, adding some form of calcium carbonate would boost calcium content.
- Studies of titratable acidity, total solids, and antioxidant activity of the developed almond milk are encouraged.
- To minimize product spoilage (to protect almond milk's freshness and flavor), it is suggested that an antioxidant is added.
- In order to determine product demand, and preferred packaging, additional sensory and microbiological testing, plus market research are suggested.
- In order to determine shelf-life stability, accelerated shelf-life testing (ASLT) is recommended

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APPENDICES

Appendix 1: IRB Approval Letter– Osteoporosis Study



Oklahoma State University Institutional Review Board

Date: 04/11/2019
Application Number: AG-19-8
Proposal Title: A Cross-Sectional Study on the Association of Calcium, Vitamin D, Physical Activity, and Bone Mineral Density in Post-Menopausal Saudi Women Based on DXA Measurements

Principal Investigator: Alaa Alharbi
Co-Investigator(s):
Faculty Adviser: Barbara Stoecker
Project Coordinator:
Research Assistant(s):

Processed as: Full Board

Status Recommended by Reviewer(s): Approved
Approval Date: 04/11/2019
Expiration Date: 04/10/2020

The IRB application referenced above has been approved. It is the judgment of the reviewers that the rights and welfare of individuals who may be asked to participate in this study will be respected, and that the research will be conducted in a manner consistent with the IRB requirements as outlined in section 45 CFR 46.

The final versions of any recruitment, consent and assent documents bearing the IRB approval stamp are available for download from IRBManager. These are the versions that must be used during the study.

As Principal Investigator, it is your responsibility to do the following:

1. Conduct this study exactly as it has been approved. Any modifications to the research protocol must be approved by the IRB. Protocol modifications requiring approval may include changes to the title, PI, adviser, other research personnel, funding status or sponsor, subject population composition or size, recruitment, inclusion/exclusion criteria, research site, research procedures and consent/assent process or forms.
2. Submit a request for continuation if the study extends beyond the approval period. This continuation must receive IRB review and approval before the research can continue.
3. Report any unanticipated and/or adverse events to the IRB Office promptly.
4. Notify the IRB office when your research project is complete or when you are no longer affiliated with Oklahoma State University.

Please note that approved protocols are subject to monitoring by the IRB and that the IRB office has the authority to inspect research records associated with this protocol at any time. If you have questions about the IRB procedures or need any assistance from the Board, please contact the IRB Office at 223 Scott Hall (phone: 405-744-3377, irb@okstate.edu).

Sincerely,
Oklahoma State University IRB

Appendix 2: Consent Form – Osteoporosis Study



Animal and Food Science

Biomedical Adult Consent Form

A Cross-Sectional Study on the Association of Calcium, Vitamin D, Physical Activity, and Bone Mineral Density in Post-Menopausal Saudi Women Based on DXA Measurements

Background Information

You are invited to be in a research study about the association of calcium and vitamin D consumption, sunlight exposure and physical activity on Bone Mineral Density. You were selected as a possible participant because one of your friends (the community leader) thought you would be interested. We ask that you read this form and ask any questions you may have before agreeing to be in the study.

Your participation is entirely voluntary.

This study is being conducted by: Alaa Salem Alharbi, Food & Agricultural Products Center, Oklahoma State University, under the supervision of Dr. Barbara Stoecker, Nutritional Science, Oklahoma State University.

Procedures

If you agree to be in this study, we would ask you to do the following things: Complete socio-demographic, health, dietary intake and physical activity questionnaires, and a 3-day Food Intake record, complete DEXA scan, wear a Letsfit watch for 3 days and provide a small blood sample.

Participation in the study involves the following time commitment: No more than 5 days.

- Day 1 will be for filling out the socio-demographic, health questionnaire, signing the consent, and distribution of the Letsfit watches that help to collect information about your activity levels. This procedure will take place in the hospital and the total duration of participant involvement in this procedure will be up to 3 hours.
- Days 2, 3 and 4 will include filling out the physical activity questionnaire and a 3-day Food Intake Record at home.
- Day 5 will be in the hospital and includes
 1. DXA scanning, this test will last take 30 minutes.
 2. Blood lab work which will last take 20 minutes.
 3. Returning the questionnaire forms and Letsfit watches and clarifying any questions on the forms.

