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The effects of dairy foods intakes on weight change and fracture risk during critical life stages in women

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THE EFFECTS OF DAIRY FOODS INTAKES ON WEIGHT CHANGE AND FRACTURE RISK DURING CRITICAL LIFE STAGES IN WOMEN

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ABSTRACT

Menopause and pregnancy are crucial events in women's lives because women experience a series of physical and psychological changes at these stages. One of the most critical challenges is excessive weight gain during both of these stages, which could contribute to various adverse health events in their later lives. In addition to weight gain, another critical health concern that women face is fragility-related factures. The rate of fragility fractures begins rising in women during their 40s and increases to the end of life. Fractures result in impaired mobility and hospitalization, which can decrease the life quality of women significantly. Identification of modifiable dietary risk factors for excessive weight gain and fracture risk is crucial. The objectives of this dissertation are to estimate the independent effects of total dairy and individual dairy foods (e.g., yogurt, milk, and cheese), alone and in combination with overall diet patterns, physical activity, and other lifestyle factors, on three outcomes among women: weight change during the menopausal transition, weight retention after pregnancy, and risk of fragility-related fractures throughout mid-life and older adult years.

Data from two prospective studies of nurses were used: Nurses' Health Study I (NHS I) and Nurses' Health Study II (NHS II). NHS II was used for both weight change analyses, while NHS was used for the fracture analyses. The first specific aim for the analysis of weight change during the menopausal transition was to investigate the effects of total dairy, yogurt, milk, and cheese intakes on menopausal weight change (N = 35,177) and risk of obesity (N = 38,892) among women in NHS II. Weights were self-reported in biennial questionnaires. Diet was assessed with food frequency questionnaires (FFQ) every 4 years. Generalized estimating equations were used to assess the adjusted mean weight change using repeated measures of weight change. Cox proportional hazards models were used to estimate risk of obesity, controlling for confounding.

The second specific aim relates to the postpartum weight change analyses and were to investigate the effects of total dairy, yogurt, milk, and cheese intakes on postpartum weight retention (N = 18,366) and risk of postpartum obesity (N = 17,126) among women in the NHS II. Generalized linear models were used to assess postpartum weight change as continuous outcomes and multivariable models with a Poisson distribution were used to estimate risk of postpartum obesity.

The third specific aim was for the fragility fracture analyses and included investigating the effects of total dairy, yogurt, milk, and cheese on fragility fractures of the hip, wrist, and vertebrae in women ages 40 years and older in NHS I. In total, there are 99,072 women included. Fractures at the wrist and hip were self-reported. For vertebral fractures, we relied on medical record confirmed cases. Proportional hazards models were used to estimate risk of first fracture (including wrist, hip, or vertebral fractures).

Results associated with the first aims suggested that more than 2 servings per week (s/w) of yogurt led to consistently less weight gain than that observed in women consuming less than 1 serving per month (s/m) throughout the menopausal transition. Further, this same yogurt intake was associated with a 31% reduced obesity risk (95% CI: 0.64 - 0.74) after adjusting for potential confounders and baseline body mass index (BMI). Higher total dairy intake was also associated with less obesity risk, but the effect was somewhat weaker than that for yogurt. There was a U-shaped relation between milk consumption and obesity risk during perimenopause. Moderate (0.5 s/d -< 1 s/d vs. < 0.5 s/d) milk consumption reduced obesity risk by 17% (95% CI: 0.78 - 0.89), while higher milk (≥1 s/d vs. < 0.5 s/d) consumption led to a marginally statistically significant 6% higher obesity risk. Cheese intake was not associated with obesity risk in perimenopausal women.

In the postpartum weight retention analyses, women who consumed moderate amounts of yogurt (1 s/m -< 2 s/w) and higher amounts of yogurt (≥ 2 s/w) had a 0.38 lb and 0.63 lb reduction in postpartum weight retention, respectively, than those who rarely consumed yogurt (< 1 s/m). Moderate and higher cheese intakes were associated with 0.30 lb and 0.64 lb less postpartum weight retention, respectively, than lower cheese intake (< 2 s/w). In the obesity analysis, moderate (1 s/m -< 2 s/w) and higher yogurt (≥ 2 s/w) intakes were associated with 20% (95%: 0.69 - 0.93) and 16% (95%: 0.69 - 1.02) reduced risks of postpartum obesity, but the association was weakened by adjusting for pre-pregnancy BMI. Women with higher levels of activity and higher yogurt intakes had a 39% (95%: 0.50 - 0.74) lower risk of obesity. Higher Alternative Healthy Eating Index 2010 (AHEI) scores alone were associated with a statistically significantly lower obesity risk.

Results from our fracture analyses found that women who consumed more than 2 s/d of total dairy had a 19% (95% CI: 0.67 - 0.98) lower fracture risk than those who consumed less than 1 s/w. In terms of individual dairy products, 2 s/d of milk were associated with a 14% (95% CI: 0.77 - 0.95) reduction in fracture risk compared with lower milk consumption (<1 s/w). Higher cheese (≥ 1 s/d vs. < 1 s/w) intake was associated with a non-statistically significant 9% (95% CI: 0.81 - 1.02) reduction in fracture risk. No association was found between yogurt consumption and fracture. In stratified analysis, the intakes of calcium, vitamin D, and protein from non-dietary sources did not modify the inverse association between total dairy or milk intake and fracture risk.

In summary, the findings of this dissertation suggested that greater yogurt consumption was inversely associated with weight change during menopausal transition and after pregnancy while intakes of total dairy and milk had beneficial effects on the risk of fragility fractures among women ages 40 years and older.

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LIST OF ABBREVIATIONS

AHEI	Alternative Healthy Eating Index 2010
BMI	Body Mass Index
BMD	Bone Mineral Density
CDC	Centers for Disease Control and Prevention
CVD	Cardiovascular Disease
CI	Confidence Interval
DHA	Docosahexaenoic Acid
ENERGY-ADJ	Energy Adjusted
EPA	Eicosapentaenoic Acid
FFQ	Food Frequency Questionnaire
FHS	Framingham Heart Study
GEE	Generalized Estimating Equation
g/d	Gram Per Day
HPFS	Health Professionals Follow-up Study
h/w	Hour Per Week
kg	Kilogram
lb	Pound
lb/yr	Pound Per Year
MetS	Metabolic Syndrome
mg/d	Milligram Per Day
NHS I	Nurses’ Health Study I

NHS II	Nurses' Health Study II
OR	Odds Ratio
RR	Relative Risk
REF	Reference
SFA	Saturated Fat
SD	Standard Deviation
s/d	Serving Per Day
s/m	Serving Per Month
s/w	Serving Per Week
SWAN	Study of Women's Health Across the Nation
SSB	Sugar Sweetened Beverages
WC	Waist Circumference
yr	Year
µg/d	Microgram Per Day

CHAPTER ONE: INTRODUCTION

1.1 Critical Periods of Women's Lives

Menopause and pregnancy are crucial events in women's lives because of the large number of physical and psychological changes that occur during these times. One of the most critical challenges is excessive weight gain during both of these stages, which could contribute to various adverse health events in their later lives. It has been reported that approximately 27% of women are overweight and 13% are obese during the menopausal transition.[1] Research has shown that perimenopausal women continue to gain fat mass and lose lean mass during this transition, which leads in turn to a shift towards higher abdominal fat distribution.[2,3] Abdominal fat is metabolically active and has been identified as an independent risk factor for cardiovascular disease (CVD) and metabolic syndrome (MetS) in perimenopausal women.[4] Excessive weight gain and retention of added weight is also a major concern for women after pregnancy. A recent study revealed that 75% of women weighed more 1 year postpartum than they did pre-pregnancy, with approximately 47.4% of them retaining more than 10 pounds (lb) of added weight, and another 24.2% over 20 lb.[5] Even for women with normal pre-pregnancy BMI, one-third became overweight or obese within 1 year postpartum.[5] Excessive maternal weight gain or retention can promote a variety of health problems including an increased risk of hypertension, diabetes, and even the development of breast cancer.[6,7] Excessive weight during pregnancy is also associated with deleterious impacts on offspring. The offspring of obese mothers suffer a higher risk of obesity, disrupted growth pattern, and congenital anomalies.[8]

In addition to weight gain, another critical health concern that women face with aging is fragility-related factures. Women have less bone and muscle mass than men, and it has been reported that women suffer from a 2 times higher risk of osteoporosis than men.[9] In line with that, women experience 4 times more forearm fractures, 2.3 times more hip fractures, and 1.6 times more vertebral fractures than men.[10] The rate of fragility fractures begins to rise for women in their 40s and continue to increase to the end of life.[11] One study has shown that the age-specific prevalence of osteopenia in women between 40 and 49 years old is as high as the prevalence in elderly women. Fractures can result in impaired mobility and have been associated with increased mortality in women. One previous study found that women with a fracture were estimated to have twice the one-year mortality rate of women of similar ages without a fracture.[12]

Given the serious health burdens caused by weight gain and fractures in women, it is imperative that we identify modifiable interventions to combat these health challenges. Dairy foods are important dietary components in the US population. The *2020-2025 Dietary Guidelines for Americans* recommend 3 servings of dairy per day, affirming the central role of dairy foods in a healthy diet.[13] Studies have shown that dairy foods may have favorable effects on weight management [14,15] and bone health [16–18]. Moreover, different dairy products may play different roles in these health outcomes. This thesis is aimed at exploring whether intake of different dairy foods could help women to tackle excessive weight gain during the menopausal transition, weight retention after pregnancy, and risk of fragility-related fractures throughout mid-life and older adult years.

1.2 Dairy Foods and Weight Gain during the Menopausal Transition

Menopause is defined as a time point 12 months after a woman's last period, and it signifies the permanent cessation of ovarian function. The median age at menopause is 51.4 years.[19] The menopausal transition, or perimenopause, marks the time around menopause. As defined by the Stages of Reproductive Aging Workshop, the menopausal transition marks the time between the onset of variations in reproductive hormone levels to the time of the cessation of menses.[20] The length of the menopausal transition varies from individual to individual, but the range is estimated to be 4 to 11 years.[21] Although menopause begins in the mid-40s in most women,[22] some may start as early as their mid-30s, and others may not experience it until their 50s.[21]

During the menopausal transition, many women experience weight gain, particularly around the abdomen. According to the data from the Centers for Disease Control and Prevention (CDC), the prevalence of obesity is 43.3% among US women aged 40–59.[23] Data from the Healthy Women Study, a longitudinal observational study, revealed that women gained 2.25 kg on average during the 3 years of the menopausal transition; 20% gained more than 4.5 kg.[24] The Study of Women's Health Across the Nation (SWAN) showed a 10% fat mass increase among women transitioning to menopause over 6 years.[2] In addition to weight gain, studies indicated that the menopausal transition contributes to the shift of body fat distribution, leading to more central fat accumulation. The women from SWAN study experienced a 6% increase in waist circumference (WC) over the menopausal transition.[2] Meanwhile, lean mass is reported to decline starting with the onset of the menopausal transition.[25]

Excessive weight gain, particularly the accumulation of visceral fat, is associated with deleterious effects including hypertension, diabetes, and CVD.[26–28] Study found that the visceral fat accumulated during menopause is an independent risk factor for diabetes, CVD, and MetS.[29] A 9-year prospective study also confirmed that the prevalence of MetS rose steadily throughout the progression of menopause.[30] Additionally, excessive weight gain is linked to other health-related outcomes such as mental health and sexual function in women around menopause.[31] Consequently, perimenopausal women tend to suffer from an increased risk of obesity-related complications, which reduces their quality of life both physically and psychosocially.

It remains unclear whether perimenopausal obesity is due to advancing aging or the changes of hormones caused by menopause. However, recent studies tended to believe that the weight gain in middle-aged women was due to aging rather than menopause status, but menopause did contribute significantly to the change of body composition.[25,32] Several human studies suggested an association between increased central body fatness and menopause, independent of age.[29,33] Other factors, including reduced physical activity, westernized diet, or psychosocial distress, may also contribute to perimenopausal weight gain.[4]

Lifestyle modification such as a healthier diet is the first-line recommendation to treat or prevent excessive weight gain.[4] Dairy foods are a critical component of the typical diet of the US population. Dairy foods are nutrient-dense and contain a variety of fatty acids, proteins, minerals, vitamin D, and other bioactive components. The *2020-2025 Dietary Guidelines for Americans* recommend 3 servings of dairy per day as part of a healthy and balanced diet. [13] However, the impact of dairy foods on weight gain and obesity remains controversial.

A 2016 meta-analysis of 27 randomized clinical trials revealed that adults with higher total dairy intake (2 s/d to 4 s/d more dairy than the lower dairy diet) as part of energy-restricted diet lost 1.16 kg more weight and 1.49 kg more fat mass than those who with a lower dairy diet over a median follow up of 16 weeks.[14] This study also demonstrated that under energy restriction conditions, higher consumption of total dairy reduced the loss of lean mass by approximately 75% compared with the low dairy diet. However, this meta-analysis found no favorable effect of dairy foods on body weight or body fat without energy restriction. On the contrary, a different conclusion was reported in a randomized trial which compared the effects of dairy foods with and without caloric restriction in obese African American adults.[34] The results of this trial suggested that although high dairy intakes only reduced body weight in conjunction with energy-restriction, higher dairy intakes significantly reduced total body fat, trunk fat and WC compared with lower dairy intakes, regardless of energy-restriction condition. Another interesting question about the effects of lifestyle on perimenopausal weight gain relates to the impact of dairy foods in combination of physical activity on obesity. Several randomized clinical trials have investigated the effects of adding dairy intake to a physical activity intervention on body weight and body composition. However, no difference in body weight or fat distribution was found between dairy group and the control group, in the context of energy restricted diets with a resistance training component.[14]

Most randomized clinical trials have only studied a single type of dairy in their intervention, thus limiting their ability to compare different dairy food products. Moreover, randomized clinical trials are often short-term (less than 1 year), and cannot reveal the long-term effects of habitual dairy foods intakes on weight change. In fact, several prospective studies have demonstrated that different dairy products may exert different long-term effects on weight changes. Data from three large cohorts of healthcare professionals including NHS I, NHS II and found that increase of yogurt intake was associated with a significant 0.82 lb less weight gain ($p < 0.001$) over 4 years, while no such association was found for other dairy products including milk and cheese.[35] In that study, increased intakes of cheese, whole milk, and low-fat or skim milk were associated with 0.02 lb ($p = 0.75$), - 0.06 lb ($p = 0.32$), and 0.06 lb ($p = 0.37$) in weight change over 4 years respectively. Similar results were observed from the Framingham Heart Study (FHS). Subjects in FHS who consumed higher yogurt more than 3 servings per week (s/w) had 50% less weight gain and 20% less increase of WC than those who consumed less yogurt (< 1 s/w) across a 13-years of follow-up.[36] This benefit was also not observed in any other subtypes of dairy foods.

Yogurt is one kind of dairy product obtained by lactic acid fermentation of milk with specific microorganisms. Because of the fermentation process, yogurt has a greater abundance of many nutrients such as protein, minerals, and vitamins, than low-fat milk.[37] Although a cup of yogurt contains a similar amount of calcium as one cup of low-fat milk, yogurt is believed to improve the bioavailability of calcium due to its acidity. Increasing calcium intake has been shown to accelerate weight and fat loss in several studies.[34,38] Thus, the improved calcium availability may account for the specific weight-reducing effects of yogurt. Yogurt also has a rich content of probiotics. Probiotics are live bacteria that boost human health through the digestive system. It has been postulated that probiotics may favorably modulate gut microbiota and prevent against obesity, which could also explain the potential benefits of yogurt on weight management.[39] Moreover, people who consume more yogurt are usually those who have an overall healthier life-style: they tend to exercise more, have a higher social-economic status and maintain a healthier general diet.[40] All these lifestyle correlations could confound the true independent effects of yogurt on weight change. Thus, more studies are warranted to refine the independent effect of yogurt intakes on weight change.

Excessive weight gain during menopausal transition presents a serious health challenge for women. There is an urgent need to identify optimal dietary approaches to manage weight across perimenopause. However, only a few studies have examined the potentially different effects of individual dairy foods on weight change in perimenopausal women. In the first part of this dissertation, we aimed to investigate the independent effects of different types of dairy foods, alone and in combination with overall diet patterns and physical activity, on weight gain and risk of obesity in perimenopausal women.

1.3 Dairy Foods and Postpartum Weight Retention

Pregnancy presents a unique vulnerability for young, adult women for excessive weight gain and weight retention following birth.[41] Research on women from 29 states reported that 68% of women had gestational weight gains outside of the recommendations set by the Institute of Medicine[42] and many of these women failed to lose the excess gestational weight gain after childbirth. A recent study revealed that 75% of women weighed more 1 year postpartum than they did pre-pregnancy, with approximately 50% of them retaining more than 10 lb of added weight.[5] Although the average estimate of postpartum weight retention ranges from 0.5 to 4 kg, some women could experience as much as 18 kg of weight retention after pregnacy.[43,44] By one year following delivery, approximately one third of women who had normal pre-pregnant BMIs became overweight or obese.[5]

Excessive weight retention after childbirth has been linked with many health problems in women, including increased risk of hypertension, diabetes, and even the development of breast cancer.[6,7] It has also been reported that the incidence of cardiometabolic risk factor, such as elevated plasma glucose and an abnormal lipid profile, significantly increased with greater postpartum weight gain.[45] In addition to adding long-term maternal health risks, excess weight gain around pregnancy has profound impacts on the development of offspring as well. The offspring of obese mothers suffer a higher risk of obesity,[8] disrupted growth patterns, and neurodevelopment disorders.[46] Since postpartum weight often becomes the pre-pregnant weight for the subsequent pregnancy, so excessive postpartum weight retention can exert several adverse outcomes for the following birth, including pre-eclampsia, gestational diabetes, and stillbirth, thus creating a vicious cycle.[47]

It is important to find an effective strategy for mitigating excessive weight retention after pregnancy to reduce both the maternal and the offspring health risk. The time around pregnancy presents an excellent opportunity to engage with women to improve their weight control, given the increased exposure to healthcare providers and improved awareness of their own health status.[48] Study suggested that life-style intervention during pregnancy, such as exercise, a healthier diet, or a self-monitoring log of weights, could significantly reduce postpartum weight gain.[49] For example, a clinical study reported that women who received regular dietary and physical activity education during their pregnancy had 2.74 kg less weight retention compared with women who only received routine care at 6 months postpartum.[50] Another clinical trial found that repeated counseling sessions with nutritionists significantly reduced the risk of overweight in women and the incidence of excess central adiposity 4 years after childbirth.[51] Similarly, a meta-analysis revealed an average of 3.25 kg less postpartum weight retention with a diet-related intervention than with usual care.[52]

To satisfy the higher nutrition and energy intake requirement, pregnant and lactation women need to consume sufficient foods with high quality. Dairy foods are rich source of protein, minerals, vitamins, and other bioactive components, and most dietary guidelines recommended the consumption of dairy products as an important part of healthy diet for women during pregnancy and early postnatal life.[13] Due to the high fat content in dairy products, the impact of dairy foods on weight change have been controversial. So far, the evidence from clinical trials and observational research tended to suggest that dairy consumption didn’t increase the body weight in long-term studies, and there was a weight-reducing effect of dairy consumption in short-term research with energy-restriction.[14,53] Different types of dairy products could exert different effects on the weight. Data from three large cohorts of healthcare professionals including indicated that the intake of yogurt was strongly associated with less weight gain, while no benefit was found for other dairy products including milk and cheese.[35]

Study with the FHS participants suggested that yogurt intake, compared with other types of dairy products, was significantly associated with lower annualized weight change and less increment of WC.[36]

However, to our knowledge, there is no study that directly examine the effects of total dairy and individual dairy products on the postpartum weight change in women. In fact, we only found a few research that reported the effects of dairy products on maternal weight change related to pregnancy. A Portuguese study found that an increase of total dairy intake from first to second trimesters was negatively associated with gestation weight gain in women.[54] By contrast, Olafsdottir et al. reported that women with excessive gestation weight gain consumed two times of dairy products than those who with optimal gestational weight gain.[55] In a study with 1,388 women from the Project Viva cohort, the author reported that total dairy consumption during pregnancy was associated with an increased risk of excessive gestational weight gain (OR: 1.08; 95% CI:1.00-1.17, per s/d).[56] It also found that this association did not appear to be driven by whole fat dairy foods only, because both low fat (OR: 1.08; 95% CI:0.98 -1.18, per s/d) and whole fat (OR: 1.06; 95% CI:0.94 -1.20, per s/d) dairy were positively associated with increased risk of excessive weight gain. However, all the associations were only slightly significant. Results from a large prospective cohort study with Chinese women reported that a maternal diet that richer in milk and milk power didn't have any effects on the gestational weight change.[57]

Given that gestational weight gain is a strong predictor of postpartum weight retention,[58] the findings of these research may shed some light on the potential effects of dairy foods intakes on the postpartum weight change. But still these studies did not directly measure the postpartum weight change and the findings of dairy foods on gestational weight gain are inconsistent. Therefore, it is necessary to have a study that directly elucidate the effects of dairy products on the postpartum weight change. In the second part of this dissertation, we aimed to investigate the independent effects of different types of dairy foods, alone and in combination with overall diet patterns and physical activity, on postpartum weight gain and risk of obesity in women.

1.4 Dairy Foods and Fragility-Related Fractures in Women Ages 40 or Over

Fragility fractures (also known as osteoporotic fractures or low-trauma factures) are a group of fractures that are induced by low trauma injuries, such as falling from a standing height without a major traumatic event. Fragility fractures result from osteoporosis, characterized by reduced bone mineral density (BMD) and destruction of bone microarchitecture.[59] The most common fragility fractures are hip fractures, forearm fractures and vertebral fractures.[10] The prevalence of fragility fractures has been estimated to exceed 2 million and the related economic costs were an additional $17 billion in 2005 in the US It is estimated that the annual incidence of fragility fractures will grow by 50% in the US by 2025 due to the aging of the population.[60] It has been reported that women suffer from much higher risks of osteoporosis and subsequent fractures than men. A 2021 meta-analysis indicated that the prevalence of osteoporosis was 23.1% in women worldwide, which is more than twice the prevalence in men (11.7%).[9] In line with that, women experienced 4 times more forearm fractures, 2.3 times more hip fractures, and 1.6 times more vertebral fractures than men.[10]

Although osteoporosis and fragility fractures are usually considered diseases of women who are aged 65 years or older, but recent studies reveal that the prevalence of osteopenia, osteoporosis, and fragility fractures in middle-aged women (40 to 64 years) is under-appreciated.[61] In fact, the study found the age-specific prevalence of osteopenia in women ages 40 to 49 years is as high as that found in women in older age groups. Furthermore, the study also found a considerably high prevalence of fractures in middle-aged women with even normal BMD values, a finding that could be related to a higher prevalence of trauma-related fractures in younger, more active women. Population-based studies show that the risk of fractures in females is low and remains stable from 20 years of age through to the early 40s, but the risk begins rising from approximately 42 years of age and continues to increase to the end of life.[11] These results suggest that fragility fractures should be considered a health challenge for women as early as 40 years of age. Among middle aged women, postmenopausal women are at a disproportionately elevated risk of developing osteoporosis and fragility fractures because of reduced estrogen levels.[62,63] Research has shown a rapid decline of BMD during the menopausal transition, with women losing an average of 1.2% of BMD annually throughout the menopausal transition.[64] As a result, postmenopausal women are found to have twice the number of hip fractures as premenopausal women of the same age.[65] Fractures cause impaired mobility and contribute to increased risks of pneumonia and thrombosis.[66,67] One previous study also revealed that women who had fractures were estimated to have twice the one-year mortality rate of women at similar ages but without fractures.[12]

Calcium and vitamin D are the two most important nutrients in dairy foods known to play a role in bone health. Calcium is an essential component of the bone that provides the skeletal structure and strength. Although calcium supplements and dietary calcium have long been considered to improve bone health and thus prevent fracture, results from longitudinal studies have not supported this claim.[68–70] A 2007 meta-analysis found no association between total calcium intake and hip fracture in women or men based on the pooled-results from 8 prospective studies, it even found an adverse effect of calcium supplementation on hip fracture based on the pooled analysis of randomized clinical trials.[68] A more recent meta-analysis summarized the findings from 44 cohort studies and 26 randomized clinical trials, and concluded that neither dietary calcium intake nor calcium supplements had protective effects against fracture.[69] One possible reason might be impaired absorption of calcium. The absorption of calcium depends on adequate levels of vitamin D. If the vitamin D level is insufficient, higher calcium may not exert its benefits. In fact, a meta-analysis has confirmed a significant lower risk of hip fracture when calcium was combined with vitamin D.[71]

Vitamin D is an important nutrient for bone health and maintenance, as it promotes calcium absorption in the intestine and regulates adequate levels of serum calcium and phosphate to ensure proper bone mineralization.[72] Vitamin D deficiency can lead to impaired calcium absorption, causing osteoporosis and fractures. Unfortunately, it is reported that approximately 42% of the US population is affected by Vitamin D deficiency.[73] Evidence from a meta-analysis with 47 randomized clinical trials supported that vitamin D with a calcium supplement significantly reduced fracture risk by 16%.[74] Previous NHS I research also supported that high calcium alone did not reduce fracture risk, while high vitamin D combined with high calcium was significantly inversely associated with hip fracture risk.[75] Although Vitamin D can be synthesized endogenously by sunlight exposure, our modern lifestyle leads to less time being spent outdoors. Fortified foods are important source of Vitamin D in the US diet.