DEXA Scan:

A DEXA is a type of x-ray used to measure bone strength. During this test, X-ray pictures of your body will measure how much fat and muscle are present. You will lie flat on a table and a machine will scan your body to estimate the bone density.



Approved:
Expires: 04/10/2020
Protocol #: AG-19-8

Biospecimen Sampling for Research:

- The blood sample will be assigned a code that does not contain your name, national ID number, date of birth or any other unique identifier. The sample will be linked to your survey responses via a random code.
- Your blood will be stored in the chemistry lab at King Abdulaziz University hospital.
- Your samples will be stored until the analyses are completed; this is expected to be done before December 2019. At that time, they will be destroyed.
- Because your samples will not be linked to your name after they are stored, you cannot withdraw your consent to the use of the samples after they are taken.
- Information or specimens collected from you will not be used for future research studies or shared with other researchers for future research. All extra biospecimens not needed for this study will be destroyed after analysis is complete.

Risks and Benefits of being in the Study**The study involves the following foreseeable risks:**

Use of private records: (such as educational or medical records) In order to assist with the offset of this risk, all data and biospecimens will be coded immediately, and will not keep your name attached.

Psychological Risks:

You may not feel comfortable answering some of the questions. If you do not wish to answer a question, you may skip it and go to the next question.

Blood Draw:

- A blood draw may lead to lightheadedness or fainting. It may also cause bruising, prolonged bleeding, and infection at the site where the blood was drawn. In order to minimize these risks, we will swab the site of the blood draw with alcohol to disinfect the area, use disposable sterile needles and tubes to collect blood, and apply pressure to the site following the blood draw to minimize bruising.
- To protect against infection, we will also provide instructions on how to care for the site and watch it for signs of infection. It is important to know that these blood tests performed in the study are strictly for research purposes. The researcher using your blood sample is not a physician and therefore not qualified to make clinical recommendations. Furthermore, no physicians will review the results of these blood tests, as the researchers are not using this test to make a diagnosis.

Use of Radiation:

- In this study, you will be exposed to a small amount of radiation called "ionizing radiation," which is like x-rays. The amount of radiation you will get in the study is (0.5 mrem) (a "mrem" is how we measure radiation dose). In comparison, one regular chest x-ray would give you 10 mrem. The natural radiation we are exposed to all the time – like from the sun – gives you about 300 mrem each year. Neither chest x-rays nor background radiation have been found to harm most healthy adults. At doses much higher



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than you will receive, radiation is known to increase the risk of developing cancer after many years. At the doses you will receive, it is unlikely that you will see any effects at all.

- Tell us now if you have been in other research studies where you had ionizing radiation. Also tell us if you have been exposed to radiation in other ways, like on your job or in radiation therapy. If you are pregnant or nursing, you cannot be in this research study because the radiation may harm your baby. If you are able to have a baby and are not pregnant now, and you want to be in this study, we will give you a free pregnancy test.

The benefits to participation are:

The benefits which may reasonably be expected to result from this study are

- The opportunity to undergo a DEXA scan.
- Increased knowledge about osteoporosis in general.

Compensation

- You will receive no payment for participating in this study.
- Your de-identified biospecimens will not be used for commercial profit.

Confidentiality

- The information that you give in the study will be handled confidentially. Your information will be assigned a code number. The list connecting your name to this code will be kept in a locked file. When the study is completed, and the data have been matched, this list will be destroyed. Your name will not be used in any report
- We will collect your information through paper survey, 3-day Food Intake record, DEXA scan, Letsfit watch result, and blood collection. The paper data will be stored in a locked filing cabinet in a locked office on King Abdulaziz University hospital. In addition, the electronic data such as blood lab result, DEXA scan result, and Letsfit watches results will be stored in fingerprint computer access, Password protected software in a locked office.
- When the study is completed, and the data have been matched, the code list linking names to study numbers will be destroyed. This is expected to occur before December 2019. This informed consent form will be kept for 3 years, and then it will be destroyed. Your data collected as part of this research project will not be used or distributed for future research studies.
- It is unlikely, but possible, that others responsible for research oversight may require us to share the information you give us from the study to ensure that the research was conducted safely and appropriately. We will only share your information if law or policy requires us to do so.

Voluntary Nature of the Study

Your participation in this research is voluntary. There is no penalty for refusal to participate, and you are free to withdraw your consent for participation in this project at any time. The alternative is to not participate. You can skip any questions that make you uncomfortable and can stop the interview/survey at any time.



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Contacts and Questions

The Institutional Review Board (IRB) for the protection of human research participants at Oklahoma State University has reviewed and approved this study. If you have questions about the research study itself, please contact the Principal Investigator at +966504611570, alaasa@okstate.edu. If you have questions about your rights as a research volunteer or would simply like to speak with someone other than the research team about concerns regarding this study, please contact the IRB at #(405) 744-3377 or irb@okstate.edu. All reports or correspondence will be kept confidential.

- *You will be given a copy of your DEXA scan and blood lab results, but the results of other research tests will not be returned to you.*
- *You will be given a copy of this information to keep for your records.*

Statement of Consent

I have read the above information. I have had the opportunity to ask questions and have my questions answered. I consent to participate in the study.

Signature: _____ Date:

Signature of Investigator: _____ Date:



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Appendix 3: Announcement– Osteoporosis Study

Announcement

Dear ladies,

I would like to announce to you an opportunity to volunteer for a scientific research project entitled "A Cross-Sectional Study on the Association of Calcium, Vitamin D, Physical Activity, and Bone Mineral Density in Post-Menopausal Saudi Women Based on DXA Measurements".

To be qualified for participation in this study, you need to be a post-menopausal woman aged 45-65 years old.

The research will take place at King Abdulaziz University hospital over a five-day period and you will go to the hospital twice.

Women who are interested in volunteering to participate in this study, and meet the criteria stated above, please call or text the Principal Investigator (Alaa Alharbi) Ph.D. student @ 0504611570 to confirm your eligibility and learn more.



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Appendix 4: Invitation Letter– Osteoporosis Study

Invitation Letter

Dear Ms./ Mrs.,

Thank you for your interest in A Cross-Sectional Study on the Association of Calcium, Vitamin D, Physical Activity with Bone Mineral Density in Post-Menopausal Saudi Women Based on DXA Measurements. I am writing to ask whether you would be willing to pass along the enclosed information to friends and/or family members who may also be interested in learning about this research study. You are under no obligation to share this information and whether you share this information will not affect your relationship with the staff at King Abdelaziz University Hospital.

Thank you for your time and consideration.

Sincerely,

Alaa Salem Alharbi



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Expires: 04/10/2020
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Appendix 5: Study Description Flyer– Osteoporosis Study

A Cross-Sectional Study on the Association of Calcium, Vitamin D, Physical Activity, and Bone Mineral Density in Post-Menopausal Saudi Women Based on DXA Measurements

Purpose of research study:

Determine whether a combination of prior exercise, dietary calcium, and vitamin D have affected bone mineral density.

Participant criteria:

- Post-Menopausal women aged 45-65 years old.
- No history of hyperparathyroidism and kidney, skeletal, or any other debilitating diseases such as malignancy, liver pathology, chronic renal failure, or cardiovascular disorders
- Not taking estrogen medicine for osteoporosis.

Study duration time:

Over a five-day period and you will go to the hospital twice.

Study procedure:

- Day 1 will be for filling out the socio-demographic and health questionnaire, signing the consent, and the distribution of the Letsfit watches that help to collect information about your activity levels. This procedure will take place in the hospital and the total duration of participant involvement in this procedure is around 3 hours.
- Day 2, 3 and 4 will be for filling out a physical activity questionnaire and a 3-day Food Intake Record at home.
- Day 5 will be in the hospital and will include:
 1. DXA scanning, this test will take about 30 minutes.
 2. Blood lab work which will take about 20 minutes.
 3. Returning the questionnaire forms and Letsfit watches

Benefits:

- The opportunity to undergo a DEXA scan.
- Increased knowledge about osteoporosis in general.