In the 1930s, the US began to fortify milk with vitamin D; most processed fluid milk in the US is now enriched with vitamin D. Because the high content of calcium, vitamin D and other nutrients including protein, phosphorus, magnesium, dairy foods have long been recommended for improving the bone health and preventing fragility fractures.[76] Although the positive association between dairy consumption and BMD has been well-documented[16–18], there is much debate about the long-term impact of dairy foods on the risk of fractures.

To our knowledge, no clinical trial has been done to examine the effects of dairy food consumption on fracture. Longitudinal observational studies that investigated the effects of total dairy consumption on fracture prevention have shown inconsistent results. Feart et al. reported that older adults (≥ 67 years old) who consumed low total dairy experienced a significantly higher risk of fracture at any site.[77] A 2018 study found one daily serving of total dairy was associated with a statistically significant 6% lower risk of hip fracture among postmenopausal women in NHS I.[78] In contrast, a prospective study of elderly Europeans suggested that consumption of total dairy had no effect on reducing hip fracture risk.[79] The inconsistent results of total dairy intake on fracture risk could be attributable to the potential different effects from individual dairy products. For example, the analysis of the NHS cohort found a protective effect (RR: 0.92; 95% CI:0.86 - 0.98, per s/d) of milk consumption on hip fracture risk but failed to find any significant reduction in hip fracture risk associated with yogurt (RR: 1.00; 95% CI: 0.97 - 1.04, per s/d) or cheese intake (RR: 0.91; 95% CI: 0.81 - 1.02, per s/d).[78] However, in a large Swedish cohort study with 61,433 middle-aged women, the authors found no reduction of fracture risk associated with milk consumption.[80] A glass of milk per day had no effect on total fracture risk (RR: 1.02; 95% CI: 1.00 - 1.04) but was associated with increased risk of hip fracture (RR: 1.09; 95% CI: 1.05 to 1.13). Conversely, women who consumed more than 400 g of yogurt per day had a statistically significant 30% lower risk of hip fracture than those who consumed less than 1 g per day. They also found that higher intake of cheese was associated with a statistically significantly lower hip fracture risk. The inconsistency of results suggested that different types of dairy foods may have different effects on fractures risk due to the differences in nutrient profiles. Although fermented dairy products such as yogurt and cheese are not widely fortified with vitamin D as milk, the presence of probiotics may provide additional benefits to bone. It has been reported that probiotics could promote bone health by improving the intestine barrier function, reducing a variety of pro-inflammatory biomarkers, and modulating gut microbiota.[81,82]

Given the high rates of fracture among women and the rapidly rising fracture rates with age combined with the inconsistent evidence on the effects of different dairy foods on fracture risk, my third specific aim is to examine the effects of total dairy, yogurt, milk, and cheese on fragility-related fractures of the hip, wrist, and vertebrate in women ages 40 years and older in the NHS cohort. Since estrogen levels decline during menopause, which has been shown to increase fracture risk,[63] this analysis will also evaluate the effects of dairy foods on fracture risk according to menopausal status. Furthermore, we also aim to elucidate the independent effects of total dairy and individual foods intakes in combination with calcium and vitamin D from non-dairy sources.

1.5 Objectives of the Dissertation

There are three specific objectives of my dissertation:

The first objective is to investigate the independent effects of total dairy, yogurt, milk, and cheese consumption, alone and in combination with overall diet patterns, physical activity, and other lifestyle factors, on weight gain and risk of obesity in perimenopausal women from NHS II.

The second is to examine the independent effects of total dairy, yogurt, milk, and cheese consumption, alone and in combination with overall diet patterns, physical activity, and other lifestyle factors, on postpartum weight gain and risk of obesity in women from NHS II.

The third objective is to estimate the independent effects of total dairy, yogurt, milk and cheese consumption, alone and in combination with overall diet patterns, physical activity, and other dietary factors (e.g., calcium and vitamin D from non-dairy source) on the risk of fragility fractures in women from NHS I.

NHS II was chosen for the first 2 aims because this cohort is relatively younger (aged 25 to 42 years old at the baseline), and thus provided more observations for both the pregnancy and menopausal transition analyses. NHS I was selected for the third aim, because this older cohort included more fracture cases, and more detailed information about fractures such the circumstance surrounding the fracture and overall medical confirmation.

CHAPTER TWO: EFFECTS OF DIFFERENT DAIRY FOODS ON WEIGHT CHANGE AND RISK OF OBESITY DURING THE MENOPAUSAL TRANSITION

2.1 Abstract

Weight gain during the menopausal transition is common. Dietary factors such as dairy intake may impact weight change during this critical period, and different types of dairy may have different effects. We investigated the associations between total dairy, yogurt, milk, and cheese on menopausal weight change (N = 35,177) and risk of obesity (N = 38,892) among women in the prospective NHS II cohort. Weights were self-reported in biennial questionnaires. Diet was assessed with food frequency questionnaires (FFQ) every 4 years. Generalized estimating equations were used to assess adjusted mean weight change using repeated measures of weight change. Cox proportional hazards models were used to estimate risk of obesity, while controlling for confounding. Women with 2 servings per week (s/w) or more of yogurt consistently had less weight gain than women with the lowest intakes (<1.0 serving per month (s/m)) averaged over 12 years. Higher yogurt intakes reduced obesity risk by 42% (95% CI: 0.54 - 0.62) while those with the highest intakes of total dairy had only a 9% (95% CI: 0.84 – 0.97) reduction in obesity risk. We explored possible joint effects of each dairy type combined with physical activity and AHEI scores. Higher activity levels and AHEI alone were associated with statistically significant reductions in risk of obesity, but higher intakes of yogurt strengthened these

beneficial effects. Moderate cheese (2 s/w -< 5 s/w) consumption was associated with a 10% reduction of obesity risk than lower cheese intake (< 2 s/w), while higher (≥ 5 s/w) cheese intake had no effects. There was a U shape relation between obesity risk and milk intake. Consuming 0.5 to 1 serving of milk per day was linked to a 15% (95% CI: 0.79 - 0.91) lower risk of obesity, but more than 1 serving per day (s/d) led to an elevated risk by 15% (95% CI: 1.08 - 1.22). However, adding baseline BMI attenuated the positive association between higher milk consumption and obesity risk. After adjusting for baseline BMI, higher milk consumption was associated with a marginally significant 6% (95% CI: 1.00 - 1.13) increase in obesity risk. In conclusion, yogurt intake was associated with reduced weight gain and lower obesity risk in women during the menopausal transition. Although higher milk consumption seemed to be associated with an increased obesity risk, this association was only slightly significantly after adjusting for baseline BMI.

2.2 Introduction

The menopausal transition, or perimenopause, marks a critical life stage for women. During perimenopause, the production of reproductive hormones varies greatly, and leads to a variety of physiological changes and metabolic disturbances. The reduction of estrogen has been reported to lead to changes in fat distribution and an increased risk of obesity in perimenopausal women.[83] According to data from the CDC, the prevalence of obesity is 43.3% among US women ages 40–59 years.[23] Results from the Healthy Women Study, a longitudinal observational study, revealed that women gained 2.25 kg on average during the 3 years of the menopausal transition; 20% of them gained more than 4.5 kg.[24] The SWAN study also showed a 10% increase in fat mass among women transitioning to menopause.[2] In addition to weight gain, studies indicated that the menopausal transition contributes to a shift in body fat distribution, leading to more central fat accumulation[2], which could further increase the risk of diabetes, or CVD, and MetS in perimenopausal women.[29]

Dairy foods are a critical part of the typical diet of the US population. The *2020-2025 Dietary Guidelines for Americans* recommend 3 servings of dairy per day, affirming the central role of dairy foods in a healthy diet.[13] Due to their high saturated fat (SFA) content, the effects of dairy foods on weight change remain controversial. However, not all types of SFA have equal effects on health. In fact, dairy fat contains a higher level of short-chain fatty acids, which have been shown to lower risk of obesity.[84] Despite their fat content, dairy foods are nutrient-dense and contribute to the intake of proteins, several minerals, vitamin D, and other bioactive components, which could play beneficial roles in managing weight. However, studies of the impact of dairy foods on weight change have reported inconsistent results. A 2016 meta-analysis of 27 randomized clinical trials revealed that adults with higher total dairy intake (2 - 4 s/d more dairy than the lower dairy diet) as part of energy-restricted diet lost 1.16 kg more weight and 1.49 kg more fat mass than those who with a lower dairy diet over a median follow up of 16 weeks.[14] This study also demonstrated that under energy restriction conditions, higher consumption of total dairy reduced the loss of lean mass by approximately 75% compared with the control diet. In contrast, Yu-Kang Tu found that an increase in total dairy intake was associated with a modest increase in weight gain in a meta-analysis of randomized controlled trials without calorie-restriction.[85] The results from long-term observational studies with free-living adults are also inconsistent. A systematic review of 24 prospective studies concluded that there was no statistically significant relation between total dairy consumption and weight change, although there was a 15% lower risk of abdominal obesity and a 13% lower risk of overweight among subjects with the highest dairy intake compared with subjects in the lowest dairy intake category.[15]

The inconsistency of the association between total dairy and weight in some studies could be attributed to the different effects of individual dairy products. The health effects of fermented dairy products such as cheese and yogurt are of increasing interest. Yogurt is a dairy product that is obtained by lactic acid fermentation of milk with specific microorganisms. Yogurt has a rich content of probiotics, which have been reported to favorably modulate gut microbiota and protect against obesity.[39] The bioactive components such as bioactive peptides in cheese are also known to benefit gut microbiota and greater intake of cheese have been shown to be associated with a lower prevalence of obesity and overweight in a cross-sectional study.[86] In fact, several prospective studies have compared the effects of individual dairy products on weight change. Data from three large cohorts of healthcare professionals including NHS I, NHS II, and The Health Professionals Follow-up Study (HPFS) found that increasing yogurt intake was associated with a statistically significant 0.82 lb lower weight gain ($p < 0.001$) over 4 years, while no such association was found for other dairy products including milk and cheese.[35] A longitudinal study with adults of a mean age of 54.5 years reported that people who consumed more yogurt (≥ 3 s/w) had 50% less weight gain and a 20% lower increase of waist circumference than those who consumed less yogurt (< 1 s/w) across 13 years of follow-up. No effects on weight change were found in milk or cheese.[87] A Swedish study examined the effects of consuming different dairy foods on weight change among women 40 to 55 years of age. These results found different associations of individual dairy foods with body weight.[88] For example, more than 1 s/d of whole or sour milk was associated with 15% lower risk of weight gain than women consumed less than 1 s/d; and cheese intake (≥ 1 s/d vs < 1 s/d) was associated with a 30% lower risk of weight gain.[15] These results altogether suggest that individual dairy foods may play different roles in weight change.

It has been shown that higher consumption of certain dairy foods such as yogurt tend to be associated with an overall healthier lifestyle, including more exercise, less smoking, and a healthier general diet.[40] These lifestyle factors could possibly confound the independent effects of individual dairy foods on weight change. For example, it has been reported that resistance training could preclude the weight-reducing effects of dairy intake under calorie-restriction.[14] Thus, more studies are warranted to refine our understanding of the independent effects of individual dairy food intakes on weight change and risk of obesity.

Due to the adverse effects of the menopausal transition on weight gain, it is important to identify optimal dietary approaches to manage weight across perimenopause. However, only a few studies have examined the potentially different effects of individual dairy foods on weight change in perimenopausal women. In this study, we aimed to investigate the independent effects of different types of dairy foods, alone and in combination with overall diet patterns and physical activity, on weight gain and risk of obesity in perimenopausal women from NHS II.

2.3 Methods

2.3.1 Population Characteristics

The NHS II was launched in June of 1989 with the enrollment of 116,429 women, ages 25-42 years, to examine the health consequences of oral contraceptive use along with other lifestyle factors in a younger cohort than the original NHS cohort.[89] Participants in NHS II came from 14 different states (California, Connecticut, Indiana, Iowa, Kentucky, Massachusetts, Michigan, Missouri, New York, North Carolina, Ohio, Pennsylvania, South Carolina, and Texas), and ~ 97% were Caucasian.

On the first questionnaire, participants were asked to provide information on their medical history, anthropometrics, and lifestyle factors such as physical activity, smoking status, and alcohol use. Every 2 years after 1989, a follow-up questionnaire was sent to participants to update their data and to identify incident diagnoses of disease. The first FFQ was sent in 1991 and subsequent FFQs were sent every 4 years. The response rate to questionnaires was approximately 85% to 90% in NHS II.

In this analysis, we included data on subjects from 1989 to 2015, although the focus of the analysis for each individual was approximately 12 years around menopause, including 6 years before and 6 years after the exam period in which menopause occurred. The exam of menopause (denoted exam x4 in Figure 2.1) was the exam when women first reported that their menstrual periods had ceased permanently (i.e., no menstrual period for at least one year). Exams 1-3 (denoted x1-x3) represent the 3 exams before exam x4. Exams 5-7 (x5-x7) represent the 3 exams after menopause, which gave us six weight change intervals (for approximately 12 years) around menopause. Exam x1 is considered the baseline exam for the current analysis. For example, if the exam of menopause for a subject was 1999, then her exam x1 was the exam at 1993 (~6 years before 1999), and her exam x7 was the exam at 2005 (~6 years after 1999). Thus, she was followed from 1993 through 2005.

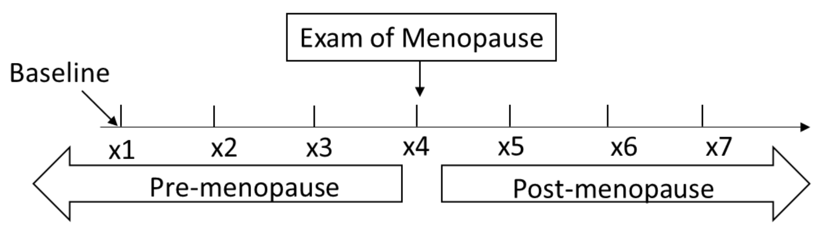

Figure 2.1 Study Timelines for Weight Change Around Menopause.

In Table 2.1, we show the baseline exclusions followed by any exam-specific exclusions. Specifically, 2,768 women were still pre-menopausal at the end of study (i.e., 2015), another 2,980 were missing age at menopause, 1,557 died before reaching menopause, and 9,854 had an unknown menopause status. In addition to these exclusions, 31,942 were excluded who had a non-natural menopause and 3,041 whose age at menopause was less than 45 years. Other baseline exclusions were those missing height (n = 85), those with prevalent cancer, diabetes, or cardiovascular diseases (n = 3,257), and women with missing data on dairy food intakes (n = 15,122). These exclusions left 45,799 possible participants for these analyses.

For each weight change interval, we allowed participants to contribute all available non-missing data. The exam-specific exclusions are also shown the lower half of Table 2.1. In each interval, we excluded participants at that interval and in all future intervals if they were diagnosed with cancer, diabetes, or CVD. We skipped data for participants in any interval in which they were missing weight change, or if weight loss was ≤5 pounds per year (lb/yr) (which may indicate the subject had intentional weight loss), or weight gain was greater than 2.5 times the interquartile range (IQR) based on the distribution of weight changes in that interval (i.e., ~13-14 lb/yr), or if BMI was ≤18 kg/m^2 or ≥40 kg/m^2. Further, we excluded data from exams in which participants were missing information on potential confounders (i.e., physical activity and smoking status). Participants who were skipped were allowed to contribute to later intervals if they met the criteria for inclusion in the later interval. The detailed numbers of exclusion at each interval were shown in Table 2.1. In the final weight change analysis, there were 35,177, 34,339, 34,603, 32,212, 28,775, and 24,506 subjects for each of the six 2-year intervals.

In the analysis of obesity risk, the exclusions are shown in Table 2.2 The first 55,501 exclusions (items 1-9) are identical to those from the weight change analyses. In addition, we excluded those missing baseline BMI (n = 3,522) and those who were obese at baseline (≥ 30 kg/m^2) (n = 11,796), or underweight at baseline (≤ 18 kg/m^2) (n = 403). We also excluded 1,351 who failed to provide any follow-up data after the baseline questionnaire, 4,901 missing data on dairy foods intakes, and 63 missing information on covariates (i.e., physical activity and smoking status) (n = 63). In total, there were 38,892 subjects included in the obesity analysis. The study protocol was approved by the Institutional Review Boards of the Brigham and Women's Hospital and the Harvard T.H. Chan School of Public Health. All analyses were approved by the Institutional Review Board of Boston University School of Medicine.

2.3.2 Dietary Assessment

The first FFQ was sent in 1991 and every 4 years thereafter. The FFQs collected information on how often the participant consumed each food on a list in the past year. Each FFQ had 131 items. The nine categories of consumption frequency included never or less than once per month, 1–3 per month, 1 per week, 2–4 per week, 5–6 per week, 1 per day, 2–3 per day, 4–5 per day, and ≥ 6 times per day. The following foods were included in the total dairy consumption in our analysis: milk (whole milk, 1 or 2 % fat milk, and skim milk), yogurt, and cheese (cottage, ricotta, and other cheese, e.g., American, Cheddar). Butter, cream, and cream cheese were excluded due to low calcium content. A standard serving size was 1 cup for milk and yogurt, 1 slice or 1 ounce serving for other cheese (e.g., American, cheddar), and 0.5 cup for cottage or ricotta cheese.

Nutrient intakes were calculated based on consumption of all the foods reported in the FFQ

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Table 2.1. The Exclusion of Weight Change Analysis.

	Baseline exclusion:		
	Start with 116,429 subjects	***Exclusion***	***Remaining N***
			116,429
1	NHS exclusion of certain subjects as not part of the study	17	116,412
2	Still pre-menopausal at end of follow-up (2015)	2,768	113,644
3	Missing age at menopause	2,980	110,664
4	Died before menopause	1,557	109,107
5	Missing menopause status	9,854	99,253
6	Not natural menopause	31,942	67,311
7	Age of menopause < 45 yr	3,041	64,270
8	Missing height	85	64,185
9	Prevalent cancer, diabetes, or CVD	3,257	60,928
10	Missing dairy (yogurt, milk, or cheese) intake	15,129	45,799
	N after baseline exclusions		***45,799***

	The exclusion below is period-dependent:						
	Interval	***x1-x2***	***x2-x3***	***x3-x4***	***x4-x5***	***x5-x6***	***x6-x7***
12	Incident cancer or diabetes or CVD (exclude once diagnosis)	513	544	939	982	1,002	896
13	Missing weight change (skip)	4,943	5,075	4,869	5,078	8,205	12,302
14	Weight loss > 5 lb/yr (skip)	3,174	3,315	3,045	3,732	3,216	2,795
15	Weight gains more than 2.5 the IQR, i.e., ~13-14 lb/yr (skip)	695	734	639	666	585	484
16	Underweight or morbid obese BMI (> 40 or < 18) (skip)	1,115	1,142	1,108	1,068	986	800
17	Missing activity, smoking (skip)	142	94	40	28	17	10
18	Missing smoking status (skip)	40	43	39	37	35	26
	N for each period	***35,177***	***34,339***	***34,603***	***32,212***	***28,775***	***24,506***

* x1-x2 indicates the yearly weight change from exam-x1 to exam-x2.

Table 2.2. The Exclusion of Obesity Analysis.

	Start with 116,429 subjects	***Exclusion***	***Remaining N***
			116,429
1	NHS exclusion of certain subjects as not part of the study	17	116,412
2	Still pre-menopausal at end of follow-up (2015)	2,768	113,644
3	Missing age at menopause	2,980	110,664
4	Died before menopause	1,557	109,107
5	Missing menopause status	9,854	99,253
6	Not natural menopause	31,942	67,311
7	Age of menopause < 45 yr	3,041	64,270
8	Missing height	85	64,185
9	Prevalent cancer, diabetes, or CVD	3,257	60,928
10	Missing baseline BMI	3,522	57,406
11	Baseline obesity (i.e., BMI ≥ 30 kg/m^2)	11,796	45,610
12	Baseline BMI ≤ 18 kg/m^2	403	45,207
13	No follow up after baseline	1,351	43,856
14	Missing dairy (yogurt, milk, or cheese) intakes	4,901	38,955
15	Missing physical activity	25	38,930
16	Missing smoking status	38	38,892
	N for obesity analysis		***38,892***

Alternative Healthy Eating Index (AHEI) scores were calculated for individual participants based on their food and nutrient intakes from each FFQ. The AHEI components and an overview of the previously-described score calculation can be found in Table 2.3.[90] Dairy products were not a component in the AHEI score.

A previous study has confirmed the reasonable accuracy of estimation of dairy consumption using the FFQ compared with a 7-day dietary record. The correlation coefficients for skim milk, whole milk, yogurt, cottage cheese and hard cheese were 0.81, 0.62, 0.94, 0.80, and 0.57, respectively.[91]

Table 2.3. The Component and Calculation of AHEI Scores.

Component	Minimum score of 0	Maximum score of 10
Vegetables, s/d	0	≥ 5
Fruit, s/d	0	≥ 4
Whole grains, g/d	0	75
SSB and fruit juice, s/d	≥ 1	0
Nuts and legumes, s/d	0	≥ 1
Red meat and processed meat, s/d	≥ 1.5	0
Trans fat, % of energy	≥ 4	≤ 0.5
Long-chain (n-3) fats (EPA + DHA), mg/d	0	250
Poly-unsaturated fatty acids, % of energy	≤ 2	≥ 10
Sodium, mg/d	highest decile	lowest decile
Alcohol, drinks/d	≥ 2.5	0.5 -1.5
TOTAL	**0**	**110**

*Derived from Nurses' Health Study coding documents
Abbreviations: DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; SSB, sugar-sweetened beverages; s/d, serving per day; s/m, serving per month; s/w, serving per week.

2.3.3 Weight Change Assessment

Heights were self-reported at enrollment and weights were self-reported at enrollment and updated in all biennial follow-up questionnaires. Weight change in our analysis was annualized based on the time interval between the adjacent exams. The weight change was calculated by subtracting weight in the current exam from the weight in the following exam, and then dividing by the time interval between the two exams to derive yearly weight change in pounds between exams. The self-reported weights were highly correlated (r =0.96) with technician-measured weights in a previous validation study.[92] BMI was calculated for each questionnaire cycle by using the self-reported weight in kilograms (kg) divided by baseline height in meters (m) squared. Overweight was defined as BMI ≥ 25 kg/m^2, and obesity was defined as BMI ≥ 30 kg/m^2.

2.3.4 Assessment of Potential Confounding

We explored a range of potential covariates including race, age, age at menopause, physical activity, AHEI score, red and processed meat, sugar-sweetened beverages (SSB), energy intake, alcohol, fiber, fruits and vegetables, *trans* fatty acid intakes, post-menopausal hormone use, parity, and smoking status. Race was ascertained in the 1989 questionnaire in 5 categories – White, Black, Amerind, Asian, and Hawaiian. Because most of participants (97%) were White, we combined all the other races and classified race into two categories (white, other races). Age at each questionnaire was calculated by subtracting the date of birth from the date of return of questionnaire. Age at menopause was asked on each biennial questionnaire. Physical activity was assessed on each biennial questionnaire by asking participants about the average time per week spent on specific activities such as jogging, running, biking, tennis, swimming, etc. We summed the hours per week (h/w) for each activity to derive total activity hours per week. Post-menopausal hormone use was determined (as never, current, past user, and unknown) on each biennial questionnaire while parity (i.e., # of pregnancies lasting more than 6 months) was asked in questionnaires between 1989 and 2009. Smoking status (missing, never smoker, past smoker, and current smoker) was asked in each questionnaire along with the number of cigarettes smoked per day among current smokers. Diet was assessed as described above every four years using a 131-item FFQ.