Study location:

King Abdulaziz University Hospital
Address: Prince Majid Rd, Jeddah 22252, Saudi Arabia



Contact information:

(Alaa Salem Alharbi) Ph.D. student:
0504611570



Approved:
Expires: 04/10/2020
Protocol #: AG-19-8

Appendix 6: Flyer– Osteoporosis Study

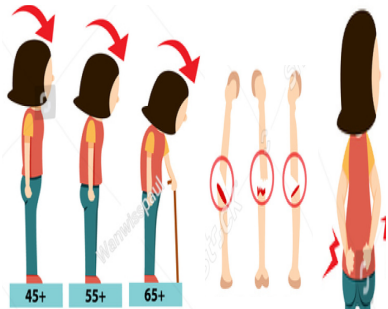


Osteoporosis:

Osteoporosis signs:

Sudden painful, back ache that is worse when standing or walking, Some relief when lying down, Pain & difficulty in twisting or bending, Loss of height, Curving of the spine

Normal hormonal changes associated with post-menopausal aging can result in reduced bone density or osteoporosis. This can lead to fractures and other skeletal changes. Without proper treatment, individuals with Osteoporosis are at risk for more fractured bones (Mayo, 2012).



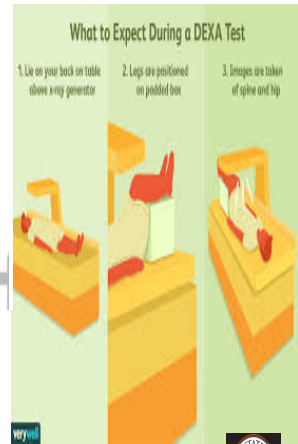
Best option:

DEXA is the favorite option for diagnosing osteoporosis, to assess an individual's risk for developing osteoporotic fractures. DXA is simple, quick and noninvasive.

DEXA or DXA, uses a very small dose of ionizing radiation to produce pictures of the inside of the body (usually the lower (or lumbar) spine and hips) to measure bone loss

How Can I Protect My Bones ?

- Get enough Calcium and Vitamin
- Eat a balanced diet, with fruits & vegetables
- Exercise Regularly
- Avoid Smoking and Alcohol
- Sun exposure
- Taking calcium and vitamin D supplement



Types of exercises to start:

Strength Training such as: free weights or water exercises, Weight-bearing, low-impact activities, Flexibility Stretches or Beginner Yoga, Stability and Balance exercises



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Appendix 7: Socio-Demographic Characteristics Questionnaire– Osteoporosis Study

Name _____

Code Number _____

Date: _____
 Age: _____
 Marital Status: Single: _____ Married: _____ Widowed: _____ Divorced: _____
 Education: No schooling completed _____ Elementary school _____ Middle school _____
 High school _____ Bachelor's degree _____ Master's degree _____ Ph.D. degree _____
 Weight: kgs: _____ or pounds: _____
 Height: cms: _____ or inches: _____

1. How many total pregnancies have you had during your reproductive life? _____

2. Have you ever taken oral contraceptives? Yes No
 If yes, for how long? months/years: _____

3. Before menopause, have you ever missed your period for 6 months or more, besides during pregnancy? Yes No

4. At what age did your menstruation end? _____

5. Have you had a hysterectomy? Yes No
 If YES, at what age? _____

6. Have you had both of your ovaries removed? Yes No
 If YES, at what age? _____

7. Have you ever taken estrogen? Yes No
 If yes, at what age and for how long? _____
 If discontinued, why? _____

8. Do you get outdoor sun exposure? Yes No
 If yes, estimate for how many minutes is your skin exposed to sunlight daily?
 Less than 10 minutes _____ 15 minutes _____ 30-50 minutes _____
 1-2 hours _____ More than 2 hours _____

9. Have you ever smoked? Yes No
 If yes, how many cigarettes per day? _____
 For how many months/years have you smoked, or did you smoke previously? _____
 If you quit, at what age did you quit? _____

10. Do you have a history of any of the following diseases?

Rheumatoid Arthritis	Yes	No
Diabetes Type I or Type II	Yes	No
Osteogenesis Imperfecta	Yes	No
Malnutrition or Eating Disorder	Yes	No
Intestinal Disorder	Yes	No
Liver Disease	Yes	No
Parathyroid Disease	Yes	No
Kidney Disease or Kidney Stones	Yes	No
Chronic renal failure	Yes	No
Cardiovascular disorders	Yes	No
Cancer	Yes	No

11. Have you ever had a bone density test? Yes No
 If YES, when and where? _____

12. Have you ever broken a bone?

Yes No

Which bone?	Simple fall?	If not a simple fall, please describe the circumstances	Age when this happened?