2.3.5 Statistical Analysis

To assess the association between dairy products and weight change over 12 years around menopause, we categorized women into three groups of reported intakes of total dairy, yogurt, milk, and cheese after first examining the trends in weight change in each of the nine categories of intake (never or less than once per month, 1–3 per month, 1 per week, 2–4 per week, 5–6 per week, 1 per day, 2–3 per day, 4–5 per day, or ≥ 6 times per day) for each type of dairy food. Categories showing similar trends in weight change were then collapsed to obtain the final 3 categories of intake for each dairy food, as follows: total dairy (<

1 s/d, 1 s/d -< 2 s/d, and ≥ 2 s/d), yogurt (< 1 s/m, 1 s/m -< 2 s/w, and ≥ 2 s/w), milk (< 0.5 s/d, 0.5 s/d -< 1 s/d, and ≥ 1 s/d), and cheese (< 2 s/w, 2 s/w -< 5 s/w, and ≥ 5 s/w) respectively. In the weight change analysis, the intakes of dairy foods were determined as the average intake over the 12 years surrounding menopause. All participants had at least two FFQs during the 12 years for use in the weight change analyses.

Generalized estimating equations (GEE) were used to assess the adjusted mean weight change using repeated measures of weight change. An exchange correlation matrix was used to account for the correlation of within-subject data (i.e., repeated measures of weights, exposures, and covariates from the same subjects in the follow-up period). A wide range of covariates were explored including race, age, age at menopause, physical activity, AHEI score, red and processed meat consumption, SSB, total energy intake, alcohol consumption, fiber, fruits and vegetables, *trans* fatty acids, post-menopausal hormone use, parity, and smoking status. We retained the covariates that changed the effect estimates by more than 5% and we also adjusted each type of dairy food by other dairy foods (e.g., milk intake model was adjusted for intakes of yogurt and cheese). The Pearson correlation coefficients among individual dairy products were low: 0.072 between yogurt and milk, 0.097 between yogurt and cheese, 0.012 between milk and cheese. The covariates retained in the final model were smoking status (never, current, past, or recent quitters), post-menopausal hormone use (never, current, past user, and unknown), parity (nulliparous, 1 and 2, or ≥ 3 births), alcohol (quintiles), SSB (quintiles), energy intake (quintiles), and other dairy foods. The exclusion criteria and number of subjects at each exam in the weight change analysis can be found in Table 2.1.

In the obesity analysis, we excluded obese (BMI ≥30 kg/m^2) subjects at the exam_x1. Cox proportional hazard models were used to estimate the relative risk (RR) and 95% confidence interval (95% CI) for becoming obese over the 12 years around menopause. All non-obese subjects were followed from the exam_x1 to the first of the following events (censoring events): incident obesity, death, diagnosis of cancer, loss to follow-up, or the end of follow-up (return date for exam x7). Dairy food intakes were calculated as average intakes from exam_x1 to the censoring events in the obesity analysis. However, we also stopped accumulating dairy intake at the exam before the diagnosis of CVD or diabetes. The covariates in the final model were the same as those in the weight change analysis. There were 38,892 subjects in the obesity analysis and the mean of follow-up time was 10.13 years.

We also explored the potential effect modification of dairy foods by physical activity and overall dietary pattern as estimated by the AHEI. We selected these potential effect modifiers because a healthy lifestyle such as healthy eating and being more physically active are important strategies for weight management and yogurt consumption is associated with healthy lifestyle.[40] To test potential effect modification of dairy foods by physical activity and overall dietary pattern (AHEI), we created combined categories of dairy products with physical activity and AHEI. Physical activity was dichotomized as higher (≥ 3 h/w) vs. lower (< 3 h/w) based the distribution of physical activity. AHEI was likewise dichotomized as higher (top 3 quintiles) vs. lower (bottom 2 quintiles) and dairy food intakes were dichotomized by combining the low and moderate groups vs. higher intakes. Subjects were then cross-classified into four groups based on these dichotomized groups. For example, the combined categories of total dairy and physical activity were: (1) lower physical activity (< 3 h/w) + lower total dairy intake (< 2 s/d), (2) lower physical activity (< 3 h/w) + higher total dairy intake (≥ 2 s/d), (3) higher physical activity (≥ 3 h/w) + lower total dairy intake (< 2 s/d), and (4) higher physical activity (≥ 3 h/w) + higher total dairy intake (≥ 2 s/d). Those in the lower dairy and lower activity category served as the referent group

Statistical significance was set at a two-tailed $p < 0.05$. SAS version 9.4 (SAS Institute) was used to analyses the data.

2.4 Results

Table 2.4. Diet and Lifestyle Characteristics of Women at Baseline by Categories of Yogurt Intake[1].

	Yogurt Intake		
	< 1 s/m	**1 s/m -< 2 s/w**	**≥ 2 s/w**
N	5768	15663	13746
		Mean (SD)	
Age (years)	45.4 (3.0)	45.7 (3.0)	46.2 (2.9)
BMI (kg/m^2)	25.1 (4.6)	25.2 (4.5)	24.8 (4.3)
Physical activity (h/w)	1.9 (2.3)	2.2 (2.4)	2.7 (2.5)
AHEI score	54.3 (11.9)	59.0 (11.3)	63.5 (11.0)
Energy intake (kcal/d)	1666 (465)	1764 (468)	1912 (474)
Energy-adj protein (g/d)	78.4 (12.9)	80.1 (11.4)	81.8 (11.0)
Energy-adj carbohydrates (g/d)	218.7 (36.8)	219.1 (31.8)	224.7 (29.6)
Energy-adj total fat (g/d)	67.2 (11.9)	66.0 (10.8)	63.0 (10.4)
Energy-adj trans fats (g/d)	3.1 (1.2)	2.8 (1.0)	2.3 (0.8)
Energy-adj alcohol (g/d)	6.2 (10.6)	6.7 (9.4)	6.6 (8.5)
Total dairy (s/d)	1.2 (0.9)	1.5 (0.9)	2.0 (0.9)
Yogurt (s/w)	0.0 (0.1)	1.0 (0.6)	4.1 (2.1)
Milk (s/d)	0.7 (0.8)	0.8 (0.8)	0.9 (0.8)
Cheese (s/w)	3.3 (2.9)	3.8 (2.9)	4.2 (3.0)
Red and processed meat (s/d)	0.7 (0.5)	0.7 (0.4)	0.6 (0.4)
Fruits and vegetables (s/d)	4.5 (2.7)	5.2 (2.4)	6.2 (2.7)
SSB (bottle/d)	0.8 (0.9)	0.6 (0.7)	0.5 (0.6)
Current smoker (%)	13.0	7.2	5.1
Nulliparous (%)	18.2	17.4	18.1

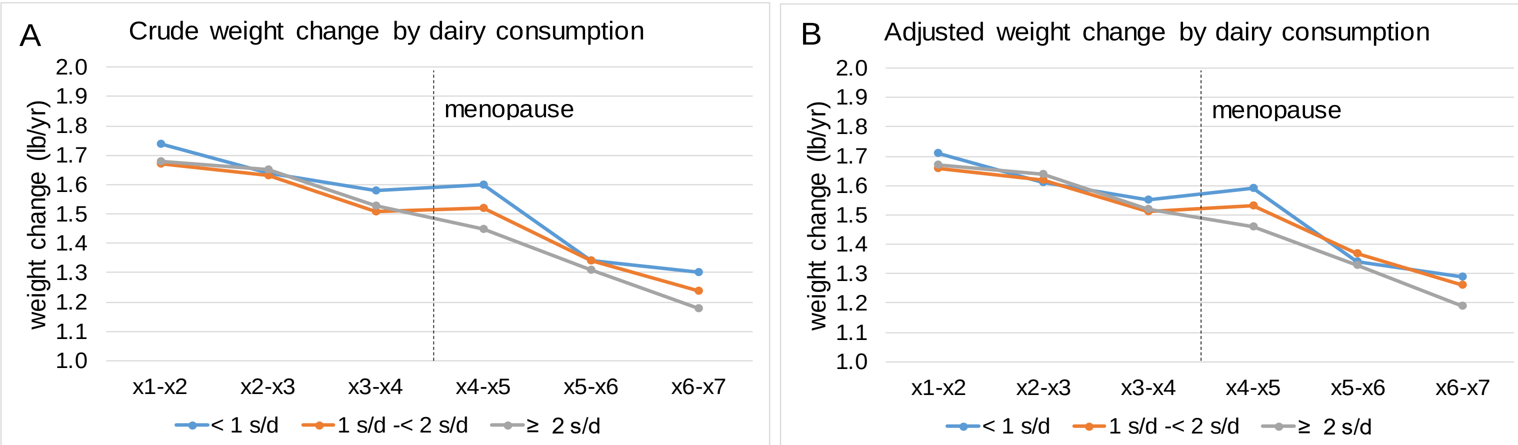
Abbreviations: AHEI, alternative healthy eating index 2010; BMI, body mass index; Energy-adj, energy adjusted; h/w, hours/week; SSB, sugar sweetened beverages; SD, standard deviation; s/d, serving per day; s/m, serving per month; s/w, serving per week.
[1] The table includes subjects at the exam_x1 in the weight change analysis.

The characteristics of 35,177 subjects at baseline are presented in Table 2.4. On average, women were approximately 46 years of age at baseline. Women with more frequent yogurt intake tended to be more active, have a healthier overall dietary pattern and consumed more total energy. In terms of nutrient intakes, women with higher yogurt intakes also consumed more total protein, carbohydrate, and alcohol but less fat. Women who consumed more yogurt were also had higher intakes of total dairy, milk, and cheese. Almost half of total dairy intake was from the consumption of milk. Participants in the lowest yogurt intake group tended to consume fruits and vegetables but more SSB, and they were more likely to be current smokers.

Table 2.5. Number of Participants in Each Interval of Weight Change Analysis.

	Dairy			**Yogurt**		
Intervals[1]	**< 1 s/d**	**1 s/d -< 2 s/d**	**≥ 2 s/d**	**< 1 s/m**	**1 s/m -< 2 s/w**	**≥ 2 s/w**
x1-x2	9,635	15,032	10,510	5,768	15,663	13,746
x2-x3	9,434	14,523	10,382	5,596	15,245	13,498
x3-x4	9,428	14,387	10,248	5,571	15,156	13,336
x4-x5	8,850	13,710	9,652	5,277	14,238	12,697
x5-x6	7,889	12,260	8,626	4,746	12,850	11,179
x6-x7	6,696	10,433	7,377	4,219	11,027	9,260
	Milk			**Cheese**		
Intervals	**< 0.5 s/d**	**0.5 s/d -< 1 s/d**	**≥ 1 s/d**	**< 2 s/w**	**2 s/w -< 5 s/w**	**≥ 5 s/w**
x1-x2	15,605	9,184	10,388	9,861	16,340	8,976
x2-x3	15,296	8,836	10,207	9,629	15,965	8,745
x3-x4	15,230	8,745	10,088	9,538	15,838	8,687
x4-x5	14,430	8,265	9,517	9,043	14,948	8,221
x5-x6	12,709	7,464	8,602	8,133	13,417	7,225
x6-x7	10,529	6,456	7,521	6,973	11,456	6,077

Abbreviations: s/d, serving per day; s/m, serving per month; s/w, serving per week.
[1] x1-x2 indicates the yearly weight change from exam-x1 to exam-x2.



* x1-x2 indicates the yearly weight change from exam-x1 to exam-x2. Panel A shows the results of unadjusted model. Panel B shows the results of multivariable adjusted model. Multivariate model was adjusted for smoking status, post-menopause hormone use, parity, alcohol, sugar sweetened beverages, and total energy intake.

Crude weight change (lb/yr)						
	x1-x2	x2-x3	x3-x4	x4-x5	x5-x6	x6-x7
< 1 s/d	1.74	1.64	1.58	1.60	1.34	1.30
1 s/d -< 2 s/d	1.67	1.63	1.51	1.52	1.34	1.24
≥ 2 s/d	1.68	1.65	1.53	1.45	1.31	1.18

Adjusted[1] weight change (lb/yr)						
	x1-x2	x2-x3	x3-x4	x4-x5	x5-x6	x6-x7
< 1 s/d	1.71	1.61	1.55	1.59	1.34	1.29
1 s/d -< 2 s/d	1.66	1.62	1.51	1.53	1.37	1.26
≥ 2 s/d	1.67	1.64	1.52	1.46	1.33	1.19

[1] Multivariate model adjusted for smoking status, post-menopause hormone use, parity, alcohol, sugar sweetened beverages, and total energy intake.

P-value[1] among groups at each interval						
	x1-x2	x2-x3	x3-x4	x4-x5	x5-x6	x6-x7
Low vs. Moderate	0.0867	0.7460	0.1275	0.1206	0.9836	0.2673
Low vs. High	0.1587	0.8264	0.3411	**0.0052**	0.5323	**0.0263**
Moderate vs. High	0.8451	0.5624	0.6045	0.1324	0.5014	0.1834

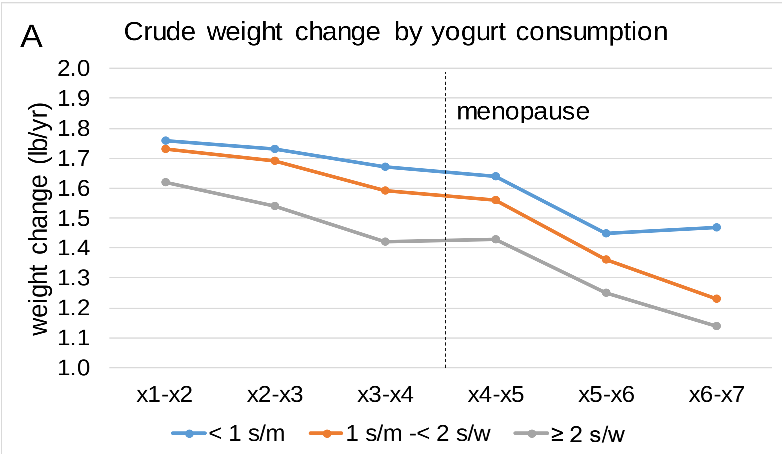
[1] P value < 0.05 is in bold font.

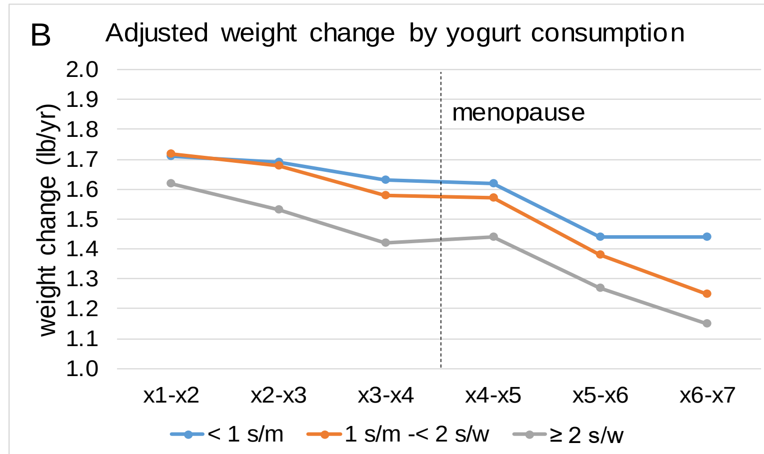
P-value[1] among groups at each interval						
	x1-x2	x2-x3	x3-x4	x4-x5	x5-x6	x6-x7
Low vs. Moderate	0.2719	0.8316	0.3179	0.2702	0.5948	0.5907
Low vs. High	0.3304	0.5734	0.5196	**0.0142**	0.8086	0.0726
Moderate vs. High	0.9729	0.0061	0.7565	0.1041	0.4097	0.1418

[1] P value < 0.05 is in bold font.

Figure 2.2 Crude and Multivariable Adjusted Yearly Weight Change of 6 Intervals During 12 Years Around Menopause by Intakes of Total Dairy.

Figure 2.2 shows the annualized weight change over 12 years (six 2-year weight change intervals) surrounding menopause according to total dairy intakes. Panel A shows the crude weight change and panel B, the multivariable-adjusted weight change. The number of subjects in each dairy food groups can be found in Table 2.5. In general, women continued to gain weight across the 12 years around menopause, but the amount of weight gain tended to decline over time. In the crude weight change graph (Panel A), there was no statistically significant difference in weight change among the three groups of total dairy intake in most time intervals, except for interval x4-x5 and x6-x7 after menopause. Women with highest total dairy intake showed 0.15 lb/yr ($p = 0.0052$) less weight gain at x4-x5, and 0.12 lb/yr ($p = 0.0263$) less weight gain at x6-x7 than women in the lowest total dairy intake category. Adjusting for potential confounding variables in Panel B led to a small amount of attenuation of the differences among total dairy groups. Higher total dairy intake only associated with a statistically significantly lower weight gain (high vs. low total dairy:1.46 lb/yr vs. 1.59 lb/yr, $p = 0.0142$) at exam x4-x5.





* x1-x2 indicates the yearly weight change from exam-x1 to exam-x2. Panel A shows the results of unadjusted model. Panel B shows the results of multivariable adjusted model. Multivariate model was adjusted for smoking status, post-menopause hormone use, parity, alcohol, sugar sweetened beverages,total energy intake, milk and cheese.

Crude weight change (lb/yr)						
	x1-x2	x2-x3	x3-x4	x4-x5	x5-x6	x6-x7
< 1 s/m	1.76	1.73	1.67	1.64	1.45	1.47
1 s/m -< 2 s/w	1.73	1.69	1.59	1.56	1.36	1.23
≥ 2 s/w	1.62	1.54	1.42	1.43	1.25	1.14

Adjusted[1] weight change (lb/yr)						
	x1-x2	x2-x3	x3-x4	x4-x5	x5-x6	x6-x7
< 1 s/m	1.71	1.69	1.63	1.62	1.44	1.44
1 s/m -< 2 s/w	1.72	1.68	1.58	1.57	1.38	1.25
≥ 2 s/w	1.62	1.53	1.42	1.44	1.27	1.15

[1] Multivariate model adjusted for smoking status, post-menopause hormone use, parity, alcohol, sugar sweetened beverages, total energy intake, milk and cheese.

P-value[1] among groups at each interval						
	x1-x2	x2-x3	x3-x4	x4-x5	x5-x6	x6-x7
Low vs. Moderate	0.5887	0.4138	0.1271	0.1585	0.1190	**0.0001**
Low vs. High	**0.0118**	**0.0003**	**<.0001**	**0.0003**	**0.0005**	**<.0001**
Moderate vs. High	**0.0069**	**0.0002**	**<.0001**	**0.0019**	**0.0084**	**0.0295**

[1] P value < 0.05 is in bold font.

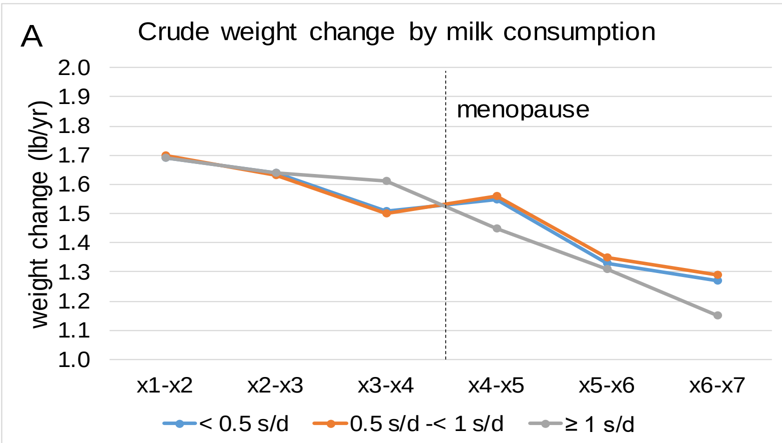
P-value[1] among groups at each interval						
	x1-x2	x2-x3	x3-x4	x4-x5	x5-x6	x6-x7
Low vs. Moderate	0.8344	0.8427	0.3362	0.4080	0.2836	**0.0011**
Low vs. High	0.0808	**0.0042**	**< .0001**	**0.0027**	**0.0044**	**< .0001**
Moderate vs. High	**0.0076**	**0.0138**	**< .0001**	**0.0026**	**0.0149**	**0.0400**

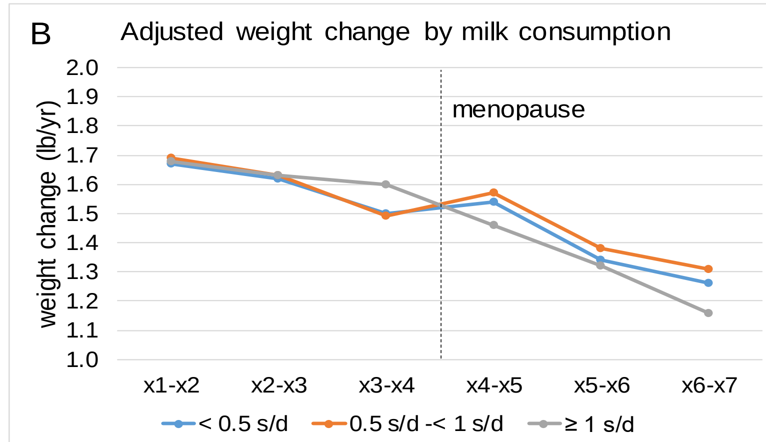
[1] P value < 0.05 is in bold font.

Figure 2.3 Crude and Multivariable Adjusted Yearly Weight Change of 6 Intervals During 12 Years Around Menopause by Intakes of Yogurt.

Figure 2.3 illustrates the trend in weight change according to the intake of yogurt during the 12 years surrounding menopause. As shown in panel A, women with the highest yogurt intake (≥ 2 s/w) had the lowest unadjusted weight gain throughout all the six intervals during menopausal transition, while women in the lowest yogurt group (< 1 s/m) consistently had the highest weight gain over 12 years. The largest difference of weight was seen during the last 2 years of follow up, where higher intake of yogurt was associated with 0.33 lb/yr ($p < 0.0001$) less weight gain compared with those who had lower yogurt intakes. On the other hand, the moderate yogurt intake (1 s/m -< 2 s/w) was not associated with a statistically significant reduction in unadjusted weight gain compared with lower yogurt intake (except in interval x6-x7). The inverse association between yogurt and weight change was slightly attenuated after adjustment for confounding factors. However, the inverse association between yogurt consumption and weight gain remained (Figure 2.3. panel B). The difference in the adjusted weight change between high vs. low yogurt intake was -0.29 lb/yr ($p < 0.0001$) in the last 2 years of follow up.

The crude and multivariable-adjusted associations of milk and cheese with weight change over 12 years of follow-up surrounding menopause are show in Figures 2.4 and 2.5 below.





* x1-x2 indicates the yearly weight change from exam-x1 to exam-x2. Panel A shows the results of unadjusted model. Panel B shows the results of multivariable adjusted model. Multivariate model was adjusted for smoking status, post-menopause hormone use, parity, alcohol, sugar sweetened beverages,total energy intake, yogurt and cheese.

Crude weight change (lb/yr)						
	x1-x2	x2-x3	x3-x4	x4-x5	x5-x6	x6-x7
< 0.5 s/d	1.69	1.64	1.51	1.55	1.33	1.27
0.5 s/d -< 1 s/d	1.70	1.63	1.50	1.56	1.35	1.29
≥ 1 s/d	1.69	1.64	1.61	1.45	1.31	1.15

Adjusted[1] weight change (lb/yr)						
	x1-x2	x2-x3	x3-x4	x4-x5	x5-x6	x6-x7
< 0.5 s/d	1.67	1.62	1.5	1.54	1.34	1.26
0.5 s/d -< 1 s/d	1.69	1.63	1.49	1.57	1.38	1.31
≥ 1 s/d	1.68	1.63	1.6	1.46	1.32	1.16

[1] Multivariate model adjusted for smoking status, post-menopause hormone use, parity, alcohol, sugar sweetened beverages, total energy intake, yogurt and cheese.