13. Has a parent or sibling had a broken hip from a simple fall or bump?

Yes No

14. Has a parent or sibling had any other type of broken bone from a simple fall or bump?

Yes No

15. How many times have you fallen with bone injury? _____

16. Have you ever had surgery of the spine, hips, legs or arms?

Yes No

17. If YES, describe what type of surgery you had and which side was affected:

18. Do you take any calcium supplements?

Yes No

19. Do you take any vitamin D supplements?

Yes No

If yes, how much and how often for each? _____

20. At what age did you begin calcium supplements? _____

At what age did you begin vitamin D supplements? _____

21. If you consume milk, milk products, or Laban, how much do you consume in an average week? _____

Appendix 8: Level of Physical Activity Questionnaire- Osteoporosis Study

Name _____

Code Number _____

Level of Physical Activity Questionnaire

Dear Volunteer,

Please answer the following questions as accurately as possible by marking the box that corresponds to your answer. This information will be kept confidential and will be retained only under your code number after initial processing. Please take note of your code number for future use.

A. Do you walk per week?

- Yes No
- How many times per week do you exercise by slow or normal speed walking?
 None Once Twice 3 times 4 times
 5 times More than 5 times
- If walking exercise is done regularly, how much time do you spend in each session?
 Less than 20 min 20 min 30 min 45 min
 One hour One hour and a half Two hours or more

B. Do you do any moderate-intensity sports, fitness or recreational (leisure) activities that cause a small increase in breathing or heart rate like

1- **Fast walking**

- Yes No
- How many times per week do you exercise by fast walking?
 None Once Twice 3 times 4 times 5 times
 More than 5 times
- If walking exercise is done regularly, how much time do you spend in each session?
 Less than 20 min 20 min 30 min 45 min
 One hour One hour and a half Two hours or more

2- **Cycling**

- Yes No
- How often do you use a bicycle per week?
 None Once Twice 3 times 4 times
 5 times More than 5 times
- If bicycle exercise is done regularly, how long is each use (on average)?
 Less than 20 min 20 min 30 min 45 min
 One hour One hour and a half Two hours or more

3- **Swimming**

- 4- Yes No
- How often do you swim per week?
 None Once Twice 3 times 4 times
 5 times More than 5 times
- If swimming is done regularly, how long is each session (on average)?
 Less than 20 min 20 min 30 min 45 min
 One hour One hour and a half Two hours or more

Appendix 9: Three Days-Food Intake Record Osteoporosis Study

Name _____

Code Number _____

3-day Food Intake Record

Date Taken: _____

INSTRUCTIONS

- Please write down all foods and beverages consumed for a 3-Days' time period.
- List the approximate time the meal was consumed, place where it was consumed (home, work, name of restaurant, church, etc.), and the type of eating occasion or meal (breakfast, lunch, dinner, snack, or other).
- List each food/beverage item you consumed, including foods eaten between meals, and all drinks, even if it is a non-caloric item like water, coffee, tea, or sugar-free gum.
- Specify details/ingredients/preparation of each food or beverage consumed. See the form for details on what to include.
- Record the amount of each food or beverage consumed. Portion sizes can be recorded in a variety of ways. Please use the method that works best for you. Portion sizes can be recorded using the following typical standard measurements:

Tablespoon = tbsp
Teaspoon = tsp
Ounce = oz

Cup = c
Pound = lb
Slice = sl

Example	Shape	Measurement Needed
meatball	sphere	diameter
meat patty	cylinder or disk	diameter x thickness
lasagna	rectangle or cube (or deck of cards)	length x height x width
pie	wedge	length x height x width of arc

Example

Time	Place	Meal	Food/Beverage Item	Details/Ingredients/Preparation	Amount
8:00am	home	breakfast	brown sugar instant oatmeal	made with water, nothing else added	2 packets
			milk	skim	8 fluid oz
			coffee	brewed, caffeinated	16 fluid oz
			coffee creamer	fat free, liquid hazelnut Coffee Mate	1 tbsp
12:00pm	home	lunch	pizza	frozen, thin crust, supreme pizza (Tony's brand)	2 slices Size D-4 each
			water	tap water, with ice	16 fluid oz

DAY ONE – DATE OF RECORD _____

Time	place	Meal	Food/Beverage Item	Details/Ingredients/Preparation	Amount

Appendix 10: Recruitment Script- Osteoporosis Study

Speech Script

Hello Everyone,

Good morning/afternoon.