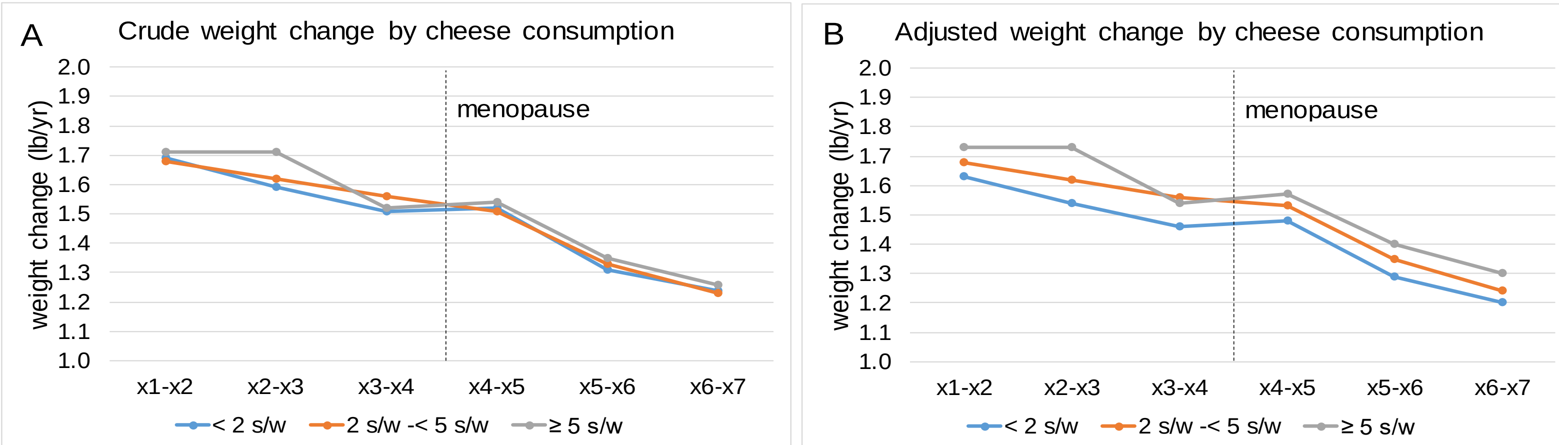
P-value[1] among groups at each interval						
	x1-x2	x2-x3	x3-x4	x4-x5	x5-x6	x6-x7
Low vs. Moderate	0.8209	0.9079	0.7543	0.8347	0.6141	0.6419
Low vs. High	0.8744	0.8536	**0.0209**	**0.0412**	0.6109	**0.0226**
Moderate vs. High	0.9454	0.7879	**0.0200**	**0.0466**	0.3620	**0.0137**

[1] P value < 0.05 is in bold font.

P-value[1] among groups at each interval						
	x1-x2	x2-x3	x3-x4	x4-x5	x5-x6	x6-x7
Low vs. Moderate	0.5957	0.8642	0.9063	0.6442	0.4665	0.4110
Low vs. High	0.7028	0.7118	**0.0154**	0.0685	0.7288	**0.0363**
Moderate vs. High	0.8823	0.8664	**0.0234**	**0.0412**	0.3271	**0.0081**

[1] P value < 0.05 is in bold font.

Figure 2.4 Crude and Multivariable Adjusted Yearly Weight Change of 6 Intervals During 12 Years Around Menopause by Intakes of Milk.



* x1-x2 indicates the yearly weight change from exam-x1 to exam-x2. Panel A shows the results of unadjusted model. Panel B shows the results of multivariable adjusted model. Multivariate model was adjusted for smoking status, post-menopause hormone use, parity, alcohol, sugar sweetened beverages,total energy intake, yogurt and milk.

Crude weight change (lb/yr)						
	x1-x2	x2-x3	x3-x4	x4-x5	x5-x6	x6-x7
< 2 s/w	1.69	1.59	1.51	1.52	1.31	1.24
2 s/w -< 5 s/w	1.68	1.62	1.56	1.51	1.33	1.23
≥ 5 s/w	1.71	1.71	1.52	1.54	1.35	1.26

Adjusted[1] weight change (lb/yr)						
	x1-x2	x2-x3	x3-x4	x4-x5	x5-x6	x6-x7
< 2 s/w	1.63	1.54	1.46	1.48	1.29	1.20
2 s/w -< 5 s/w	1.68	1.62	1.56	1.53	1.35	1.24
≥ 5 s/w	1.73	1.73	1.54	1.57	1.40	1.30

[1] Multivariate model adjusted for smoking status, post-menopause hormone use, parity, alcohol, sugar sweetened beverages, total energy intake, milk and yogurt.

P-value[1] among groups at each interval						
	x1-x2	x2-x3	x3-x4	x4-x5	x5-x6	x6-x7
Low vs. Moderate	0.9508	0.5338	0.2329	0.9673	0.6954	0.8042
Low vs. High	0.6280	**0.0221**	0.8460	0.6371	0.4345	0.7077
Moderate vs. High	0.5520	0.0526	0.3533	0.5750	0.6265	0.5150

[1] P value < 0.05 is in bold font.

P-value[1] among groups at each interval						
	x1-x2	x2-x3	x3-x4	x4-x5	x5-x6	x6-x7
Low vs. Moderate	0.2308	0.0689	**0.0211**	0.2808	0.1774	0.3695
Low vs. High	0.0546	**0.0003**	0.1411	0.0776	**0.0391**	0.0767
Moderate vs. High	0.3172	**0.0189**	0.5666	0.3498	0.3141	0.2609

[1] P value < 0.05 is in bold font.

Figure 2.5. Crude and Multivariable Adjusted Yearly Weight Change of 6 Intervals During 12 Years Around Menopause by Intakes of Cheese.

In the weight change graphs by milk categories, there is no clear association between milk intake and weight change in that the trends in weight change in the low and moderate milk consumption groups were almost the same results (Figure 2.4. panel A). However, women with low or moderate milk consumption experienced an increase of weight gain in the 2 years following menopause (i.e., x4-x5), but this was not the case for women with higher milk intakes. In both the univariable and multivariable models, there was no consistent association between higher milk intake and weight change during the menopausal transition. While we included the same covariates as shown in the analyses above, this led to little change in the results.

Figure 2.5. Panel A shows the crude weight change by the three groups of cheese intake. While there was no association between cheese intake and unadjusted weight change, after adjusting for confounding, women with the highest cheese intakes tended to have greater weight gain than women with lower cheese intakes (although not all of these interval-specific differences were statistically significant. However, women who consumed the lowest level of cheese demonstrated consistently lower weight gains in all 12 years.

Table 2.6. Relative Risk (95% CI) for Obesity Risk According to Different Dairy Foods Intakes During 12 Years Around Menopause.

	N	Case	Rate[1]	Multivariable Model[2]	Multivariable Model + Baseline BMI
Total dairy					
< 1 s/d	11,343	1,904	16.75	1.00 (Ref)	1.00 (Ref)
1 s/d -< 2 s/d	16,093	2,431	14.83	0.94 (0.89 - 1.00)	0.91 (0.85 - 0.96)
≥ 2 s/d	11,456	1,763	15.14	0.91 (0.84 - 0.97)	0.88 (0.81 - 0.94)
Yogurt					
<1 s/m	7,916	1,728	22.78	1.00 (Ref)	1.00 (Ref)
1 s/m -< 2 s/w	16,633	2,632	15.46	0.77 (0.73 - 0.82)	0.84 (0.79 - 0.90)
≥ 2 s/w	14,343	1,738	11.74	0.58 (0.54 - 0.62)	0.69 (0.64 - 0.74)
Milk					
<0.5 s/d	18,006	2,824	15.68	1.00 (Ref)	1.00 (Ref)
0.5 s/d -< 1 s/d	8,818	1,130	12.15	0.85 (0.79 - 0.91)	0.83 (0.78 - 0.89)
≥ 1 s/d	12,068	2,144	17.73	1.15 (1.08 - 1.22)	1.06 (1.00 - 1.13)
Cheese					
< 2 s/w	12,211	2,118	17.45	1.00 (Ref)	1.00 (Ref)
2 s/w -< 5 s/w	16,834	2,399	13.79	0.90 (0.85 - 0.96)	0.84 (0.79 - 0.90)
≥ 5 s/w	9,847	1,581	16.02	1.01 (0.94 - 1.09)	0.96 (0.89 - 1.03)

Abbreviations: CI, confidence interval; Ref, reference; RR, relative risk; s/d, serving per day; s/m, serving per month; s/w, serving per week.
[1]Per 1000 person years.
[2]Model adjusted for race, smoking status, post-menopausal hormone use, parity, sugar sweetened beverage, alcohol, calorie, (yogurt, cheese and milk were adjusted for each other).

In addition to the weight change analysis, we also explored the association between dairy food intakes and risk of obesity during the 12 years around menopause. After excluding women with obesity at the baseline, there were 38,892 women for these analyses. Of these women, 6,098 developed incident obesity during the follow up. Higher yogurt intake (≥ 2 s/w) was associated with a statistically significant 42% (95% CI: 0.54 - 0.62) lower obesity risk than lower yogurt intake (<1 s/m), while moderate yogurt intake (1 s/m -< 2 s/w) was associated a 23% (95% CI: 0.73 - 0.82) reduced risk of obesity compared with lower intakes. There was an inverse trend between total dairy consumption and obesity risk, but only higher total dairy consumption was associated with a statistically significant reduction in risk of becoming obese (RR: 0.91; 95% CI: 0.84 - 0.97). There appeared to be a U-shaped relation between milk consumption and obesity risk. Consuming half to 1 serving of milk per day was linked to a 15% (95% CI: 0.79 - 0.91) lower risk of obesity, while consuming more than 1 s/d was associated with a 15% (95% CI: 1.08 - 1.22) increased risk. In terms of cheese consumption, only moderate cheese intake (2 s/w -< 5 s/w) was associated with a reduced (RR: 0.90; 95% CI: 0.85 - 0.96) obesity risk; there was no association (RR: 1.01; 95% CI: 0.94 - 1.09) with higher cheese intake.

Adding baseline BMI to the main model attenuated the inverse association between yogurt intakes with obesity risk, but still, both moderate and higher yogurt consumption led to significant reduction of obesity risk. Interestingly, the positive association between higher milk intakes and obesity was weakened with adjustment of baseline BMI. In the model with BMI, higher milk was only associated with a marginally significant 6% (95% CI: 1.00 - 1.13) increased risk of becoming obese. Still, no association (RR: 0.96; 95% CI: 0.89 - 1.03) was observed between higher cheese intakes and obesity after adjusting for baseline BMI.

Table 2.7. Relative Risk (95% CI) for Obesity Risk According to Combined Categories of Physical Activity (or AHEI) and Dairy Foods.

	Activity < 3 h/w	Activity ≥ 3 h/w	Activity < 3 h/w	Activity ≥ 3 h/w
	N of subjects		RR (95% CI)[1]	
Total dairy				
< 2 s/d	18,769	8,667	1.00 (Ref)	0.75 (0.70 - 0.80)
≥ 2 s/d	7,421	4,035	0.94 (0.88 - 1.01)	0.72 (0.66 - 0.80)
Yogurt				
< 2 s/w	17,478	7,071	1.00 (Ref)	0.81 (0.76 - 0.87)
≥ 2 s/w	8,712	5,631	0.73 (0.68 - 0.78)	0.52 (0.48 - 0.57)
Milk				
< 1 s/d	18,037	8,787	1.00 (Ref)	0.78 (0.72 - 0.83)
≥ 1 s/d	8,153	3,915	1.20 (1.13 - 1.28)	0.97 (0.88 - 1.06)
Cheese				
< 5 s/w	19,864	9,181	1.00 (Ref)	0.81 (0.76 - 0.87)
≥ 5 s/w	6,326	3,521	1.12 (1.04 - 1.20)	0.81 (0.73 - 0.89)
	Lower AHEI [2]	**Higher AHEI**	**Lower AHEI [2]**	**Higher AHEI**
	N of subjects		RR (95% CI)[1]	
Total dairy				
< 2 s/d	10,903	16,533	1.00 (Ref)	0.48 (0.45 - 0.52)
≥ 2 s/d	4,653	6,803	0.91 (0.85 - 0.99)	0.47 (0.43 - 0.51)
Yogurt				
< 2 s/w	11,596	12,953	1.00 (Ref)	0.54 (0.50 - 0.57)
≥ 2 s/w	3,960	10,383	0.81 (0.74 - 0.87)	0.38 (0.35 - 0.41)
Milk				
< 1 s/d	9,988	16,836	1.00 (Ref)	0.52 (0.48 - 0.56)
≥ 1 s/d	5,568	6,500	1.13 (1.05 - 1.21)	0.64 (0.59 - 0.70)
Cheese				
< 5 s/w	11,978	17,067	1.00 (Ref)	0.51 (0.48 - 0.55)
≥ 5 s/w	3,578	6,269	1.01 (0.94 - 1.10)	0.59 (0.54 - 0.64)

Abbreviations: AHEI, alternative healthy eating index 2010; CI, confidence interval; Ref, reference; RR, relative risk; s/d, serving per day; s/m, serving per month; s/w, serving per week.
[1] Model adjusted for race, smoking status, post-menopausal hormone use, parity, sugar sweetened beverage, alcohol, calorie, (yogurt, cheese and milk were adjusted for each other).
[2] Lower vs. Higher AHEI was defined by the bottom 2 quintiles vs. the upper 3 quintiles.

We also explored the joint effects of physical activity with dairy intakes as well as AHEI scores and dairy food intakes on the development of obesity in women across menopausal transition. Among less active women, only higher yogurt intake (but not other forms of dairy) was associated with a statistically significantly lower risk of obesity (HR: 0.73; 95% CI: 0.68 - 0.78). Higher milk intake and higher cheese intakes, respectively, were associated with 20% (95% CI: 1.13 - 1.28), and 12% (95% CI: 1.04 - 1.20) higher risks of developing obesity in less active women. Greater consumption of total dairy combined with higher activity resulted to a 29% lower obesity risk, although most of the effect seems to be driven by higher activity, rather than total dairy. In contrast, while active women who consumed less yogurt only had a 19% (95% CI: 0.76 - 0.87) lower obesity risk, active women who consumed more yogurt had a 48% (95% CI: 0.48 - 0.57) lower obesity risk than the reference group. This suggests that the beneficial association of yogurt consumption with obesity risk is further modified by physical activity levels. and yogurt consumption on weight change. Among active women, neither higher milk intake (RR: 0.97; 95% CI: 0.88 - 1.06) nor higher cheese intake (RR: 0.81; 95% CI: 0.73 - 0.89) had any adverse association with obesity risk.

In the analysis of joint effects of AHEI and dairy foods, higher total dairy and higher yogurt intakes were associated with lower risks of obesity risk (HR: 0.91; 95% CI: 0.85 - 0.99 and HR: 81; 95% CI: 0.74 - 0.87, respectively) among women with less healthy overall diets. A higher AHEI score alone was associated with approximately 50% less obesity risk. Among these women with a healthier overall diet pattern (higher AHEI scores), increased intake of total dairy had no additional beneficial effect on obesity risk, but higher yogurt intake strengthened the benefit of AHEI. Women with higher yogurt intake and higher AHEI showed 62% (95% CI: 0.35 - 0.41) lower obesity risk, while women with higher AHEI alone only had 46% lower obesity risk (95% CI: 0.50 - 0.57). Higher milk consumption was associated with a 13% increased risk of obesity (RR: 1.13; 95% CI: 1.05 - 1.21) among women with lower AHEI scores, while those with higher AHEI scores who consumed more milk had a 36% lower risk (95% CI: 0.59-0.70) of obesity. There was no association between cheese consumption and obesity risk among women with lower AHEI scores. As with milk, women with higher AHEI scores who consumed more cheese had a 41% lower risk of obesity (95% CI: 0.54-0.64).

2.5 Discussion

In this prospective study of perimenopausal women, participants with higher yogurt intake had consistently less weight gain than those with lower yogurt intake across 12 years around menopause. Moreover, higher yogurt intake led to a lower risk of obesity in women during the menopausal transition. Total dairy intake was also associated with less obesity risk, but the effect was not as strong as that of yogurt. Although more than 1 serving of milk per day led to an elevated obesity risk, but the association was only slight significant after adjusting for baseline BMI. Activity alone and higher AHEI scores alone were associated with statistically significant reductions in obesity risk. However, the addition of a diet characterized by higher yogurt intakes led to an even greater reduction in obesity risk than either factor alone.

The average weight gain has been reported to be 1.5 lb/yr around the menopausal transition.[93] In this study, the unadjusted mean weight change over 12 years around menopause was 1.53 lb/yr, which is in line with these previous results. Studies have shown that even modest weight gain (e.g., >2.5 kg) is associated with significantly increased risks of CVD, diabetes, obesity-related cancers, and total mortality in middle-aged adults.[94] Therefore, the consistent weight gain over 12 years in perimenopausal women that observed in this study could lead to serious additional health burden for women in their later lives. We also observed a decline in the rate of the weight gain over time and this trend has been observed in other prospective studies of middled-aged adults.[36,95] This attenuated weight gain could be attributable to the concurrent loss of lean mass during the menopausal transition, which could promote adverse changes in body composition and contribute to the added health burden for women after menopause.[3]

Several short-term interventions have investigated the effects of total dairy on weight change and the results from these clinical trials tended to suggest an inverse association between total dairy intake and weight change under hypocaloric conditions.[14] Data from longitudinal studies examining the effects of habitual dairy intakes on weight change are sparse. Our analyses suggest that there was there is beneficial effect of total dairy consumption on the risk of obesity during the perimenopausal period. This finding was somewhat different from the results in the Women's Health Study, in which Rautiainen et al. found a significant inverse association between the total dairy consumption and the weight change of women aged ≥ 45 years during a mean follow-up of 11 years.[95] They also reported a significant inverse relation between total dairy and the risk of obesity or overweight in age-adjusted model only; this association was eliminated after adjusting for other lifestyle and dietary covariates. There are two potential reasons why Rautiainen et al. observed a stronger effect of total dairy intake on weight change than our analysis. First, only subjects with a normal BMI were included in the weight change analysis in the Women's Health Study, and it was reported in their study that the favorable effect of total dairy was stronger in women with lower BMIs. Secondly, the highest category was ≥ 3 s/d of total dairy in Women's Health Study (while ours was ≥ 2 s/d) and it was the women with ≥ 3 s/d of total dairy that had the lowest weight change in the Women's Health Study. The trend observed in the Women's Health Study analysis might be driven by the women with this particularly high total dairy (≥ 3 s/d) intake. It is worth noting that Rautiainen et al. failed to find a benefit of yogurt consumption in their study, and even reported an increased risk of overweight or obesity with higher yogurt intake. In their analysis, women who consumed more than 1 s/d of yogurt had a

16% (RR: 1.16, 95% CI: 1.02 -1.31) higher risk of becoming overweight or obese than non-yogurt consumers. There were, however, few subjects (605 of 7,442) in the category with ≥ 1 s/d of yogurt (605 in yogurt ≥ 1 s/d vs. 7442 in the referent category) which could lead to uncontrolled confounding in that category. Moreover, they used only the baseline intake of yogurt as the exposure, which could lead to misclassification if subjects change their yogurt consumption in the follow-up.

There are a few other longitudinal studies that investigated the role of yogurt intake on weight change, and several are in agreement with our current NHS II analyses. In a previous study with participants from NHS I, NHS II and HPFS, yogurt was the only individual dairy product that was associated with a statistically significant reduction in weight gain. It was reported that 1 serving of yogurt per day was associated with 0.82 lb less weight gain (95% CI: -0.99, -0.67) over 4 years after adjusting for other lifestyle and dietary variables.[35] Results from the FHS suggested that both yogurt and total dairy intake were significantly associated with lower annualized weight change, while only yogurt intake was associated with a statistically significant 20% lower increase in waist circumference.[36] No association was found for other types of dairy products in the FHS. Given that weight and waist circumference were measured by the trained professionals in the FHS, their results add important evidence of an inverse association between yogurt intake and weight change. In addition, the FHS included both men and women and at baseline, they were approximately 6 years older than participants in our current NHS II analyses, which could explain the lower annualized weight gain (0.60 lb/yr) in the FHS. Data from the Québec Family Study also supported that yogurt consumers not only have lower weight, BMI, and percent body fat, but also a better glycemic profile compared with non-consumers.[96]

The mechanisms by which yogurt improves weight change and risk of obesity are not clear but there are several possibilities. Calcium has been postulated to play a role in the fat metabolism. Increased calcium intake is reported to inhibit lipogenesis, stimulate lipolysis, promote lipid oxidation, and increase the excretion of fecal fat.[97,98] Increasing calcium intake has been shown to accelerate weight and fat loss in several studies.[34,38] Although a cup of yogurt has similar amount of calcium as one cup of milk, yogurt is believed to improve the bioavailability of calcium due to its acidity.[99] Thus, the improved bioavailability may account for the specific weight-reducing effects of yogurt. Yogurt is a unique dairy product with a significant abundance of probiotics. Probiotics are live bacteria that boost human health through the digestive system. It has been postulated that probiotics may favorably modulate gut microbiota and prevent against obesity.[39] Moreover, the semi-solid matrix of yogurt provides a more satiating feeling and may thus lower hunger.[100] Furthermore, yogurt intake is an indicator of an overall healthier lifestyle, including more exercise, less smoking, and a healthier general diet,[40] which might promote the beneficial effect of yogurt intake on weight change or obesity. However, our joint effect analysis revealed that the favorable effects of yogurt on preventing obesity were additive to independent effects of physical activity and AHEI scores. This finding indicates that the associated healthy lifestyle does not explain of all the benefits of yogurt on weight management.

In the current analysis, we did not observe any clear association between the weight gain with the consumption of milk. Moreover, we observed a null association between milk intake and weight change and a U- or J-shaped association between milk intake and obesity risk. However, higher intake of milk was only associated with a slightly increased obesity risk (RR: 1.06, 95% CI: 1.00 – 1.13) after adjusting for baseline BMI in the model. In previous studies, the effects of milk on body weight have been inconsistent. In previous analyses of NHS I, NHS II and HPFS data, investigators failed to find any association between whole milk or skim milk intake and weight change, which is consistent with our findings on weight change during perimenopause.[35] In the Women's Health Study, researchers reported that more than 1 serving of skim milk per day increased the risk of obesity and overweight (RR: 1.11; 95% CI: 1.04 - 1.19), but that association was eliminated after adding baseline BMI to the model. The attenuation with adjustment of baseline BMI was also observed in our analyses of milk and obesity risk. There was no association between the consumption of whole milk and risk of obesity and overweight in that study.[95]

Results from a 10-year prospective study of Spanish adults demonstrated that neither whole milk nor skim milk (≥ 200 ml vs. non-consumer) had statistically significant effects on abdominal obesity. However, substitution of 100 kilocalories of soft drinks with 100 kilocalories of whole milk was associated with a significant 1.34 cm (95% CI: 0.31 - 2.36) reduction in WC; substitution with skim or low-fat milk had no effect on WC.[101] Furthermore, in a longitudinal analysis with 19,532 perimenopausal Swedish women, Rosell et al. explored the effects of three different types of milk on the risk of a weight gain of more than 1 kg/yr over 9 years. They found that the intake of whole milk was inversely associated with weight gain (OR:0.85; 95% CI: 0.73 - 0.99), while neither medium-fat (1.5 % fat) milk nor low-fat (0.5 % fat) milk had favorable effects on weight change.[88]

Considering the mixed results from different cohort studies, it is difficult to draw a clear conclusion on the impact of milk consumption on weight change. However, several factors could affect the association between the milk intake and weight change. Given that whole milk (vs. skim milk) had favorable effects on weight change and WC in some studies, it is possible that different sub-types of milk may have different effects.[88,101] In fact, recent studies suggest that certain dairy fatty acids such as conjugated linoleic acid, butyric acid, and palmitoleic acid may have beneficial effects on adiposity. Further, fatty acids from dairy have been shown to be associated with lower obesity risk, perhaps by lowering the chronic inflammation and oxidative stress.[102] Secondly, baseline BMI may play a role as well since the adjustment for baseline BMIs eliminated the elevated obesity risk from skim milk in the Women's Health Study.[95] In addition, the effect of milk intake may also depend on which food or beverage with which it is replaced.[101] In the stratification analysis, we also observed that higher milk intake only had adverse effects on obesity in less active women or women with a less healthy diet in general (lower AHEI score). Finally, it is important to note that total dairy intake had a beneficial effect on obesity risk and since milk is the primary source of dairy in these women, we conclude that more studies are needed to explore the effect of milk intake on obesity risk in perimenopausal women

Several large longitudinal research studies with US cohorts reported no association between cheese intake and weight change or obesity.[35,87,95] Results from a previously-described Swedish study found that regular intake of cheese (≥ 1 s/d vs. < 1s/d) led to a statistically significant 30% (95% CI:0.59 - 0.84) lower risk of weight gain in both normal weight women and overweight women.[88] Cheese intake in this cohort was much higher than in the NHS II cohort in that 74% of these Swedish women consumed more than 1 s/d (with women consuming <1 s/d serving as the referent group).[103] This difference in consumption levels makes it difficult to compare our results directly with the Swedish study results. Thus, we cannot evaluate whether a higher cheese intake among a US population would have similar effects. Among the study with Spanish adult women, cheese consumption was inversely associated with BMI as well as prevalence of obesity and overweight.[86] The current analysis failed to find lower weight gain associated with higher cheese intake. In contrast, women who consumed more than 5 serving of cheese per week had greater weight gain throughout the 12 years around menopause. However, in the obesity analysis, moderate cheese intake (2 s/w -< 5 s/w) was associated with a protective effect against obesity, and higher consumption (≥ 5 s/w) had no effect on obesity risk in women. It may suggest that cheese intake is not detrimental in non-obese perimenopausal women.[35]

The strength of the present study included its particular focus on women during the menopausal transition, which has been shown to be a population that is particularly susceptible to unfavorable weight change and the consequences of excessive weight gain. To our knowledge, this is the first long-term study to investigate the effects of individual dairy foods on weight change in the time surrounding menopause. Although some studies have examined weight change in middle-aged women, these analyses were not specific to the menopausal transition. Another strength if this study is the availability of repeated FFQs and multiple self-reported weights in NHS II. Therefore, we were able to update dairy intakes for each weight change interval by calculating the cumulative average intake starting at baseline. This method could better capture the long-term intakes for each individual and minimized the potential misclassification associated with using just a single FFQ. Moreover, this study provided not only the effects of total dairy intake but also intake of individual dairy foods on weight change, allowing us to examine different effects among these different dairy foods.

There are also some limitations of this study. First, the FFQ is subject to measurement error, although a previous validation study has shown high levels of validity for self-reported dairy food consumption.[91] In this study, weight was self-reported and is therefore subject to both biased reporting and random error. While previous analyses have shown a high correlation ($r = 0.96$) between self-reported weights in NHS and measured weights in a subset of participants, the self-reported weights were found to be lower on average than the measured weights.[92] Another limitation of this study is the generally low level of consumption of yogurt in this cohort. Therefore, further studies are warranted to determine whether a higher level (e.g., ≥ 2 s/d) of yogurt consumption would still exert beneficial effects on weight change. Finally, NHS II participants were largely Caucasian nurses with higher education levels. These women may be more health-consciousness than the general population of perimenopausal women, which could lead to some biased reporting and also limit the generalizability of these results.

In conclusion, this study suggests that higher intake of yogurt is associated with less weight gain and reduced obesity risk among perimenopausal women. Incorporating yogurt intake as a component in a healthy dietary pattern may have beneficial effects on weight during this critical life period.

CHAPTER THREE: EFFECTS OF DIFFERENT DAIRY FOODS INTAKES ON POSTPARTUM WEIGHT RETENTION AND RISK OF OBESITY

3.1 Abstract

Pregnancy is a trigger for obesity for women of reproductive age. Excessive postpartum weight retention puts women at higher risks for long-term obesity and subsequent health problems, including diabetes and CVD. Dairy intake may have beneficial effects on weight, and different types of dairy products may have different roles on weight maintenance and change. We investigated effects of total dairy, yogurt, milk, and cheese on postpartum weight retention (N = 18,366) and risk of postpartum obesity (N = 17,126) among women in the prospective NHS II cohort. Weights were self-reported in biennial questionnaires. Diet was assessed with a FFQ every 4 years. Generalized linear models were used to assess postpartum weight change and multivariable models with a Poisson distribution were used to estimate risk of postpartum obesity. After controlling for relevant covariates, women who consumed moderate yogurt (1 s/m -< 2 s/w) and higher yogurt (≥ 2 s/w) had 0.38 lb and 0.63 lb reductions in postpartum weight retention respectively than those who rarely consumed yogurt (< 1 s/m). Moderate (2 s/w -< 5 s/w) and higher (≥ 5 s/w) cheese intakes were also associated with 0.30 lb and 0.64 lb less postpartum weight retention than lower cheese intake (< 2 s/w). There was a non-statistically significant inverse association between total dairy consumption and postpartum weight retention. No clear trend was observed between milk intake and postpartum weight

retention. Women with higher activity and higher yogurt intake had 0.92 lb less weight retention postpartum than women with lower activity and lower yogurt intake. Similar trends were observed for cheese. Higher cheese and higher activity together led to 0.95 lb less postpartum weight retention than found in sedentary women with lower cheese intake. In the obesity analysis, moderate and higher yogurt intakes were associated with 20% (95%: 0.69 - 0.93) and 16% (95%: 0.69 - 1.02) reduced risks of postpartum obesity, respectively, but the association was weakened by adjusting for pre-pregnancy BMI. The combination of higher activity and higher yogurt consumption was associated with a 39% (95%: 0.50 - 0.74) lower risk of postpartum obesity. A higher AHEI score alone was associated with a statistically significantly lower obesity risk. No additional benefits were observed with higher yogurt intake in women with higher AHEI.

In conclusion, higher yogurt and cheese intakes were associated with significantly reduced postpartum weight retention. Moderate and higher yogurt intakes led to a reduction in postpartum obesity, but the association became non-statistically significant after adjusting for pre-pregnant BMI.

3.2 Introduction

Pregnancy presents a unique vulnerability for young, adult women for excessive weight retention. Thus, this period may promote long-term health problems for women.[41] In the US, the obesity prevalence for women between 20 and 39 years of age was 39.7% in 2017 – 2018.[23] Research on women from 29 states reported that 68% of women had gestational weight gains outside of the recommendations set by the Institute of Medicine[42] and many of these women fail to lose the excess gestational weight gain after childbirth. A recent study revealed that 75% of women weighed more one year postpartum than they did before pregnancy, with approximately 47% of them retaining more than 10 lb of added weight, and 24% over 20 lb.[5] Even for women with a normal pre-pregnancy BMI, one-third of them became overweight or obese one year postpartum.[5] Although postpartum weight retention on average ranges from 0.5 to 3 kg, the variability among women was high. Women who were obese or overweight before pregnancy are at a much greater risk of substantial postpartum weight gain.[43] Furthermore, postpartum weight retention was a predictor of long-term obesity in later life. Women who lost their pregnancy weight within 6 months gained an average of 2.4 kg one decade later, while those who failed to lose their pregnancy weight gained 8.3 kg.[104] Evidence suggested the weight retained from childbearing was associated with preferential accumulation of visceral fat, which adversely impacted the metabolic health of women postpartum.[105] Research has linked excessive postpartum weight retention with many health problems in women, including increased risk of hypertension, diabetes, and even the development of breast cancer.[6,7,106] In addition to maternal health risks, excess weight gain around pregnancy also has profound negative impacts on the development of offspring. The offspring of obese mothers suffer a higher risk of obesity, disrupted growth patterns, and congenital anomalies.[8]

Pregnant and lactating women need more nutrients and energy than other women of their age to support the fetal development. To satisfy the higher nutrition and energy intake requirements, pregnant and lactating women need to consume sufficient high-quality foods. Dairy foods are rich source of protein, minerals, vitamins, and other bioactive components, which are vital for the optimal growth and development for fetus. The *Dietary Guidelines for Americans* recommend 3 servings of dairy foods per day for women during pregnancy and lactation.[13] Due to the high fat content of dairy products, the impact of dairy foods on weight change has been controversial. So far, the evidence from clinical trials and observational research tend to suggest that dairy consumption does not increase body weight in long-term studies, but there is a weight-reducing effect of dairy consumption in short-term research with energy-restriction.[53] Different types of dairy products could exert different effects on weight. Data from three large cohorts of healthcare professionals including NHS I, NHS II, and HPFS indicated that the intake of yogurt was strongly associated with less weight gain, while no benefit was found for other dairy products including milk and cheese.[35] Results from the FHS suggested that yogurt intake, compared with other types of dairy, was associated with lower annualized weight change and less incremental gains in waist circumference.[36] Limited studies have investigated the effects of dairy products on maternal weight change related to pregnancy and the results are inconsistent. One study found that increasing total dairy intake was inversely associated with gestational weight gain in Portuguese women.[54] In contrast, Olafsdottir et al. reported that women with excessive gestational weight gain consumed two times more dairy products than those who with optimal gestational weight gain.[55] Results from a large prospective cohort study with Chinese women reported that a maternal diet that is richer in milk and milk power had no effects on gestational weight change.[57] Given that gestational weight gain is a strong predictor of postpartum weight change,[58] the findings of these studies may suggest that dairy foods intakes have the potential to influence postpartum weight change. However, to our knowledge there is no study that directly examines the effects of total dairy and individual dairy products on the postpartum weight change.

Excessive postpartum weight retention has been shown to increase both maternal and fetal health risks. It is imperative to identify modifiable interventions to combat these serious health burdens. Dairy foods are typically important components of the maternal diet during pregnancy and lactation. Since previous research has suggested potential weight-reducing effects of dairy products, we chose to examine these questions. The purpose of this study was to examine the independent effects of total dairy as well as individual dairy product intakes, alone and in combination with overall diet patterns, physical activity, and other lifestyle factors, on postpartum weight retention and risk of obesity using data from women in NHS II.

3.3 Methods

3.3.1 Population Characteristics

NHS II was launched in June of 1989 with the enrollment of 116,429 women, ages 25-42 years, to examine the health consequences of oral contraceptive use along with other lifestyle factors in a younger cohort than was available in the original NHS I cohort.[89] Participants in NHS II came from 14 different states (California, Connecticut, Indiana, Iowa, Kentucky, Massachusetts, Michigan, Missouri, New York, North Carolina, Ohio, Pennsylvania, South Carolina, and Texas), and ~ 97% were Caucasian.

On the first questionnaire, participants were asked to provide information on their medical history, anthropometrics, and lifestyle factors such as physical activity, smoking status, and alcohol use. Every 2 years after 1989, a follow-up questionnaire was sent to participants to update their data and to identify incident diagnoses of disease. The first FFQ was sent in 1991 and subsequent FFQs were sent every 4 years. The response rate to questionnaires was approximately 85% to 90%.

In each biennial questionnaire until 2009, subjects were asked the year of any pregnancies that had occurred since the last questionnaire and whether they were currently pregnant. In the 2009 questionnaire, women were asked about the year, length, outcomes, and complications in all the previous pregnancies that lasted at least 20 weeks. There were 164,129 pregnancies lasting at least 20 weeks in NHS II. Options for the outcomes of pregnancy included: single live birth, twins/triplets (live birth), miscarriage/still birth, induced abortion, and tubal or ectopic pregnancy. In our analyses, we only included single live birth with at least 20 weeks of gestation time. Given that questionnaires were sent every two years, we did not have pregnancy information from each year, which means if a childbirth happened in a year with questionnaire, we only had pre-pregnancy information for two years before the childbirth, and postpartum information two years after the childbirth. Therefore, we chose to focus on a 4-year period from the pre-pregnancy to postpartum. The pre-pregnancy exam and the postpartum exam were defined according to whether the childbirth happened in a year with questionnaire (Figure 3.1). If the birth was happened in a year with questionnaire, then the two adjacent exams were defined as the pre-pregnancy and postpartum exams. For example, as shown in Figure 3.1. Panel A, if the child was born in 1995 (a year with a questionnaire), the pre-pregnancy exam would be 1993, and the postpartum exam would be 1997. If the delivery happened in a year without questionnaire, then the first following exam was defined as the postpartum exam, and the exam three years before childbirth was defined as the pre-pregnancy exam. For example, if the delivery happened in 1996 (not a year with a questionnaire), the pre-pregnancy exam would be set to 1993, and the postpartum exam would be 1997 (Figure 3.1. Panel B). The rationale for this approach was first the time from pre-pregnancy to postpartum would be constant (~ 4 years) for all births, regardless of whether it happened in the year with questionnaire or not. Secondly, for women who gave births in a non-questionnaire year (e.g., 1996), most were already pregnant by the end of the year before (e.g., 1995); therefore, we decided that the exam 3 years before the delivery (e.g., 1993) would be more appropriate than the year before the delivery. We also checked the pregnancy status in the years designated as the pre-pregnancy and postpartum exam periods to make sure the subjects were not pregnant in any of the two exams.

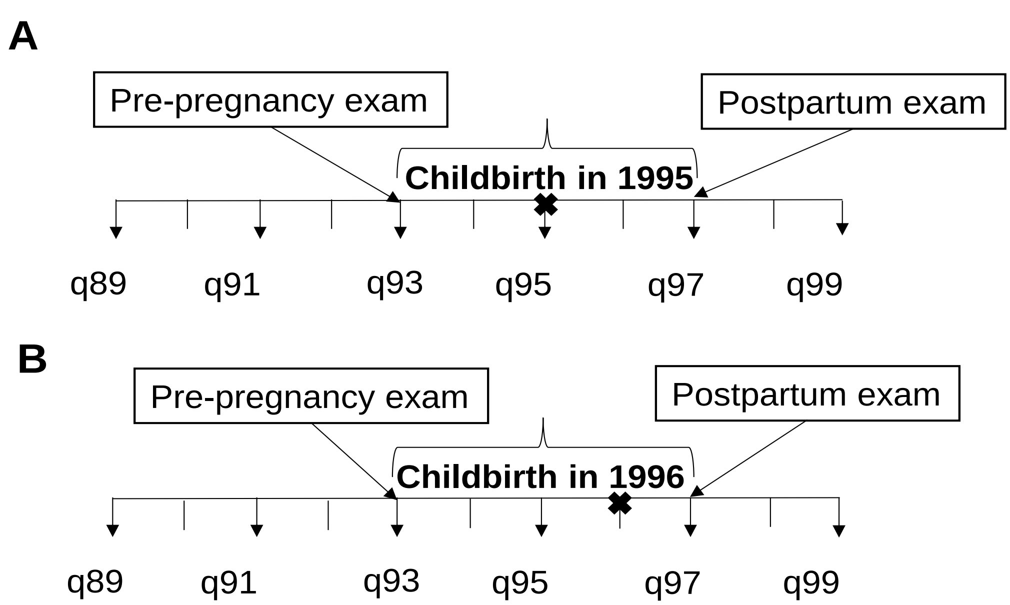


Figure 3.1 Timeline of 4 Years Surrounding Childbirth.

Table 3.1 shows the detailed exclusion criteria. There were 164,129 pregnancies lasting at least 20 weeks in women from the NHS II cohort. Of these, we excluded the following: 127,023 pregnancies that occurred before or at enrollment (i.e., 1989); 826 multiple pregnancies (e.g., twins); 430 stillbirths; 165 pregnancies with missing outcomes. These exclusions left 35,685 eligible singleton live pregnancies. We further excluded 13,791 pregnancies with missing pre-pregnancy or postpartum weights; 2,083 pregnancies with missing dairy foods intakes; 213 pregnancies in women with more than 6 servings of total dairy servings per day; 186 pregnancies with missing covariates; 469 pregnancies with prevalent or incident cancer, CVD, or diabetes; 145 pregnancies with extreme pre-pregnancy BMI (< 16 kg/m^2 or > 40 kg/m^2); 18 pregnancies in which maternal age more than 50 yrs; and 414 pregnancies with extreme postpartum weight change (the top or bottom 1% of postpartum weight change). These exclusions left a sample of18,366 pregnancies in the analyses for postpartum weight change. In the obesity analyses, we further excluded 1,240 pregnancies in women who were already obese (BMI ≥ 30 kg/m^2) at the exam prior to pregnancy, leaving 17,126 pregnancies for the analysis of incident obesity. The study protocol was approved by the Institutional Review Boards of the Brigham and Women's Hospital and the Harvard T.H. Chan School of Public Health. All analyses were approved by the Institutional Review Board of Boston University School of Medicine.

Table 3.1. Exclusion Criteria for Weight Change Analysis.

Criteria	**Exclusions**	**Remaining N**
Start with 164,129 pregnancies lasting ≥ 20 weeks:		164,129
Childbirth before or at 1989 (i.e. no pre-pregnancy weight)	127,023	37,106
Twin/triplets	826	36,280
Still birth	430	35,850
Missing births outcome	165	35,685
Missing pre-pregnant or postpartum weights	13,791	21,894
Missing cross-sectional dairy food intakes	2,083	19,811
Total dairy intakes > 6 s/d	213	19,598
Missing height	17	19,581
Missing activity	148	19,433
Missing smoking	21	19,412
Prevalent or incident cancer during follow-up	259	19,153
Prevalent or incident CVD during follow-up	94	19,059
Prevalent or incident diabetes during follow-up	116	18,943
Baseline BMI < 16 kg/m^2	5	18,938
Baseline BMI > 40 kg/m^2	140	18,798
Age at childbirth > 50 yrs	18	18,780
Postpartum weight gains > top 1%, i.e., 45 lb	208	18,572
Postpartum weight loss < bottom 1%, i.e., 20 lb	206	18,366
Final sample size for postpartum weight change analyses		**18,366**

3.3.2 Dietary Assessment

The first FFQ was sent in 1991 and every 4 years thereafter. The FFQs collected information on how often the participant consumed each food on a list in the past year. Each FFQ had 131 items. The nine categories of consumption frequency included never or less than once per month, 1–3 per month, 1 per week, 2–4 per week, 5–6 per week, 1 per day, 2–3 per day, 4–5 per day, and ≥ 6 times per day. The following foods were included in the total dairy consumption in our analyses: milk (whole milk, 1 or 2 % fat milk, and skim milk), yogurt, and cheese (cottage, ricotta, and other cheese, e.g., American, Cheddar). Butter, cream, and cream cheese were excluded due to low calcium content. A standard serving size was 1 cup for milk and yogurt, 1 slice or 1 ounce serving for other cheese (e.g., American, cheddar), and 0.5 cup for cottage or ricotta cheese. Nutrient intakes were calculated based on consumption of all the foods reported in the FFQ.

AHEI scores were calculated for individual participants based on their food and nutrient intakes from each FFQ. The AHEI components and an overview of the previously-described score calculation can be found in Table 2.3.[90] Dairy products were not a component in the AHEI score.

A previous study has confirmed the reasonable accuracy of the estimation of dairy consumption using the FFQ compared with 7-day dietary records. The Pearson correlation coefficients for skim milk, whole milk, yogurt, cottage cheese and hard cheese were 0.81, 0.62, 0.94, 0.80, and 0.57, respectively.[91]

3.3.3 Postpartum Weight Retention Assessment

Heights were self-reported at enrollment and weights were self-reported at enrollment and updated on all biennial follow-up questionnaires. Postpartum weight retention was calculated by subtracting the weight reported at the pre-pregnancy exam from the weight of the postpartum exam. It is a continuous variable, and since it is the change of weights between 2 exams, it can be positive, zero, or negative. The self-reported weights have been shown to be highly correlated (r =0.96) with technician-measured weights in a previous validation study.[92] BMI was calculated for each questionnaire cycle by using the weight divided by the baseline height, squared. Overweight was defined as BMI ≥ 25 kg/m^2, and obesity was defined as BMI ≥ 30 kg/m^2.

3.3.4 Assessment of Potential Confounding

We explored a range of potential covariates including age at childbirth, the order of childbirth, breast-feeding, physical activity, AHEI score, red and processed meat, SSB, energy intake, alcohol, fiber, fruits and vegetables, *trans* fatty acid intakes, and smoking status. Year of childbirth was reported from biennial questionnaires. Age at childbirth was calculated by subtracting the year of birthday (of the mother) from the year of childbirth. Birth order (first, second, third, etc.) of each live born delivery was self-reported on the 2009 questionnaire. Breast feeding history was assessed retrospectively in 1997 and 2003. Women were asked whether they breast fed and the duration of breast feeding for each child. Physical activity was assessed on each biennial questionnaire by asking participants about the average time per week spent on specific activities such as jogging, running, biking, tennis, swimming, etc. We summed the hours per week for each activity to derive total activity hours per week. Smoking status (missing, never smoker, past smoker, and current smoker) was asked in each questionnaire along with the number of cigarettes smoked per day among current smokers. Diet was assessed as described above every four years using a 131-item FFQ.

3.3.5 Statistical Analysis

To assess the association between the intakes of dairy products and postpartum weight retention, we used the mean of dairy foods intakes from the 4-year period surrounding the delivery (from the pre-pregnancy exam to the postpartum exam) as the exposure. We categorized women into three groups by the intakes of total dairy, yogurt, milk, and cheese after first examining the trends in postpartum weight change in each of the nine categories of intake (never or less than once per month, 1–3 per month, 1 per week, 2–4 per week, 5–6 per week, 1 per day, 2–3 per day, 4–5 per day, or ≥ 6 times per day) for each type of dairy food. Categories showing similar trends in postpartum weight change were then collapsed to obtain the final 3 categories of intake for each dairy food, as follows: total dairy (< 1 s/d, 1 s/d -< 2 s/d, and ≥ 2 s/d), yogurt (< 1 s/m, 1 s/m -<2 s/w, and ≥ 2 s/w), milk (< 1 s/d, 1 s/d - <2 s/d, and ≥ 2 s/d), and cheese (< 2 s/w, 2 s/w - <5 s/w, and ≥ 5 s/w). Linear models were used to estimate the postpartum weight retention as a continuous variable. GEE methods with an exchange correlation matrix were used to account for the correlation of within-subject data (i.e., exposures and covariates from childbirths that contributed from the same women). The outcome is weight change as a continuous variable. A wide range of covariates were explored including age at childbirth, the order of the pregnancy, breast-feeding, physical activity, AHEI score, red and processed meat, SSB, energy intake, alcohol, fiber, fruits and vegetables, *trans* fatty acid intakes, and smoking status. We retained the covariates that changed the effect estimates by more than 5% and we also adjusted each type of dairy food by other dairy foods (e.g., milk intake model was adjusted for intakes of yogurt and cheese). The Pearson correlation coefficients among individual dairy products were low in the current analyses: 0.041 between yogurt and milk, 0.055 between yogurt and cheese, 0.059 between milk and cheese. The covariates retained in the final model were age at childbirth (continuous), order of pregnancy (first childbirth, second childbirth, and third or later childbirth), *trans* fats (continuous), fruits and vegetables (continuous), alcohol (continuous), and SSB (continuous); intakes of milk, yogurt or cheese were adjusted for each other. Tests for linear trend across increasing categories of individual dairy food intake were performed by assigning the median value of the respective categories of exposure and entering this continuous variable into models.

In the incident postpartum obesity analyses, women who were obese (n = 1,240) before pregnancy were excluded. There were 17,126 subjects in the obesity analyses. Multivariable models with a Poisson distribution were used to estimate the relative risk and 95% confidence interval for becoming obese at the postpartum exam. The BMIs of the middle exam between the pre-pregnancy and postpartum exams were ignored because it was too close the time of childbirth. The covariates in the final model were the same as those in the weight change analyses.

We also explored the potential effect modification of dairy foods by pre-pregnant overweight status, physical activity, and overall dietary pattern as estimated by the AHEI. We selected activity and AHEI as potential effect modifiers because a healthy lifestyle such as healthy eating and being more physically active are important strategies for weight management and yogurt consumption is associated with healthy lifestyle.[40] We also selected pre-pregnant overweight status as an effect modifier because it has been previously shown that women who were overweight before pregnancy have a much higher risk of excess weight retention postpartum.[43]

To test potential effect modification of dairy foods by pre-pregnant overweight status, physical activity, and overall dietary pattern (i.e., AHEI), we created combined categories of each dairy products with each other variable (i.e., pre-pregnant overweight status, physical activity and AHEI score). Pre-pregnant overweight status was dichotomized as non-overweight (pre-pregnant

BMI <25 kg/m^2) vs. overweight (pre-pregnant BMI ≥25 kg/m^2). Physical activity was dichotomized as higher (≥2 h/w) vs. lower (<2 h/w) based the distribution of physical activity. AHEI was likewise dichotomized as higher (top 3 quintiles) vs. lower (bottom 2 quintiles) and dairy food intakes were dichotomized as follows: total dairy (<2 s/d vs. ≥2 s/d), yogurt (<1 s/m vs. ≥1 s/m), milk (<1 s/d vs. ≥1 s/d), and cheese (<2 s/w vs. ≥2 s/w). Subjects were then cross-classified into four groups based on these dichotomized groups. For example, the combined categories of total dairy and physical activity were: (1) lower physical activity (<2 h/w) + lower total dairy intake (<2 s/d), (2) lower physical activity (<2 h/w) + higher total dairy intake (≥2 s/d), (3) higher physical activity (≥2 h/w) + lower total dairy intake (<2 s/d), and (4) higher physical activity (≥2 h/w) + higher total dairy intake (≥2 s/d). Those in the lower categories for both dichotomous variables served as the referent group

Statistical significance was set at a two-tailed $p < 0.05$. SAS version 9.4 (SAS Institute) was used to analyses the data.

3.4 Results

Table 3.2. Diet and Lifestyle Characteristics of Women in the Postpartum Weight Retention Analysis by Categories of Yogurt Intake throughout the 4 Years Surrounding Pregnancy[1].