My name is Alaa. I am a Ph.D. student in the Department of Food Science at Oklahoma State University. Firstly, I would like to thank you all for taking out time to participate in my Ph.D. dissertation experiment, which is titled “A Cross-Sectional Study on Association of Calcium, Vitamin D, and Physical Activity with Bone Mineral Density in Post-menopausal Saudi Women Based on DXA Measurements.” I hope you have signed in at the entrance of this room with your name and contact information.

The study will be divided into five days. On the first day, you will be given a general questionnaire, a physical activity questionnaire, and a 3-Day Food Intake Record, as well as a Letsfit watch. You will need to complete the general questionnaire here which is based on socio-demographic characteristics like age in the hospital, while the physical activity questionnaire and the 3-Day Food Intake Record are to be completed at home starting from day 2 until day 4, and bring it back along with the Letsfit watches in day 5, the day that is assigned for blood lab work and DXA scans to assess the bone mineral density. There will be an assistant fluent in the Arabic language to explain all the questionnaires.

The Letsfit watch will monitor your physical activity, which will provide me with information such as the number of steps you have walked, the frequency, the distance traveled, sleep duration, and calories burned. Through the physical activity questionnaire, we are interested in finding out about the kinds of physical activities that people do. The questions will ask you about the time you spent being physically active in a week. Please answer each question even if you do not consider yourself to be an active person. Please think about the activities you do at work, as part of your house and yard work, to get from place to place, and in your spare time for recreation, exercise, or sports.

Think about all the vigorous and moderate activities that you did in a week. Vigorous physical activities refer to activities that take hard physical effort and make you breathe much harder than normal. Moderate activities refer to activities that take moderate physical effort and make you breathe somewhat harder than normal. The 3-Day Food Intake Record questionnaire asks you for the different food items you had in 3 days, including the amounts. BMD will be calculated at the femoral neck and lumbar spine, mainly the lumbar vertebrae L1 to L4. You will receive a copy of the DEXA and lab work results as soon as they are available, and you can discuss it with your

We will make every effort to be sure that no personal information will be disclosed. I also assure you that there will be no injury or ill effects to your health as a result of participating in this study.

If you have any questions, I will be happy to answer them now or at any time. Now let's proceed with enrolling you for the study. If you would like to sign the consent forms, or if you would prefer to think it over and sign the consent forms later, we will provide you the contact information for doing that later. Also, if you would like flyers to share with your friends, please let me know.

Thank you again for your participation

Appendix 11: IRB Approval Letter- Sensory Analysis Study



Oklahoma State University Institutional Review Board

Date: 11/12/2019
Application Number: AG-19-52
Proposal Title: Developing a Calcium-Rich Almond Milk

Principal Investigator: Alaa Alharbi
Co-Investigator(s):
Faculty Adviser: Barbara Stoecker
Project Coordinator:
Research Assistant(s):

Processed as: Exempt
Exempt Category:

Status Recommended by Reviewer(s): Approved

The IRB application referenced above has been approved. It is the judgment of the reviewers that the rights and welfare of individuals who may be asked to participate in this study will be respected, and that the research will be conducted in a manner consistent with the IRB requirements as outlined in 45CFR46.

This study meets criteria in the Revised Common Rule, as well as, one or more of the circumstances for which continuing review is not required. As Principal Investigator of this research, you will be required to submit a status report to the IRB triennially.

The final versions of any recruitment, consent and assent documents bearing the IRB approval stamp are available for download from IRBManager. These are the versions that must be used during the study.

As Principal Investigator, it is your responsibility to do the following:

1. Conduct this study exactly as it has been approved. Any modifications to the research protocol must be approved by the IRB. Protocol modifications requiring approval may include changes to the title, PI, adviser, other research personnel, funding status or sponsor, subject population composition or size, recruitment, inclusion/exclusion criteria, research site, research procedures and consent/assent process or forms.
2. Submit a request for continuation if the study extends beyond the approval period. This continuation must receive IRB review and approval before the research can continue.
3. Report any unanticipated and/or adverse events to the IRB Office promptly.
4. Notify the IRB office when your research project is complete or when you are no longer affiliated with Oklahoma State University.

Please note that approved protocols are subject to monitoring by the IRB and that the IRB office has the authority to inspect research records associated with this protocol at any time. If you have questions about the IRB procedures or need any assistance from the Board, please contact the IRB Office at 405-744-3377 or irb@okstate.edu.

Sincerely,
Oklahoma State University IRB

Appendix 12: Consent Form- Sensory Analysis Study



Animal and Food Science

Developing a Calcium-Rich Almond Milk

Background Information

You are invited to be in a research study about developing a calcium-rich almond milk containing additional fruits. This product has been developed especially for Middle Eastern women and you were selected as a possible participant because you are from the Middle East and might like to get the opportunity to explore the product and taste it. We ask that you read the consent form first and ask any questions you may have before agreeing to be in the study and signing the consent form.

Your participation is entirely voluntary.

This study is being conducted by: Alaa Salem Alharbi, Animal and Food Science, Oklahoma State University, under the supervision of Dr. Barbara Stoecker, Nutritional Sciences, Oklahoma State University.

Procedures

If you agree to be in this study, we would ask you to do the following things,

- 1- Reading and signing the consent.
- 2- Getting two samples (1 market almond milk sample and 1 developed almond milk sample), each with a copy of the sensory evaluation questionnaire.
- 3- Tasting one almond milk sample and rate it on the sensory scales.
- 4- Eating unsalted crackers and drink water to separate the taste from the first sample.
- 5- Tasting the other almond milk sample and rate it on the sensory scales.
- 6- The procedure will take place at a meeting hall in the Islamic Society of Greater Oklahoma (Masjid An-Nasr) Oklahoma, USA.

Participation in the study involves the following time commitment: No more than 1 hour

Risks and Benefits of being in the Study

If you are **not** allergic to NUTS, there are no other known risks or embarrassment associated with this project which are greater than those ordinarily encountered in daily life. If you are allergic to nuts, you should not participate in the study.

The benefits to participation are:

1. Learning about the benefits of the developed almond milk and having the opportunity to taste it.
2. Findings can be used to help consumers who do not tolerate cow's milk, soymilk, and other dairy products to be able to drink milk without having any concerns.



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Compensation

You will receive no payment for participating in this study.

Confidentiality

- The records of this study will be kept private. Any written results will not include information that will identify you.
- Research records will be stored in a locked office on a password-protected computer and only the main researcher will access to the records.
- Data will be destroyed after the study has been completed and this is expected to occur by December 2019. This informed consent form will be kept for 3 years, and then it will be destroyed.

Voluntary Nature of the Study

Your participation in this research is voluntary. There is no penalty for refusal to participate, and you are free to withdraw your consent for participation in this project at any time. The alternative is to not participate, and you can stop the tasting at any time.

Contacts and Questions

The Institutional Review Board (IRB) for the Protection of Human Research Participants at Oklahoma State University has reviewed and approved this study. If you have questions about the research study itself, please contact the Principal Investigator at 2063568662, or at alaasa@okstate.edu. If you have questions about your rights as a research volunteer or would simply like to speak with someone other than the research team about concerns regarding this study, please contact the IRB at #(405) 744-3377 or irb@okstate.edu. All reports or correspondence will be kept confidential.

Statement of Consent

I have read the above information. I have had the opportunity to ask questions and have my questions answered. I consent to participate in the study.

Signature: _____ Date: _____

Signature of Investigator: _____ Date: _____



Approved: 11/12/2019
Protocol #: AG-19-52

Appendix 13: Announcement– Sensory Analysis Study

Announcement

Dear ladies,

I would like to announce to you an opportunity to volunteer for a scientific research project entitled " Developing a Calcium-Rich Almond Milk".

To be qualified for participation in this study, you need to be not allergic to nuts and aged 25-65 years old.

The research will take place at a meeting hall in the Islamic Society of Greater Oklahoma (Masjid An-Nasr) Oklahoma, USA.

Women who are interested in volunteering to participate in this study, and meet the criteria stated above, please contact the Principal Investigator

(Alaa Alharbi) Ph.D. student

Phone # 206-356-8662.

Email: alaasa@okstate.edu

Appendix 14: Recruitment Script-- Sensory Analysis Study

Hello Everyone,

Good morning/afternoon.