	Cross-sectional Yogurt Intake		
	1 s/m	1 s/m -< 2 s/w	≥ 2 s/w
N of live births	5734	8868	3764
		Mean (SD)	
Age at birth (years)	34.7 (3.8)	35.2 (3.8)	35.6 (3.9)
Pre-pregnant BMI (kg/m^2)	23.3 (4.0)	23.2 (3.8)	23.0 (3.7)
Activity (h/w)	2.0 (2.5)	2.4 (2.7)	3.1 (2.9)
AHEI score	44.1 (10.3)	47.8 (10.0)	51.3 (10.2)
Energy intake (kcals/d)	1825 (538)	1903 (515)	2045 (513)
Energy-adj protein (g/d)	83.6 (14.0)	85.0 (12.7)	86.4 (12.8)
Energy-adj carbohydrate (g/d)	226.7 (33.8)	232.2 (29.9)	242.0 (28.9)
Energy-adj total fat (g/d)	63.7 (11.4)	60.7 (10.2)	55.9 (10.0)
Energy-adj trans-fat (g/d)	3.3 (1.2)	3.0 (1.0)	2.6 (0.9)
Energy-adj alcohol (g/d)	2.5 (5.3)	2.9 (5.2)	3.2 (5.5)
Total dairy (s/d)	1.8 (1.2)	2.1 (1.2)	2.7 (1.2)
Yogurt (s/w)	0.0 (0.0)	0.8 (0.5)	4.1 (1.9)
Milk (s/d)	1.3 (1.1)	1.4 (1.1)	1.5 (1.1)
Cheese (s/d)	0.5 (0.5)	0.6 (0.4)	0.6 (0.5)
Red and processed meat (s/d)	0.9 (0.5)	0.8 (0.5)	0.7 (0.5)
Fruits and vegetables (s/d)	3.9 (2.2)	4.6 (2.2)	5.6 (2.6)
SSB (bottles/d)	1.4 (1.4)	1.3 (1.2)	1.2 (1.2)
Pre-pregnant current smoker (%)	9.9	7.0	6.5
First birth (%)	23.7	25.6	32.1

Abbreviations: AHEI, alternative healthy eating index 2010; BMI, body mass index; Energy-adj, energy adjusted; SD, standard deviation; SSB, sugar sweetened beverages; s/d, serving per day; s/m, serving per month; s/w, serving per week.

[1] All the dietary variables and activities variables are cross-sectional; only BMI and smoking status are pre-pregnant.

The characteristics of subjects in the postpartum weight retention analysis were presented in Table 3.2. Women with higher yogurt intake were slightly older when giving birth, and tended to be more active, have a healthier overall diet, and be non-smokers. It was also more likely for higher yogurt consumers to be giving birth for the first time. In terms of nutrients intakes, women of higher yogurt group consumed more calories and more energy-adjusted intakes of protein, carbohydrates, and alcohol but less fat. There was a positive association between yogurt intake with fruits and vegetables. The consumption of SSB and red & processed meat consistently were inversely associated with yogurt intake. There were no apparent differences in milk or cheese consumption across the three groups of yogurt intake.

Table 3.3 Postpartum Weight Retention by Intakes of Different Types of Dairy Foods.

	N	Unadjusted	Multivariable[1]	Multivariable + pre-pregnant BMI
		Mean of Postpartum Weight Retention (lb)		
Total dairy				
< 1 s/d	3,567	6.66	6.53	6.55
1 s/d -< 2 s/d	6,197	6.40	6.35	6.33
≥ 2 s/d	8,602	6.19	6.30	6.28
per 1 day		-0.17	-0.07	-0.09
p-trend:		***0.032***	*0.349*	*0.269*
Yogurt				
<1 s/m	5,734	6.76	6.67	6.65
1 s/m -< 2 s/w	8,868	6.25	6.29	6.28
≥ 2 s/w	3,764	5.98	6.04	6.04
per 1 week		-0.20	-0.17	-0.16
p-trend:		***0.003***	***0.022***	***0.026***
Milk				
< 1 s/d	7,677	6.55	6.43	6.45
1 s/d - < 2 s/d	4,769	6.16	6.22	6.21
≥ 2 s/d	5,920	6.25	6.37	6.34
per 1 day		-0.12	-0.01	-0.03
p-trend:		*0.180*	*0.897*	*0.692*
Cheese				
< 2 s/w	5,485	6.65	6.67	6.63
2 s/w -< 5 s/w	7,516	6.33	6.37	6.32
≥ 5 s/w	5,365	6.09	6.03	6.10
per 1 week		-0.10	-0.13	-0.10
p-trend:		***0.006***	***0.002***	***0.011***

Abbreviations: BMI, body mass index; lb, pound; Ref, reference; s/d, serving per day; s/m, serving per month; s/w, serving per week.
$p < 0.05$ is in bold font.
[1] Adjusted for the age at childbirth, the order of childbirth, *trans* fat, fruit and vegetable, alcohol, sugar sweetened beverages, yogurt, cheese, and milk were adjusted for each other.

Table 3.3 demonstrates postpartum weight retention by dairy food intakes. In the unadjusted model, there was a statistically significant inverse association between the postpartum weight retention and intakes of total dairy, yogurt, and cheese. Women with higher total dairy, yogurt, and cheese intakes showed 0.47 lb (p-trend = 0.032), 0.78 lb (p-trend = 0.003), and 0.56 lb (p-trend = 0.006) less weight retention than women in the lower categories, respectively. After adjusting for potential confounders, the statistically significant inverse association with post-partum weight change remained only for yogurt and cheese intakes. Women who consumed more than 2 s/w of yogurt had 0.63 lb (p-trend = 0.022) less weight retention then women who consumed less than 1 s/m. Higher cheese (≥ 5 s/w vs. < 2 s/w) intake was linked with 0.64 lb (p-trend = 0.002) less weight retention. When we added pre-pregnancy BMI to the multivariable model, the favorable effects of yogurt and cheese on weight retention remained and were only very slightly attenuated (p-trend = 0.026 for yogurt intake; p-trend = 0.011 for cheese intake). There was no clear effect of milk intake on the postpartum weight retention in any model.

Table 3.4. Independent and Combined Effects of Dairy Food Intakes with Overweight Status, Physical Activity, and AHEI Score on Postpartum Weight Retention.

	Adjusted Mean of Postpartum Weight Retention (lb)[1]					
	BMI ≥ 25	BMI < 25	Lower activity[2]	Higher activity	Lower AHEI[3]	Higher AHEI
Dairy < 2 s/d	8.97	5.64	6.76	5.93	7.06	6.06
Dairy ≥ 2 s/d	9.03	5.44	6.38	6.17	6.44	6.16
P-value	*0.880*	*0.214*	*0.069*	*0.306*	***0.014***	*0.596*
Yogurt < 1 s/m	9.71	5.69	6.80	6.48	7.23	6.13
Yogurt ≥ 1 s/m	8.65	5.48	6.48	5.88	6.42	6.08
P-value	***0.017***	*0.247*	*0.150*	***0.032***	***0.001***	*0.832*
Milk < 1 s/d	9.03	5.67	6.78	5.98	7.17	6.03
Milk ≥ 1 s/d	8.98	5.45	6.44	6.10	6.49	6.15
P-value	*0.893*	*0.193*	*0.119*	*0.629*	***0.010***	*0.553*
Cheese < 2 s/w	8.82	5.97	6.85	6.41	7.07	6.43
Cheese ≥ 2 s/w	9.08	5.37	6.47	5.90	6.61	5.96
P-value	*0.568*	***0.001***	*0.095*	***0.049***	*0.102*	***0.034***

Abbreviations: AHEI, alternative healthy eating index 2010; lb, pound; lb, pound; s/d, serving per day; s/m, serving per month; s/w, serving per week.
[1] Adjusted for the age at childbirth, the order of childbirth, *trans* fat, fruit and vegetable, alcohol, sugar sweetened beverages, yogurt, cheese, and milk were adjusted for each other.
[2] Lower vs higher physical activity is defined as < 2 vs ≥ 2 hours of physical activity per week.
[3] Lower vs higher AHEI is defined as the lower 2 quintiles vs the upper 3 quintiles of AHEI score.

To explore the potential effect modifiers, we estimated the weight retention by the categories of individual dairy foods with pre-pregnancy weight status, physical activity, and AHEI score (Table 3.4). Women who were overweight before the pregnancy had 35-40% higher weight retention than those who were not overweight. In the women who were overweight before the pregnancy, higher intake of yogurt was associated with 1.06 lb (p = 0.017) less weight retention than those consuming less yogurt. In contrast, among women who were not overweight before pregnancy, higher cheese intake was linked with 0.60 lb ($p=0.001$) less postpartum weight retention.

We next stratified by physical activity (Table 3.4) and found that while higher activity alone and higher yogurt intake alone were both associated with 0.32 lb less weight retention, the combination of higher yogurt intake and higher activity resulted in a reduction in weight retention of 0.92 lb compared with that in the reference category (lower activity & lower yogurt). These results suggest a synergistic effect of yogurt and activity that is more than additive. Similarly, the combined effects of higher cheese intake with higher activity levels seems to have a synergistic effect on weight retention. Higher AHEI scores alone were statistically significantly associated with less weight retention regardless of dairy intake. Among women with lower AHEI scores, higher intakes of all dairy foods were associated with less postpartum weight retention. However, the effect of AHEI and dairy foods appeared to be synergistic only for cheese.

Table 3.5. Relative Risk (95% CI) for Postpartum Obesity According to Different Dairy Foods Intakes

	N	Case	Multivariable[1]	Multivariable + pre-pregnant BMI
Total dairy				
< 1 s/d	3,300	163	1.00 (Ref)	1.00 (Ref)
1 s/d -< 2 s/d	5,787	309	1.13 (0.94 - 1.36)	1.06 (0.90 - 1.25)
≥ 2 s/d	8,039	382	1.07 (0.89 - 1.28)	1.08 (0.92 - 1.26)
Yogurt				
<1 s/m	5,297	317	1.00 (Ref)	1.00 (Ref)
1 s/m -< 2 s/w	8,292	374	0.80 (0.69 - 0.93)	0.90 (0.79 - 1.01)
≥ 2 s/w	3,537	163	0.84 (0.69 - 1.02)	0.93 (0.79 - 1.10)
Milk				
< 1 s/d	7,131	363	1.00 (Ref)	1.00 (Ref)
1 s/d -< 2 s/d	4,459	230	1.05 (0.90 - 1.24)	1.06 (0.92 - 1.22)
≥ 2 s/d	5,536	261	1.01 (0.85 - 1.19)	1.03 (0.90 - 1.18)
Cheese				
< 2 s/w	5,116	256	1.00 (Ref)	1.00 (Ref)
2 s/w -< 5 s/w	6,977	363	1.04 (0.89 - 1.22)	1.05 (0.92 - 1.20)
≥ 5 s/w	5,033	235	0.92 (0.77 - 1.10)	0.96 (0.82 - 1.12)

Abbreviations: BMI, body mass index; lb, pound; Ref, reference; s/d, serving per day; s/m, serving per month; s/w, serving per week.
[1] Adjusted for the age at childbirth, the order of childbirth, *trans* fat, fruit and vegetable, alcohol, sugar sweetened beverages, yogurt, cheese, and milk were adjusted for each other.

We explored the effects of dairy foods intakes on postpartum obesity risk in Table 3.5. Moderate and higher yogurt intakes were linked to 20% (95% CI: 0.69 - 0.93) and 16% (95% CI: 0.69 - 1.02) reduced risks of developing obesity, respectively. Higher cheese intake (≥ 5 s/w) was associated with a non-statistically significant 8% (95% CI: 0.77 - 1.10) lower obesity risk compared with lower cheese intake (< 2 s/w). No clear trend was observed between total dairy or milk and obesity risk. Adding pre-pregnant BMI attenuated the favorable effects of yogurt on obesity. There were only 10% (95% CI: 0.79 - 1.01) and 7% (95% CI: 0.79 - 1.01) reduced risks of becoming obesity in women with moderate and higher yogurt intakes, respectively, after adjusting for pre-pregnant BMI.

Table 3.6. Relative Risk (95% CI) for Postpartum Obesity According to Intake of Different Dairy Foods, Stratifying by Physical Activity and AHEI[1].

	Lower Activity[2]	Higher Activity	Lower Activity	Higher Activity
	N of subjects		RR (95% CI)	
Dairy < 2 s/d	5,213	3,874	1.00 (Ref)	0.71 (0.58 - 0.86)
Dairy ≥ 2 s/d	4,468	3,571	0.95 (0.80 - 1.12)	0.73 (0.60 - 0.90)
Yogurt < 1 s/m	3,404	1,893	1.00 (Ref)	0.79 (0.62 - 1.00)
Yogurt ≥ 1 s/m	6,277	5,552	0.84 (0.71 - 1.00)	0.61 (0.50 - 0.74)
Milk < 1 s/d	4,019	3,112	1.00 (Ref)	0.72 (0.58 - 0.89)
Milk ≥ 1 s/d	5,662	4,333	1.01 (0.85 - 1.20)	0.75 (0.62 - 0.92)
Cheese < 2 s/w	2,887	2,229	1.00 (Ref)	0.82 (0.64 - 1.05)
Cheese ≥ 2 s/w	6,794	5,216	1.05 (0.87 - 1.26)	0.73 (0.59 - 0.91)
	Lower AHEI[3]	**Higher AHEI**	**Lower AHEI**	**Higher AHEI**
	N of subjects		RR (95% CI)	
Dairy < 2 s/d	3,284	5,803	1.00 (Ref)	0.63 (0.51 - 0.78)
Dairy ≥ 2 s/d	3,567	4,472	0.82 (0.67 - 1.00)	0.68 (0.54 - 0.85)
Yogurt < 1 s/m	2,828	2,469	1.00 (Ref)	0.59 (0.46 - 0.75)
Yogurt ≥ 1 s/m	4,023	7,806	0.70 (0.57 - 0.85)	0.58 (0.47 - 0.72)
Milk < 1 s/d	2,536	4,595	1.00 (Ref)	0.58 (0.46 - 0.73)
Milk ≥ 1 s/d	4,315	5,680	0.83 (0.68 - 1.02)	0.69 (0.55 - 0.86)
Cheese < 2 s/w	1,794	3,322	1.00 (Ref)	0.70 (0.53 - 0.91)
Cheese ≥ 2 s/w	5,057	6,953	0.95 (0.77 - 1.19)	0.69 (0.54 - 0.88)

Abbreviations: AHEI, alternative healthy eating index 2010; CI, confidence interval; RR, relative risk; s/d, serving per day; s/m, serving per month; s/w, serving per week.

[1] Adjusted for the age at childbirth, the order of childbirth, trans fat, fruit and vegetable, alcohol, sugar sweetened beverages, yogurt, cheese, and milk were adjusted for each other.

[2] Lower vs higher physical activity is defined as < 2 vs ≥ 2 hours of activity per week.

[3] Lower vs higher AHEI is defined as the lower 2 quintiles vs the upper 3 quintiles of AHEI score.

In Table 3.7, higher activity alone (among those with lower dairy intakes) was associated with lower risks of obesity for all types of dairy. Higher yogurt alone reduced the risk of obesity by 16% (95% CI: 0.71 - 1.00), while higher yogurt and activity together led to a statistically significant 39% (95% CI: 0.50 - 0.74) lower risk of becoming obese postpartum. No statistically significant reductions of obesity risk were observed by total dairy, milk, or cheese intake alone. However, there did appear to be a synergistic effect between physical activity and higher consumption of both yogurt and cheese. There were no synergistic effects between dairy foods and AHEI scores with respect to obesity risk. However, higher AHEI scores alone were associated with statistically significant reductions in risk of postpartum obesity.

3.5 Discussion

The current study finds that higher intakes of yogurt and cheese were associated with less postpartum weight retention. Further, yogurt intake was associated with a 16% (95% CI: 0.71 - 1.00) lower obesity risk in sedentary women, and a 30% (95% CI: 0.57 - 0.85) lower risk in women with a less healthy diet (with lower AHEI scores).

Higher activity levels and higher AHEI scores were both independently associated with less post-partum weight retention. Further, both yogurt and cheese intakes had additive synergistic beneficial effects on weight change when combined with higher activity levels. Finally, we found that among women with less healthy diets, total dairy, milk, and yogurt were all associated with less postpartum weight retention. However, among those with a healthy diet, only higher cheese consumption was associated with less weight retention.

To our knowledge, this was the first study that focused on the association between postpartum weight and dairy food intakes. Our study provides important evidence that total dairy or individual dairy foods do not lead to increased postpartum weight retention or obesity risk. In contrast, incorporating cheese or yogurt in particular into the maternal diet could have favorable effects on attenuating postpartum weight retention or retention and improving the health of women at this critical stage.

Postpartum weight retention plays an important role in contributing to the incidence of obesity in women of reproductive age. Although a number of studies have examined the patterns of postpartum weight change, the results are highly variable, perhaps due to different sociodemographic characteristics of the various study cohorts, issues of study power and sample size, non-comparable methods of weight measurement, and the length of postpartum follow-up.[5,43,107–109] Endres et al. reported a mean of 11.1 lb of weight retention 12 months after childbirth, with 47% women retaining more than 10 lb and 24% retaining more than 20 lb.[5] In an analysis of pregnant women recruited from North Carolina between 2001 and 2005, the average postpartum weight retention was 9.4 lb at 3 months following delivery. By the end of 12 months postpartum, the average weight retention was 5.7 lb, with 27% of participants having retained at least 10 lb.[108] In a recent study of 183 women from Lebanon and Qatar, the mean weight retention was 7.3 lb and 6.0 lb at 4 and 6 months postpartum, respectively.[109] Although we did not have the exact dates of the childbirth in the current study, the estimated average length between the delivery and the postpartum exam was 20 months, with a mean postpartum weight change at that time of 6.34 lb. This result was in concordance with the result of a study with similar postpartum follow-up. Janney et al. reported a mean postpartum weight retention of 6.6 lb at 18 months in 110 women from Ann Arbor, Michigan.[110] Previous evidence has also shown that women who were overweight or obese before pregnancy had a 3-fold higher risk of postpartum weight retention.[5] In the current analyses, we observed the weight retention of women with a pre-gravid BMI ≥25 kg/m^2 was about 40% higher than the weight retention of women whose pre-gravid BMI was less than 25 kg/m^2. This result supports the finding that women who are already overweight or obese at the time that they become pregnant are susceptible to greater weight retention in the postpartum period.

Dairy foods are excellent source of essential nutrients for fetal growth and studies found that maternal consumption of dairy foods has positive effects on the fetal femur growth, and infant birthweight.[111] It has been reported that maternal consumption of dairy products can also prevent the risk of infantile eczema, and asthma.[112] The Dietary Guidelines recommend the consumption of dairy products as an important part of healthy and balanced diet for women during pregnancy and early postnatal life.[13] It is possible that at least part of the beneficial effects of dairy foods on the offspring of pregnant women are a result of intermediate effects on body weight. We were unable to find research that investigated the potential impact of dairy foods on the postpartum weight change directly. However, a few studies have reported the association between dairy intake and gestational weight retention. In one prospective analysis of pregnant Portuguese women, Abreu et al. measured the dietary intake of milk, cheese, and yogurt of pregnant women using 3-day food diaries during both the first and the second trimesters. After adjusting for potential confounding, change of total dairy intakes from the first to the second trimesters had a significant inverse association with gestational weight gain. Moreover, the increase in consumption of individual dairy products including milk, yogurt and cheese were all inversely associated with gestational weight gain, although these associations were not statistically significant.[54] In a 2019 study, Wei et al. classified 5,733 pregnant Chinese women into six dietary patterns. They found that women who had a dietary pattern that was richer in fish, beans, nuts, and yogurt had significantly lower risk of inadequate gestation weight gain compared with women in the reference dietary pattern (richer in cereal). They also reported that women with a dietary pattern that was richer in milk and milk power had no difference in gestational weight gain than the reference dietary pattern.[57] However, in a study of 1,388 women from the Project Viva cohort, the authors reported that total dairy consumption during pregnancy was associated with an increased risk of excessive gestational weight gain (OR: 1.08; 95% CI:1.00 - 1.17, per s/d).[56] They also found that this association did not appear to be driven by whole fat dairy foods, because both low fat (OR: 1.08; 95% CI:0.98 -1.18, per s/d) and whole fat (OR: 1.06; 95% CI:0.94 -1.20, per s/d) dairy foods were similarly associated with increased risk of excessive gestational weight gain.

Gestational weight gain is a strong predictor of postpartum weight retention. A meta-analysis of 9 observational studies found that women with excessive gestational weight gain retained significantly more weight postpartum.[58] Our results suggested that increased consumption of yogurt and cheese were significantly associated with reduced postpartum weight retention, and yogurt intake was also associated with a lower risk of postpartum obesity. Research in adult populations has supported favorable effects of yogurt consumption on weight management. Higher yogurt intake was reported to be linked with lower BMI, lower body weight gain, smaller WC and lower body fat in the general adult population.[113] In a previous study of participants from NHS I, NHS II and HPFS, 1 serving of yogurt per day was associated with 0.82 lb less weight gain over 4 years after adjusting for other lifestyle and dietary variables.[35] Notably, yogurt intake was also reported to have significant effects on attenuating central adiposity, an important component of metabolic syndrome. In a large prospective study from Spain, adults with higher yogurt consumption had a 15% lower risk of developing central adiposity over 6 years.[114] Moreover, the consumption of yogurt was found to be statistically significantly associated with a reversal of abdominal obesity in a clinical trial with 4,545 subjects who were at high risk for CVD.[115] Results from the FHS also found that participants who consumed more yogurt (≥ 3 s/w vs. < 1 s/w) had a 0.13 cm smaller annualized increase in WC (p-trend = 0.008).[36] Evidence suggests that childbearing preferentially contributes to the increase of visceral fat. In a study that compared the changes in adiposity between women with and without childbirth over 5 years, the women who had experienced childbirth had 40% more accumulation of visceral fat than their baseline level, while women who did not experience childbirth only had 14% increase in visceral fat. On the other hand, there was no difference in weight change or total fat change between women who delivered a child versus the those who did not.[105] Visceral fat is metabolically active and contributes to a variety of adverse health outcomes such as insulin resistance, diabetes, and CVD.[116] Given the increased visceral fat in postpartum women, the weight-reducing effects of yogurt could provide important benefits for women at this critical stage.

Although cheese has long been perceived to promote weight gain due to its high fat content, most studies so far have suggested that it either has no effect or a protective effect on weight gain. A previous study of three large cohorts of healthcare professionals (NHS I & II and HPFS) found a null association between cheese intake and weight change. Similarly, in the FHS, higher cheese intake (≥ 3 s/w vs. <1 s/w) did not lead to any difference in weight change or WC in 3,440 participants.[87] A cross-sectional study of Spanish adult women found that cheese consumption was inversely associated with BMI as well as prevalence of obesity and overweight.[86] Moreover, in the analysis of subtypes of cheese, the inverse association held for both fresh cheese and processed cheese intakes. Rosell et al. found that women who regularly consumed more than 1 s/d of cheese had a 30% (95% CI:0.59 - 0.84) lower risk of weight gain compared with those who consumed less than 1 s/d; this association was statistically significant in both normal weight and overweight women.[88] However, it is worth noting that in the current analysis, the weight-reducing benefit of cheese intake was not found in women who were overweight before pregnancy. It is possible that women who were already overweight before pregnancy may have a history of having difficulty maintaining their weight or shedding small amounts of weight retention, which makes them have particular difficulty losing weight retained during pregnancy.[43]

The mean cheese intake was 2.5 s/d in the high cheese group in the study of Rosell et al., while the mean cheese intake was only 0.6 s/d in the high cheese group in our current analysis. This may limit our ability to evaluate the effects of higher cheese intake on weight change in women.