My name is Alaa. I am a Ph.D. student in the Department of Food Science at Oklahoma State University. Firstly, I would like to thank you all for taking out time to participate in my Ph.D. dissertation experiment, which is titled "Developing a Calcium-Rich Almond Milk."

The study time commitment will be approximately 1 hour, and the study procedure will be as follows:

1. Getting two samples (1 market almond milk sample and 1 developed almond milk sample), each with a copy of the sensory evaluation.
2. Tasting one almond milk sample and rate it on the sensory scales.
3. Eating unsalted crackers and drink water to separate the taste from the first Sample.
4. Tasting the other almond milk sample and rate it on the sensory scales.

We will make every effort to be sure that any written results will not include information that will identify you. I also assure you that there will be no injury or ill effects to your health as a result of participating in this study.

If you have any questions, I will be happy to answer them now or at any time. Now let's proceed with enrolling you for the study. If you would like to sign the consent forms, or if you would prefer to think it over and sign the consent forms later, we will provide you the contact information for doing that later.

Thank you again for your participation

Appendix 15: Study Description Flyer– Sensory Analysis Study



DEVELOPMENT OF A CULTURALLY ACCEPTABLE FOOD PRODUCT

- Purpose: To develop a healthy milk that is plant-based milk and free of dairy and soy ingredients.
- Method: Taste a new and healthy plant-based almond milk that has a mixture of fruits.
- Time required for completion: Approximately 1 hour.
- Benefits: Opportunity to taste a healthy plant-based milk.
- Risks: Minimal (if you are not allergic to nuts, there is no more risk than those ordinarily encountered in daily life).
- Place: a meeting hall in the Islamic Society of Greater Oklahoma (Masjid An-Nasr) Oklahoma, USA.

Contact Information

Alaa Alharbi
+1(206)356-8662
alaasa@okstate.edu

Appendix 16: Sensory Evaluation Questionnaire– Sensory Analysis Study

Sensory Evaluation Questionnaire

Sample 1

Date: _____ Sample Code: _____

Please taste the first sample and rate how much you like the following characteristics of the sample:

	Dislike extremely 1	Dislike very much 2	Dislike moderately 3	Dislike slightly 4	Neither like nor dislike 5	Like slightly 6	Like moderately 7	Like very much 8	Like extremely 9
Color									
Flavor									
Sweetness									
odor									
Texture									
Overall acceptance									

Sample 2

Date: _____ Sample Code: _____

Please eat a cracker. Then, taste the second sample and rate how much you like the following characteristics of the sample:

	Dislike extremely 1	Dislike very much 2	Dislike moderately 3	Dislike slightly 4	Neither like nor dislike 5	Like slightly 6	Like moderately 7	Like very much 8	Like extremely 9
Color									
Flavor									
Sweetness									
odor									
Texture									
Overall acceptance									

What is your age group?

25-35 years old 36-45 years old 46-55 years old 56-65 years old

VITA

ALAA SALEM ALHARBI

Candidate for the Degree of

Doctor of Philosophy

Thesis: DIET, PHYSICAL ACTIVITY AND BONE MINERAL DENSITY OF POST-MENOPAUSAL SAUDI WOMEN AND DEVELOPMENT OF A CULTURALLY ACCEPTABLE FOOD PRODUCT

Major Field: Food Science

Biographical:

Education:

Completed the requirements for the Doctor of Philosophy in Food Science at Oklahoma State University, Stillwater, Oklahoma in 2020.

Completed the requirements for the Master of Science in Nutrition & Food Management at University of Central Oklahoma, Edmond, Oklahoma in 2014.

Completed the requirements for the Bachelor of Science in Nutrition & Food Science at King Abdulaziz University, Jeddah, Saudi Arabia in 2009.

Experience:

Technician, King Abdul Aziz University Hospital, Jeddah, Saudi Arabia, Aug 2009-Sept 2009.

Dietitian, King Abdul Aziz University Hospital, Jeddah, Saudi Arabia, Jun 2009-Jul 2009.

Technician, National Food Industries Company, Ltd., Saudi Arabia, Oct 2008-Jan 2009.

Professional Memberships:

Honor Society membership since 2018.

IFT student membership since 2016.