The mechanisms by which yogurt or cheese may benefit weight change are not clear, but several explanations have been proposed. The bacteria present in fermented dairy products have anti-inflammatory properties. Some evidence indicates that fermented dairy foods have stronger effects on lowering chronic inflammation than non-fermented dairy foods, and these findings could alleviate the risk of obesity.[117] Also, the products derived from the fermentation of milk such as bioactive peptides and probiotics have been reported to play a role in weight management. Bioactive peptides have been shown to be effective in preventing obesity by suppressing appetite, regulating adipocyte differentiation, and impacting the activity of lipase.[118,119] Probiotics are live bacteria that boost human health through the digestive system. It has been postulated that probiotics may favorably modulate gut microbiota and prevent against obesity. Increased calcium has also been found to inhibit lipogenesis, stimulate lipolysis, promote lipid oxidation, and increase the excretion of fecal fat.[97,98] Although a cup of yogurt has similar amount of calcium as one cup of milk, yogurt is believed to improve the bioavailability of calcium due to its acidity.[99] Thus, the improved bioavailability may account for the specific weight-reducing effects of yogurt. Further, despite the fact that cheese has been perceived as a food that promotes weight gain due to its high content of fat, recent studies suggested that certain dairy fatty acids such as conjugated linoleic acid, butyric acid, and palmitoleic acid have positive effects on reducing adiposity.[102]

We also explored the combined effects of dairy food consumption combined with physical activity and diet quality on postpartum weight retention. Because of the need to feed and nurse infants, women are thought to be more sedentary during pregnancy and the early postnatal years. Increased physical activity might be an effective strategy to reduce postpartum weight retention. However, a previous clinical trial showed that 12 weeks of a moderate aerobic exercise intervention did not promote weight loss in lactating women.[120] In addition, Lovelady et al. found that a 10-week program combining moderate physical activity and calorie restriction was associated with weight loss in women during the postpartum period.[121] In the current results, we found that the women with higher physical activity alone had less weight retention as well as a reduced risk of obesity. The physical activity in the current study represents long-term activity instead of a short-term intervention as in the previous clinical trial. This could explain why physical activity had weight-reducing effects in the current study but not in some earlier studies. Interestingly, the current analysis also found that yogurt and cheese intakes further reduced weight retention in active women. Moreover, the combination of activity with yogurt or cheese intake resulted in an even lower postpartum obesity risk than activity alone. These results together suggested that adequate exercise combined with yogurt or cheese intakes may be effective strategies for women to combat postpartum weight retention and obesity risk.

Boghossian et al. investigated the association between the AHEI score and weight change in 1136 lactating women. They observed apparent differences in the postpartum weight change trajectory associated with different AHEI scores. Specifically, women with higher AHEI scores tended to have lower weight retention over the time. However, they failed to find any difference in postpartum weight retention associated with AHEI scores at 14 months after childbirth.[122] The authors assumed that the shorter follow-up time could be the reason why they failed to detect a significant weight change in association with the AHEI. In our analysis, we observed that a higher AHEI itself contributed to decreased postpartum weight retention and reduced risk of obesity. These results could be due to our larger sample size or the relatively longer follow-up time than was used in the study of Boghossian et al. We also found that the intakes of total dairy and yogurt were linked to significantly less weight retention as well as obesity risk in women with a lower AHEI. This suggests that women who had less healthy overall dietary pattern may benefit more by increasing their consumption of total dairy and yogurt to manage their postpartum weight. Considering that it is much easier and more practical to change the consumption of a single food than changing the overall dietary pattern, our finding provides an effective strategy for women with a less than optimal holistic diet to manage their weights after pregnancy.

There are several strengths of the present study. First, it was the first study that illustrated the effects of different dairy foods on weight in postpartum period, a critical stage during which women are prone to the development of obesity. Secondly, the current analysis was based on the large NHS II cohort, so we had a larger sample size than most previous studies that focused on postpartum women. Thirdly, this study provided not only the effects of different types of dairy food on postpartum weight change but also the effects in combination with other risk factors including pre-pregnancy BMI, physical activity and AHEI scores.

However, the findings of our study should also be assessed in light of the limitations of the data. First, do not have the specific month and day of birth, which prevented us from calculating the exact postpartum time length. Secondly, the dietary data were collected using an FFQ, which may be subject to measurement error. However, a validation study has shown there to be high levels of validity of self-reported dairy foods with the FFQ.[91] Thirdly, the weights were self-reported in our study. Although the previous study suggested a high correlation (r = 0.96) between self-reported weights and measured weights, reported weights were relatively lower than the measured weights, which may lead to greater misclassification of the outcome.[92] However, the potential bias associated with the reported pre-pregnancy weights should be similar to the bias in the reporting of postpartum weights. Thus, the weight change between those two weights should be less biased than either reported weight alone. However, we cannot be certain that this is true. Finally, NHS II participants were largely Caucasian nurses. It has been suggested that black women are more prone to excess weight retention of postpartum weight than white women.[123] Therefore more research is needed to explore the effects of dairy foods in minority women during postpartum period.

In conclusion, our results suggest that higher consumption of yogurt and cheese are associated with less postpartum weight gain and retention. Women with higher physical activity levels and higher intakes of yogurt had a 39% lower risk of incident postpartum obesity. The AHEI alone was also associated with less postpartum weight gain and lower obesity risk. Incorporating yogurt or cheese intake into diet may be an effective strategy for women to manage their weight during the postpartum period, especially for those who have less healthy dietary patterns in general.

CHAPTER FOUR: EFFECTS OF DIFFERENT DAIRY FOODS INTAKES ON FRAGILITY FRACTURES IN WOMEN AGED 40 AND OVER IN NHS I

4.1 Abstract

The rate of fragility fracture begins to rise in women in their 40s, presenting great health challenges for both middle-aged and elderly women. It is important to find modifiable risk factors for the prevention of fragility fractures in women. Dairy products are nutrient-dense foods that provides many bone-beneficial nutrients such as calcium, vitamin D, and protein. Individual dairy foods may exert different effects on bone health due to differences in their nutrient profiles. For example, fermented dairy products contain probiotics, which might provide benefits to bone health. However, few studies have examined the different effects of individual types of dairy foods on fracture risk. The current study investigated effects of total dairy, yogurt, milk, and cheese on fragility fracture of the hip, wrist, of vertebrae in women at 40 years of age or older in the prospective NHS I cohort. In total, there are 99,072 women aged 40 or over in the current analysis. We relied on the self-reported wrist and hip fractures while for vertebral fractures, we used medical record confirmed cases. Proportional hazards models were used to estimate risk of first fracture (of the wrist, hip, or vertebrae). After controlling for relevant covariates, each additional serving of total dairy per day was associated with 5% (95% CI: 0.92 - 0.98) less fracture risk. Specifically, consumption of two or more servings of total dairy per day (vs. < 1 s/w) led to an 19% reduction in fracture risk (RR: 0.81; 95% CI: 0.67-0.99). In

terms of individual dairy products, each serving of milk was associated with 6% (95% CI: 0.91 - 0.97) lower fracture risk while there was no statistically significant effect of either yogurt or cheese intake. In categorical analyses, two servings of milk per day were associated with a 14% reduction in fracture risk (95% CI: 0.77 - 0.95). There was no association between yogurt intake categories and fracture risk and the highest intake of cheese (≥ 1 s/d) was associated with a non-statistically significant 9% reduction in fracture risk. We also stratified this analysis by the intakes of calcium, vitamin D, and protein from non-dietary sources and found that none of these minerals modified the effects of total dairy or milk with fracture risk. Higher total dairy (> 1 s/d vs. ≤ 1 s/d) intake alone (among leaner women) was associated with a 12% lower fracture risk (95% CI: 0.81 - 0.95); a higher BMI alone (regardless of dairy intake) was also associated with lower fracture risk (RR: 0.78; 95% CI: 0.71 - 0.85). However, the strongest fracture risk reduction (RR: 0.71; 95% CI: 0.65 - 0.77) was found among overweight women with higher dairy intakes. Higher milk intake (> 1 s/d vs. ≤ 1 s/d) alone and in combination with BMI, was associated with fracture risks that were essentially the same as that observed for total dairy. In the postmenopausal women, both total dairy and milk intakes were associated with approximately 10% less risk of developing a fracture. These risks were much lower among premenopausal women. Finally, cheese intake (≥ 5 s/w vs. < 5 s/w) was associated with lower fracture risk, but only among premenopausal women. On the basis of these analyses, we conclude that total dairy and milk intakes were associated with significantly lower risks of fracture in women ages 40 years and older. In contrast, consumption of yogurt and cheese had little effect on fracture risk. None of these effects were observed in or modified by non-dairy intakes of calcium, vitamin D, or protein.

4.2 Introduction

Fragility fractures are a group of fractures that are induced by moderate or low trauma injuries, such as falling from a standing height without a major traumatic event. Fragility fractures are typically a sign of underlying osteoporosis, characterized by reduced BMD and destruction of bone microarchitecture.[59] The most common fragility fractures are hip fractures, forearm fractures and vertebral fractures.[10] The prevalence of fragility fractures exceeded 2 million in the US in 2005, and the related economic costs were about $17 billion. It is estimated that the annual incidence of fragility fractures will grow by 50% in the US by 2025 as a result of the aging of the population.[60] It has been reported that women suffer from a much higher risk of osteoporosis and associated fractures than men. A 2021 meta-analysis indicated that the prevalence of osteoporosis was 23.1% in women worldwide, which is more than twice the prevalence observed in men (11.7%).[9] In line with that, women experience 4 times more forearm fractures, 2.3 times more hip fractures, and 1.6 times more vertebral fractures than men.[10] Osteoporosis and fragility fractures are usually considered diseases of women ages 65 or older, but a recent study revealed that the prevalence of osteopenia, osteoporosis, and fragility fractures in middle-aged women (40 to 64 years) are underrecognized.[61] In fact, the study found the prevalence of osteopenia was consistently high in women aged 40 years or older, and there was no significant difference in the osteopenia prevalence between women 40 to 49 years of age and women in older age groups. Furthermore, the study also found that middle-aged women with normal BMD values had a high prevalence of fractures. Population-based studies also illustrate that the risk of fracture in females is low and remain stable from ages 20 years to the early 40s, but the risk begins to rise after the age of 42 years to the end of life.[11] These results pointed out that fragility fractures should be considered a health challenge for women as young as 40 years of age to avoid adverse sequelae.

Dairy foods provide a rich source of nutrients such as calcium, protein, phosphorus, magnesium, and potassium that are critical for bone health.[76] In the 1930s, the US began to fortify milk with vitamin D; most processed fluid milk in the US is now enriched with vitamin D. Given that vitamin D fortification can facilitate calcium absorption, it makes dairy foods an even more attractive food group for promoting bone health. Despite accumulated evidence of a positive association between dietary dairy and BMD,[16–18] there is much debate about the long-term impact of dairy foods on fracture prevention. Studies that investigated the effects of total dairy consumption on preventing fractures have shown inconsistent results. Feart et al. reported that older adults (≥ 67 years old) who consumed low total dairy experienced a significantly higher risk of fracture at any site.[77] A 2018 study found one additional daily serving of total dairy, of which milk contributed about half, was associated with a statistically significant 6% lower risk of hip fracture among postmenopausal women in NHS I.[78] In contrast, a prospective study of elderly Europeans suggested that consumption of total dairy had no effect on hip fracture risk.[79] The inconsistent results of total dairy intake on fracture risk could be attributed to the possibility that individual dairy products may have different effects on fracture risk. A growing body of research has suggested that milk, yogurt, and cheese might play different roles in preventing fractures.[78,80,124] However, the reported effects of individual dairy products on fracture risk varied from study to study. For example, results from the FHS suggested that milk and yogurt intakes were positively associated with hip BMD, while cheese or cream intakes had no impact on hip BMD in middle-aged men and women (aged 55 years at baseline). This study also found a non-statistically significant protective effect of milk or yogurt intakes, but not cheese or cream intakes, against hip fracture.[124] In a large Swedish cohort study with more than 60,000 women, a dose-dependent positive association was found between the intake of milk and the risk of any fracture as well as hip fracture. Women who consumed ≥ 3 servings of milk per day experienced a 60% higher risk of hip fracture compared with those who consumed < 1 s/d. Interestingly, the authors also found that consumption of fermented dairy products (both yogurt and cheese) was associated with reduced hip fracture risk in the same study.[80] In contrast, previous results from post-menopausal women in NHS I failed to find a favorable effect of yogurt on the risk of hip fracture, but supported a reduced hip fracture risk associated with increased milk intake.[78] Given the highly varied results from previous research, it is hard to draw a conclusion on the association between total dairy as well as individual dairy products and fracture risk.

Moreover, most of the previous research focused solely on elderly or postmenopausal women, and many of them only examined risk of hip fracture.

Considering that the rate of fracture begins to increase in women in their early 40s, and that the effects of total dairy as well as individual dairy products on women are largely unknown, the current analyses aimed to investigate the association of total dairy as well as individual dairy products on with the risk of fracture in women ages 40 years and older. We further examined possible effect modification by menopausal status to account for the adverse effects of the drop in estrogen levels following menopause that is known to increase fracture risk.[63] Further, we examined the independent effects of total dairy and individual types of dairy, alone and in combination with baseline BMI, physical activity, and other dietary factors on fracture risk in women ages 40 and older from the NHS I cohort.

4.3 Methods

4.3.1 Population Characteristics

The NHS I was initiated in June of 1976 with 121,700 registered nurses between 30 to 55 years of age from the 11 most populous states (California, Connecticut, Florida, Maryland, Massachusetts, Michigan, New Jersey, New York, Ohio, Pennsylvania, and Texas). Nurses were selected because their health knowledge would help them to provide more accurate information about different diseases and lifestyle factors than women in general. The first questionnaire was sent in June of 1976 and participants were asked to provide information on their medical history, anthropometric levels, and lifestyle factors such as physical activity, smoking status, and alcohol use. Subsequent questionnaires were distributed every 2 years after 1976 to update lifestyle and medical information. FFQs, starting in 1980 and then repeated roughly every four years were used to collect information on how often the participant consumed each of 131 different types of food in the past year.

The current analysis included follow-up from 1980 through 2004, because 2004 was the last year in which wrist fractures were assessed. In these analyses, the baseline exam was defined as 1980 (if subjects reached 40 years old) or the first subsequent exam cycle when they were 40 years of age or older. Table 4.1 shows the details of exclusion. At the baseline (1980 or the exam when subjects first reached 40 years of age or older), women were excluded for the following reasons: were missing information of date of birth (N = 173), were Asian or black (N = 3,653), were missing heights (N = 147), failed to return any follow-up questionnaires (N = 1,037), died before or at baseline (N = 806), had prevalent fragility fractures before or at baseline (N = 648), had prevalent osteoporosis (N = 1,648), were missing information on dairy food intakes or consumed more than 6 s/d of dairy products (N = 12,149), were missing information of BMIs (N = 43), had extreme BMIs (≤ 16 kg/m^2 or ≥ 40 kg/m^2) (N = 660), were missing information of physical activity (N = 899), were missing information of menopausal status (N = 765). In total, 99,072 women contributed to the current study. The study protocol was approved by the Institutional Review Boards of the Brigham and Women's Hospital and the Harvard T.H. Chan School of Public Health. All analyses were approved by the Institutional Review Board of Boston University School of Medicine.

Table 4.1. The Exclusion of Fracture Analysis.

	Start with 121,700 subjects at baseline[1]	**Exclusion**	**Remaining N**
			121,700
1	Missing birthday	173	121,527
2	Asian or black	3,653	117,874
3	Missing heights	147	117,727
4	Failed to return any follow-up questionnaires	1,037	116,690
5	Death before or at baseline	806	115,884
6	Non-traumatic fractures before or at baseline	648	115,236
7	Osteoporosis before or at baseline	1,648	113,588
8	Missing dairy foods or dairy foods > 6 s/d	12,149	101,439
9	Missing BMIs	43	101,396
10	Extreme BMIs (≤ 16 kg/m^2 or ≥ 40 kg/m^2)	660	100,736
11	Missing activity	899	99,837
12	Missing menopause status	765	99,072
	Remaining N for analysis		***99,072***

4.3.2 Dietary Assessment

The FFQs were sent in 1980,1984,1986 and every 4 years thereafter. The FFQ asked about diet over the past year. There were nine possible response choices for each of 131 foods on the list, ranging from less than once per month ≥ 6 times per day. The nine categories of consumption included never or less than once per month, 1–3 per month, 1 per week, 2–4 per week, 5–6 per week, 1 per day, 2–3 per day, 4–5 per day, or ≥ 6 per day. We converted each food item to be on an appropriate continuous scale in serving per day, week, or month for the analysis. The following foods were included in total dairy consumption in our analysis: milk (whole, skim, low-fat and reduced fat), yogurt, and cheese (hard cheese, cottage, or ricotta cheese). Butter, cream, and cream cheese were excluded due to low calcium content. The standard serving size was 1 cup for milk and yogurt consumption, 1 slice for hard cheese, and 0.5 cup for cottage or ricotta cheese. Nutrient intakes such as protein, calcium, vitamin D from dairy and non-dairy source were derived from dairy and non-dairy food consumption reported in the FFQ. Supplement use of calcium and vitamin D were asked in biennial questionnaires. Subjects were asked about the frequency, dosage, and brand of the supplement they used; this information was linked with data files containing information on the nutrient content of each supplement brand to derive total supplemental nutrient intake.

AHEI scores were created for individual participants based on their reported intakes of foods and nutrients from each FFQ. The criteria and calculation of AHEI has been previously described in detail.[90] Dairy products were not a component of AHEI score. A previous study has confirmed reasonable accuracy of estimation of dairy consumption with the FFQ compared with a 7-day dietary record. Correlation coefficients between the FFQ and diet records for skim milk, whole milk, yogurt, cottage cheese and hard cheese were 0.81, 0.62, 0.94, 0.80, 0.57, respectively.[91]

4.3.3 Fracture Assessment

Cases in this study included the first occurrence of self-reported wrist fractures (fractures of the distal radius), self-reported hip fractures (fractures of the proximal femur), and medical record confirmed vertebral fractures that were not caused by high trauma events (e.g., skiing, horseback riding, motorized vehicle accidents). Women were asked about the life history of hip and wrist fractures (date, bone site, and circumstance leading to the fracture) in 1982 and incident hip or wrist fractures were reported in the biennial follow-up questionnaires. The last year in which incident wrist fractures were assessed was 2004. For hip or wrist fractures, we relied on self-reported data because as nurses, they were deemed to be likely to report these events accurately. A previous validation study of 30 reported hip or wrist fractures showed all to be confirmed by medical records.[125] Life history of vertebral fractures were first asked in 1988 and incident of vertebral fractures were assessed in the subsequent biennial questionnaires. Supplemental questionnaires were sent to participants who reported vertebral fractures to obtain permission to access their medical records to verify the fracture. For those who gave consent to obtain their medical records and their medical records were available, vertebral fractures were confirmed by radiology report (e.g., x-ray, computed tomography scan, or magnetic resonance imaging) or medical/surgical report (e.g., clinic visit note, operative note, hospital discharge summary). Only confirmed vertebral fractures were included in the analysis.

4.3.4 Assessment of Potential Confounding

Information about age, height, weight, menopausal status, use of post-menopausal hormones, smoking status, diagnosis of cancer, CVD, diabetes, and osteoporosis, use of thiazide diuretics, use of furosemide-type diuretics, use of oral steroids, and physical activity was collected from the self-reported biennial questionnaires. Age at each questionnaire was calculated by subtracting the date of birth from the date of return of questionnaire. Height was self-reported at the recruitment and weights were reported at each biennial questionnaire. Menopause status (as premenopausal, post-menopausal, and missing) and post-menopausal hormone use (as never, current, past user, and unknown) were asked on each biennial questionnaire. Smoking status (as missing, never smoker, past smoker, and current smoker) was asked in each questionnaire along with the number of cigarettes smoked per day among current smokers. Diagnoses of cancer, CVD, diabetes, and osteoporosis were assessed in each questionnaire. Use of thiazide diuretics was self-reported in 1980, 1982, 1988, and every questionnaire since 1994. Use of furosemide-type diuretics and use of oral steroids were reported since 1994. Physical activity was assessed on each biennial questionnaire by asking participants about the average time per week spent on specific activities such as jogging, running, biking, tennis, swimming, etc. We summed the hours per week (h/w) for each activity to derive total activity hours per week. on each biennial questionnaire.

4.3.5 Statistical Analysis

Participants contributed person-time from the return date of the 1980 questionnaire (for women ≥40 years of age at 1980) or the return date of the questionnaire of the exam when women became 40 years old or over. Follow-up for these women was censored at the first of the following dates: (1) the first occurrence of fracture (self-reported hip fracture, self-reported wrist fracture, or confirmed vertebral fracture), (2) death, or (3) end of follow up (June of 2004). Dairy food intakes were calculated as the mean of the two most recent FFQs because at least one previous study has shown that current diet has more important effect on fracture risk than earlier diet.[78] Participants in this study were required to have at least one of the two most recent FFQs to enter the study. Dairy intakes and covariates were updated over time and person-time were allocated to the appropriate category at the beginning of every 2-y follow-up cycle. Subjects did not contribute person-time in a questionnaire cycle in which they were missing information on dairy food intakes. Cox proportional hazards models were used to estimate the RR and 95% CI for fractures in each dietary intake category compared with those in the referent group (the lowest intake category). Two models were used. The basic models were adjusted for age and questionnaire cycle. Multivariable models were used to adjust for age, questionnaire cycle, total energy intake, BMI, height, physical activity, smoking status, use of postmenopausal hormones, diagnosis of cancer, diabetes or CVD, and each form of dairy was adjusted for intakes of the other forms of dairy. We also explored other potential confounders such as retinol from supplements, vitamin K, caffeine, alcohol, milk intake during teenage years, use of thiazide diuretics, use of furosemide-type diuretics, and use of oral steroids; these factors were not retained in the models because adding them to the models did not change the current result.

To test the combined effects of dairy foods intakes with other dietary factors (e.g., non-dairy source calcium, non-dairy source vitamin D, non-dairy source protein, and AHEI scores) and non-dietary factors (e.g., physical activity, BMI, and menopausal status), we cross-classified dairy intakes with these other factors. First, we dichotomized each factor at approximately the median value.[78] The dichotomized categories included the following: non-dairy calcium intake (< 500 mg/d vs. ≥ 500 mg/d), non-dairy vitamin D intake (< 6 µg/d vs. ≥ 6 µg/d), non-dairy protein intake (< 60 g/d vs. ≥ 60 g/d), AHEI scores (< 50 vs. ≥ 50), physical activity (< 2.5 h/w vs. ≥ 2.5 h/w), and menopausal status (pre-menopause vs. post-menopause). Intakes of dairy products were dichotomized as higher vs. lower as follows: total dairy (< 1 s/d vs. ≥ 1 s/d), milk (< 1 s/d vs. ≥ 1 s/d), yogurt (< 1 s/w vs. ≥ 1 s/w), and cheese (< 5 s/w vs. ≥ 5 s/w). Subjects were then divided into four groups based on these dichotomized categories. For example, the combined categories of total dairy and physical activity were as follows: (1) lower physical activity (< 2.5 h/w) & lower total dairy intake (< 1 s/d), (2) lower physical activity (< 2.5 h/w) & higher total dairy intake (≥ 1 s/d), (3) higher physical activity (≥ 2.5 h/w) & lower total dairy intake (< 1 s/d), and (4) higher physical activity (≥ 2.5 h/w) & higher total dairy intake (≥ 1 s/d).

Statistical significance was set at a two-tailed $p < 0.05$. SAS version 9.4 (SAS Institute) was used to analyses the data.

4.4 Results

In total, 99,072 women aged 40 years or older were included in the current study. In the maximum follow-up time of 24 years (median 15.2 years), there were 5,348 fracture events. The characteristics of subjects at baseline were presented in Table 4.2. Women consuming more total dairy intake were more physical active, consumed more total calories, had lower AHEI scores but consumed more fruits and vegetables; they were also less likely to be current smokers. Women who consumed more total dairy also tended to consume more protein from non-dairy sources. Non-dairy calcium in particular was observed to rise along with the increasing level of total dairy intake. The total intakes of calcium and vitamin D in the highest dairy group were considerably higher than that of the lowest category. Women with higher total dairy intake consumed more milk, yogurt, and cheese.

Table 4.2. Diet and Lifestyle Characteristics of Women by Categories of Total Dairy Intake at Baseline[1].

	< 1 s/w	1 s/w	2 – 4 s/w	5 – 6 s/w	1 s/d	≥ 2 s/d
N	2152	7311	13,811	9985	38,261	27,552
			Mean (SD)			
Age (years)	51.1 (8.2)	49.3 (7.3)	49.2 (7.3)	49.3 (7.2)	49.4 (7.1)	49.7 (7.1)
Height (cm)	163.1 (6.4)	163.3 (6.2)	163.6 (6.0)	163.7 (6.1)	163.9 (6.1)	164.2 (6.2)
BMI (kg/m^2)	24.4 (4.4)	24.7 (4.3)	24.7 (4.3)	24.8 (4.3)	24.8 (4.3)	25.0 (4.4)
Activity (hr/week)	2.7 (2.5)	2.8 (2.5)	3.0 (2.6)	3.2 (2.6)	3.4 (2.6)	3.5 (2.7)
AHEI2010	42.7 (12.1)	41.6 (10.9)	40.8 (10.8)	40.5 (10.9)	39.6 (11.1)	37.9 (11.3)
Energy intake (kcal/d)	1316.0 (501.6)	1336.6 (473.5)	1436.2 (472.7)	1497.3 (470.8)	1613.7 (485.9)	1887.5 (511.5)
Dairy protein (g/d)	2.5 (3.1)	4.1 (3.0)	6.6 (2.8)	9.3 (2.5)	13.9 (3.4)	27.7 (7.7)
Non-dairy protein (g/d)[2]	54.2 (24.0)	53.8 (21.4)	56.5 (20.6)	58.0 (20.9)	60.3 (21.3)	64.5 (22.5)
Dairy calcium (mg/d)	78.7 (101.2)	113.8 (99.2)	185.0 (88.7)	254.0 (84.3)	395.4 (106.2)	829.1 (255.1)
Non-dairy calcium (mg/d)[2]	362.8 (344.0)	327.7 (287.1)	338.0 (275.0)	348.7 (279.0)	359.5 (267.9)	390.7 (265.9)
Dairy vitamin D (µg/d)	0.4 (0.9)	0.5 (0.9)	0.7 (0.8)	1.0 (0.8)	2.0 (1.0)	5.1 (2.6)
Non-dairy vitamin D (µg/d)[2]	5.2 (6.0)	4.7 (5.5)	4.9 (5.4)	5.2 (5.8)	5.7 (5.7)	6.3 (6.4)
Total dairy (s/d)	0.0 (0.0)	0.2 (0.1)	0.5 (0.1)	0.8 (0.1)	1.4 (0.3)	3.0 (0.8)
Yogurt (s/w)	0.0 (0.1)	0.1 (0.2)	0.2 (0.5)	0.4 (0.8)	0.7 (1.3)	1.2 (2.3)
Milk (s/d)	0.0 (0.0)	0.0 (0.1)	0.1 (0.2)	0.3 (0.2)	0.7 (0.4)	1.8 (1.0)
Cheese (s/d)	0.0 (0.0)	0.1 (0.1)	0.3 (0.2)	0.5 (0.3)	0.6 (0.4)	1.0 (0.8)
Red & processed meat (s/d)	0.8 (0.7)	0.9 (0.6)	1.0 (0.6)	1.0 (0.6)	1.0 (0.6)	1.0 (0.6)
Fruits and vegetables (s/d)	3.4 (2.5)	3.3 (2.1)	3.6 (2.0)	3.8 (2.1)	4.2 (2.1)	4.8 (2.4)
Current smoker (%)	31.6	33.7	29.2	27.6	24.6	23.6
Premenopausal (%)	44.3	53.3	53.9	53.7	52.8	50.7

Abbreviations: AHEI, alternative healthy eating index 2010; BMI, body mass index; SD, standard deviation; s/d, serving per day; s/w, serving per week.

[1] Baseline is 1980 or the first subsequent exam cycle when they were 40 years of age or older

[2] Include intakes from both non-dairy food source and supplements

Table 4.3. Relative Risk (95% CI) of Fragility Fractures in Women Aged 40 Years and Older According to Intakes of Dairy Products.

Variables	Fractures	Rate[1]	RR (95% CI) Basic Model[2]	RR (95% CI) Multi-variable Model[3]
Total dairy				
< 1 s/w	125	429	1 (Reference)	1 (Reference)
1 s/w	329	391	0.98 (0.80 - 1.20)	1.00 (0.81 - 1.23)
2 - 4 s/w	861	369	0.90 (0.75 - 1.09)	0.94 (0.77 - 1.13)
5 - 6 s/w	756	378	0.93 (0.77 - 1.12)	0.96 (0.79 - 1.16)
1 s/d	2024	347	0.85 (0.71 - 1.02)	0.88 (0.73 - 1.06)
≥ 2 s/d	1253	330	0.80 (0.67 - 0.96)	0.81 (0.67 - 0.98)
per 1 day			*0.95 (0.93 - 0.98)*	*0.95 (0.92 - 0.98)*
Milk				
< 1 s/w	1209	362	1 (Reference)	1 (Reference)
1 s/w	645	382	1.03 (0.94 - 1.13)	1.04 (0.95 - 1.15)
2 - 4 s/w	1056	350	0.92 (0.84 - 0.99)	0.93 (0.86 - 1.01)
5 - 6 s/w	586	363	0.95 (0.86 - 1.04)	0.96 (0.87 - 1.07)
1 s/d	1265	344	0.90 (0.83 - 0.98)	0.91 (0.84 - 0.98)
≥ 2 s/d	587	331	0.86 (0.78 - 0.95)	0.86 (0.77 - 0.95)
per 1 day			*0.94 (0.91 - 0.98)*	*0.94 (0.91 - 0.97)*
Yogurt				
< 1 s/m	2605	373	1 (Reference)	1 (Reference)
1 s/m-< 1 s/w	1329	329	0.91 (0.85 - 0.97)	0.93 (0.87 - 1.00)
1 s/w	702	340	0.95 (0.87 - 1.03)	0.97 (0.89 - 1.05)
2 - 4 s/w	518	350	0.98 (0.89 - 1.08)	1.00 (0.91 - 1.10)
≥ 5 s/w	194	364	1.03 (0.89 - 1.19)	1.02 (0.88 - 1.18)
per 1 day			*1.03 (0.91 - 1.16)*	*1.03 (0.91 - 1.16)*
Cheese				
< 1 s/w	748	403	1 (Reference)	1 (Reference)
1 s/w	1263	355	0.95 (0.87 - 1.04)	0.97 (0.89 - 1.07)
2 - 4 s/w	2038	354	0.95 (0.87 - 1.03)	0.98 (0.90 - 1.07)
5 - 6 s/w	725	339	0.92 (0.83 - 1.02)	0.95 (0.85 - 1.05)
≥ 1 s/d	574	319	0.89 (0.80 - 1.00)	0.91 (0.81 - 1.02)
per 1 day			*0.95 (0.89 - 1.01)*	*0.95 (0.89 - 1.01)*

Abbreviations: CI, confidence interval; RR, relative risk; s/d, serving per day; s/m, serving per month; s/w, serving per week.
[1] Rate is per 100k person years.
[2] Model adjusted for age, questionnaire cycle.
[3] Model adjusted for age, questionnaire cycle, total energy intake, body mass index, height, physical activity, smoking, menopause status and use of postmenopausal hormones, diagnosis of cancer, diabetes, and cardiovascular disease, (yogurt, cheese and milk were adjusted for each other).

In Table 4.3, total dairy and each type of dairy food is first classified into six categories. At the end of each section, the linear regression coefficient for each continuous dairy variable is then given. The number of fractures and rate of fracture per category of intake are shown in the first two data columns followed by the effect estimates for the relative risks (and 95% CIs) for fracture in the last two data columns. In both the basic model and the multivariable model, both total dairy and milk consumptions were significantly associated with lower risks of fracture as intake levels increased. In the highest intake categories for both total dairy and milk, consuming two or more servings per day was associated with statistically significant 19% and 14% reductions in fracture risk, respectively, in the multivariable models. There was no clear association between yogurt consumption and fracture risk in this cohort while for cheese, there were weak, non-statistically significant inverse associations observed. For the continuous analyses, each additional serving of total dairy per day was linked with a statistically significantly 5% lower (95% CI: 0.92 - 0.98) facture risk, while one additional serving of milk per day was associated with a 6% (95% CI: 0.91 - 0.97) lower facture risk. Once again, yogurt was not associated with fracture risk.

Table 4.4. Possible Effect Modification of Total Dairy and Milk by Other Dietary Factors on Risk of Fragility Fractures in Women Aged 40 Years and Older.

	TOTAL DAIRY INTAKE			
	< 1 s/d	≥ 1 s/d	< 1 s/d	≥ 1 s/d
	Case		RR (95% CI)[1]	
NON-DAIRY CALCIUM				
Low (< 500 mg/d)	1,303	1,860	1.00 (Ref)	0.88 (0.82 - 0.95)
High (≥ 500 mg/d)	768	1,417	0.95 (0.87 - 1.04)	0.88 (0.81 - 0.96)
NON-DAIRY VIT D				
Low (< 6 µg/d)	1,249	1,747	1.00 (Ref)	0.89 (0.83 - 0.96)
High (≥ 6 µg/d)	822	1,530	0.99 (0.91 - 1.08)	0.90 (0.83 - 0.97)
NON-DAIRY PROTEIN				
Low (< 60 g/d)	1,245	1,490	1.00 (Ref)	0.90 (0.83 - 0.97)
High (≥ 60 g/d)	826	1,787	1.02 (0.93 - 1.13)	0.92 (0.84 - 1.01)
AHEI SCORES				
Low AHEI (< 50)	1,064	1,685	1.00 (Ref)	0.91 (0.84 - 0.98)
High AHEI (≥ 50)	1,007	1,592	1.01 (0.92 - 1.10)	0.90 (0.82 - 0.97)
	MILK INTAKE			
	< 1 s/d	≥ 1 s/d	< 1 s/d	≥ 1 s/d
	Case		RR (95% CI)[2]	
NON-DAIRY CALCIUM				
Low (< 500 mg/d)	2,077	1,086	1.00 (Ref)	0.91 (0.84 - 0.98)
High (≥ 500 mg/d)	1,419	766	0.98 (0.91 - 1.05)	0.90 (0.82 - 0.98)
NON-DAIRY VIT D				
Low (< 6 µg/d)	1,980	1,016	1.00 (Ref)	0.94 (0.87 - 1.01)
High (≥ 6 µg/d)	1,516	836	1.02 (0.95 - 1.09)	0.90 (0.83 - 0.98)
NON-DAIRY PROTEIN				
Low (< 60 g/d)	1,877	858	1.00 (Ref)	0.93 (0.86 - 1.01)

High (≥ 60 g/d)	1,619	994	1.04 (0.96 - 1.12)	0.93 (0.84 - 1.02)
AHEI SCORES				
Low AHEI (< 50)	1,748	1,001	1.00 (Ref)	0.96 (0.89 - 1.04)
High AHEI (≥ 50)	1,748	851	1.04 (0.96 - 1.11)	0.89 (0.81 - 0.97)

Abbreviations: AHEI, alternative healthy eating index 2010; CI, confidence interval; RR, relative risk; s/d, serving per day; Vit D, vitamin D.
[1] Model adjusted for age, questionnaire cycle, total energy intake, body mass index, height, physical activity, smoking status, menopause status and use of postmenopausal hormones, cancer, diabetes, and cardiovascular disease.
[2] Model adjusted for age, questionnaire cycle, total energy intake, body mass index, height, physical activity, smoking status, menopause status and use of postmenopausal hormones, cancer, diabetes, cardiovascular disease, yogurt, and cheese.

To explore potential effect modification, we estimated first the independent and combined effects of total dairy as well as individual dairy types with other dietary factors (Table 4.4). Higher calcium from non-dairy sources was not, by itself, associated with reduced risk of fracture. However, greater total dairy intake (≥ 1s/d) was associated with 12% significantly less fracture risk, regardless of the level of calcium consumed from other sources. Similar results were observed with other dietary factors, including non-dairy sources of vitamin D, non-dairy source protein, and AHEI scores. There were no independent effects of these variables on fracture risk and none of them altered the favorable effect of total dairy consumption on fracture risk.

Table 4.4 also shows that women who consumed more than 1 s/d of milk had approximately 10% lower fracture risks than those who consumed less than 1 serving per day, independent of the intake of non-dairy calcium. Women with both higher level of vitamin D from non-dairy sources and higher intakes of milk tended to have a slightly greater reduction (RR: 0.90; 95% CI: 0.83 - 0.98) in fracture risk than higher milk intake alone (RR: 0.94; 95% CI: 0.87 - 1.01). Levels of non-dairy source protein did not modify the association between milk intake and fracture risk. Finally, in the analysis of AHEI scores, higher milk consumption was not associated (RR: 0.96; 95% CI: 0.89 - 1.04) with fracture risk in women with a less healthy diet, but the combination of higher milk intake and a better overall diet was associated with a statistically significant 11% (95% CI: 0.81 - 0.97) reduction in risk of developing fracture.

We also explored the effects of total dairy and milk intakes in combination with non-dietary risk factors including BMI, physical activity, and menopausal status. These results are shown in Table 4.5.

Table 4.5. Possible Effect Modification of Total Dairy and Milk by Non-Dietary Factors on Risk of Fragility Fractures in Women Aged 40 Years and Older.

	TOTAL DAIRY INTAKE			
	< 1 s/d	≥ 1 s/d	< 1 s/d	≥ 1 s/d
	Case		RR (95% CI)[1]	
ACTIVITY LEVEL				
Low (< 2.5 h/w)	1,288	1,773	1.00 (Ref)	0.89 (0.83 - 0.96)
High (≥ 2.5 h/w)	783	1,504	1.00 (0.92 - 1.10)	0.91 (0.84 - 0.98)
BMI				
< 25 kg/m^2	1,151	1,755	1.00 (Ref)	0.88 (0.81 - 0.95)
≥ 25 kg/m^2	920	1,522	0.78 (0.71 - 0.85)	0.71 (0.65 - 0.77)
MENOPAUSAL STATUS				
Post-menopause	1,900	3,000	1.00 (Ref)	0.89 (0.84 - 0.95)
Pre-menopause	171	277	0.47 (0.39 - 0.56)	0.39 (0.33 - 0.46)
	MILK INTAKE			
	< 1 s/d	≥ 1 s/d	< 1 s/d	≥ 1 s/d
	Case		RR (95% CI)[2]	
ACTIVITY LEVEL				
Low (< 2.5 h/w)	2,044	1,017	1.00 (Ref)	0.92 (0.85 - 1.00)
High (≥ 2.5 h/w)	1,452	835	1.02 (0.96 - 1.10)	0.92 (0.84 - 1.00)
BMI				
< 25 kg/m^2	1,930	976	1.00 (Ref)	0.89 (0.82 - 0.96)
≥ 25 kg/m^2	1,566	876	0.78 (0.73 - 0.83)	0.73 (0.67 - 0.79)
MENOPAUSAL STATUS				
Post-menopause	3,216	1,684	1.00 (Ref)	0.90 (0.85 - 0.96)
Pre-menopause	280	168	0.43 (0.37 - 0.50)	0.44 (0.36 - 0.53)

Abbreviations: BMI, body mass index; CI, confidence interval; RR, relative risk; s/d, serving per day.

[1] Model adjusted for age, questionnaire cycle, total energy intake, BMI (dropped in BMI analysis), height, physical activity (dropped in activity analysis), smoking status, menopause status and use of postmenopausal hormones, cancer, diabetes, and cardiovascular disease.

[2] Model adjusted for age, questionnaire cycle, total energy intake, BMI (dropped in BMI analysis), height, physical activity (dropped in activity analysis), smoking status, menopause status and use of postmenopausal hormones, cancer, diabetes and cardiovascular disease, yogurt, and cheese.

Physical activity itself was not associated with fracture risk, and it did not alter the relation between fracture risk and the intake of total dairy or milk in Table 4.5. Women who were overweight (BMI ≥ 25 kg/m^2) had a 22% lower risk of fracture even when they had low intakes of milk or total dairy. However, in this analysis, the women with the lowest fracture risk were those who were overweight and who consumed at least 1 s/d of total dairy (or milk). Post-menopausal women had more than twice the fracture risk of premenopausal women. Consuming more total dairy (RR: 0.89; 95% CI: 0.84 - 0.95) or milk (RR: 0.90; 95% CI: 0.85 - 0.96) was associated with significantly reduced fracture risk in postmenopausal women.

Table 4.6. Possible Effect Modification of Yogurt and Cheese by Dietary Factors on Risk of Fragility Fractures in Women Aged 40 Years and Older.

	YOGURT INTAKE			
	< 1 s/w	≥ 1 s/w	< 1 s/w	≥ 1 s/w
	Case		RR (95% CI)[1]	
NON-DAIRY CALCIUM				
Low (< 500 mg/d)	2,489	674	1.00 (Ref)	1.03 (0.94 - 1.12)
High (≥ 500 mg/d)	1,445	740	0.99 (0.92 - 1.06)	0.98 (0.90 - 1.07)
NON-DAIRY VIT D				
Low (< 6 µg/d)	2,365	631	1.00 (Ref)	1.00 (0.91 - 1.09)
High (≥ 6 µg/d)	1,569	783	0.99 (0.93 - 1.06)	1.01 (0.93 - 1.10)
NON-DAIRY PROTEIN				
Low (< 60 g/d)	2,075	660	1.00 (Ref)	1.05 (0.96 - 1.14)
High (≥ 60 g/d)	1,859	754	1.03 (0.96 - 1.12)	1.01 (0.92 - 1.12)
AHEI SCORES				
Low AHEI (< 50)	2,223	526	1.00 (Ref)	0.99 (0.90 - 1.09)
High AHEI (≥ 50)	1,711	888	0.98 (0.92 - 1.05)	1.01 (0.93 - 1.10)
	CHEESE INTAKE			
	< 5 s/w	≥ 5 s/w	< 5 s/w	≥ 5 s/w
	Case		RR (95% CI)[2]	
NON-DAIRY CALCIUM				
Low (< 500 mg/d)	2,386	777	1.00 (Ref)	0.94 (0.86 - 1.02)
High (≥ 500 mg/d)	1,663	522	0.98 (0.91 - 1.04)	0.94 (0.85 - 1.04)
NON-DAIRY VIT D				
Low (< 6 µg/d)	2,275	721	1.00 (Ref)	0.94 (0.86 - 1.02)
High (≥ 6 µg/d)	1,774	578	0.99 (0.93 - 1.06)	0.96 (0.87 - 1.05)
NON-DAIRY PROTEIN				
Low (< 60 g/d)	2,231	504	1.00 (Ref)	0.92 (0.83 - 1.01)
High (≥ 60 g/d)	1,818	795	1.01 (0.93 - 1.09)	0.98 (0.89 - 1.08)

AHEI SCORES				
Low AHEI (< 50)	1,990	759	1.00 (Ref)	0.95 (0.87 - 1.03)
High AHEI (≥ 50)	2,059	540	1.00 (0.93 - 1.07)	0.95 (0.86 - 1.05)

Abbreviations: AHEI, alternative healthy eating index 2010; CI, confidence interval; RR, relative risk; s/d, serving per day; Vit D, vitamin D.
[1] Model adjusted for age, questionnaire cycle, total energy intake, body mass index, height, physical activity, smoking status, menopause status and use of postmenopausal hormones, cancer, diabetes, cardiovascular disease, milk, and cheese intake.
[2] Model adjusted for age, questionnaire cycle, total energy intake, body mass index, height, physical activity, smoking status, menopause status and use of postmenopausal hormones, cancer, diabetes, cardiovascular disease, milk, and yogurt intake.

We next examined the same stratification analyses with yogurt and cheese. In Table 4.6, we failed to find any beneficial effects of higher yogurt intakes. Nor did we find any modification of yogurt consumption by other dietary factors. Similarly, higher cheese intake failed to lower fracture risk, regardless of the additional intakes of non-dairy calcium, vitamin D, or protein. Further, there was no effect modification by AHEI scores in these analyses.

Table 4.7. Possible Effect Modification of Yogurt and Cheese by Non-Dietary Factors on Risk of Fragility Fractures in Women Aged 40 Years and Older.

	YOGURT INTAKE			
	< 1 s/w	≥ 1 s/w	< 1 s/w	≥ 1 s/w
	Case		RR (95% CI)[1]	
ACTIVITY LEVEL				
Low (< 2.5 h/w)	2,332	729	1.00 (Ref)	1.00 (0.92 - 1.09)
High (≥ 2.5 h/w)	1,602	685	1.01 (0.94 - 1.07)	1.03 (0.94 - 1.13)
BMI				
< 25 kg/m^2	2,157	749	1.00 (Ref)	0.99 (0.91 - 1.07)
≥ 25 kg/m^2	1,777	665	0.78 (0.73 - 0.83)	0.82 (0.75 - 0.89)
MENOPAUSAL STATUS				
Post-menopause	3,597	1,303	1.00 (Ref)	1.00 (0.93 - 1.06)
Pre-menopause	337	111	0.44 (0.38 - 0.51)	0.47 (0.38 - 0.58)
	CHEESE INTAKE			
	< 5 s/w	≥ 5 s/w	< 5 s/w	≥ 5 s/w
	Case		RR (95% CI)[2]	
ACTIVITY LEVEL				
Low (< 2.5 h/w)	2,368	693	1.00 (Ref)	0.99 (0.91 - 1.08)
High (≥ 2.5 h/w)	1,681	606	1.04 (0.98 - 1.11)	0.93 (0.85 - 1.03)
BMI				
< 25 kg/m^2	2,198	708	1.00 (Ref)	0.94 (0.86 - 1.02)
≥ 25 kg/m^2	1,851	591	0.79 (0.74 - 0.84)	0.76 (0.69 - 0.83)
MENOPAUSAL STATUS				
Post-menopause	3,718	1,182	1.00 (Ref)	0.97 (0.90 - 1.04)
Pre-menopause	331	117	0.48 (0.41 - 0.56)	0.36 (0.29 - 0.44)

Abbreviations: BMI, body mass index; CI, confidence interval; RR, relative risk; s/d, serving per day.

[1] Model adjusted for age, questionnaire cycle, total energy intake, BMI (dropped in BMI analysis), height, physical activity (dropped in activity analysis), smoking status, menopause status and use of postmenopausal hormones, cancer, diabetes, cardiovascular disease, milk, and cheese intake.

[2] Model adjusted for age, questionnaire cycle, total energy intake, BMI (dropped in BMI analysis), height, physical activity (dropped in activity analysis), smoking status, menopause status and use of postmenopausal hormones, cancer, diabetes, cardiovascular disease, milk, and yogurt intake.

With the stratification by non-dietary factors (Table 4.7), there was no effect on fracture risk observed for yogurt consumption. There may, however, be some very weak effect modification of cheese consumption by some non-dietary factors. For example, the combination of higher physical activity and higher intake of cheese was associated with a non-statistically significant 7% (95% CI: 0.85 - 1.03) lower risk of developing fracture (an effect that although weak, was higher than either independent effect alone). Secondly, Table 4.7 shows a weak protective effect (RR: 0.94; 95% CI: 0.86 - 1.02) of cheese intake on fracture risk in non-overweight women. Finally, we stratified by menopausal status. Here, postmenopausal women had a 52% lower risk of fracture than premenopausal women. When higher intakes of cheese intake were added in these postmenopausal women, fracture risk was reduced by 64%.

4.5 Discussion

The current study demonstrated that higher intakes of both total dairy and milk were associated with lower risk of fragility fracture in women aged 40 years or older with up to 24 years of follow-up (median 15.2 years) in NHS I. The beneficial effects were independent of the intakes of calcium, vitamin D, or protein from non-dairy sources. There was no association observed between yogurt intake and fracture risk, but a weak non-statically significant inverse association between cheese intake and fracture risk.

Three previous studies have examined the role of dairy products consumption on hip fracture in women from NHS I. The earliest study was conducted in 1997 with 12 years of follow-up (median: 8.3 years) of women aged 34 to 59 years at baseline.[126] This study concluded that higher consumption of milk did not prevent hip or wrist fracture. In fact, women who consumed more than 2 servings of milk per day showed a non-statistically significant 45% (RR: 1.45, 95% CI: 0.87 - 2.43) higher risk of hip fracture than those who consumed less than 1 serving per week. However, the study was limited by the small number of fracture cases because the cohort was still relatively young at the end of the 12 years follow-up (48 to 71 years old). As the authors mentioned in the discussion, the results were most directly applicable to younger middle-aged women only. In 2003, a second study investigated the effects of calcium and milk, as well as vitamin D on fracture risk, but this study focused only on the hip fracture in postmenopausal women with 18 years follow-up.[75] Still, there was no evidence showing that milk consumption conferred benefits to the prevention of hip fracture, and the effects of calcium intake on hip fracture risk were also null. However, there was a statistically significant inverse association between vitamin D intake and hip fracture among postmenopausal women. Finally, a 2018 paper revisited the effect of milk intakes on hip fracture in postmenopausal women from NHS I. [78] This study extended the follow-up duration to 32 years and examined the effects of not only milk but also total dairy, yogurt, and cheese intakes on hip fracture. This study revealed an 8% (RR: 0.92, 95% CI: 0.86 - 0.97) lower hip fracture risk associated with each additional daily serving of milk. Postmenopausal women who consumed more than 2 servings of milk per day had a 23% (RR: 0.77, 95% CI: 0.64 - 0.94) lower hip fracture risk than women who only consumed less than 1 serving of milk per week. Meanwhile, each additional serving of total dairy was also found to reduce the risk of hip fracture risk by 7% (RR: 0.93, 95% CI: 0.88 - 0.98). The risk reductions associated with milk and total dairy were not altered by the intake of calcium, vitamin D, or protein from non-dairy sources. A non-statistically significant inverse association was observed between hip fracture and higher cheese intake, but no association was found between yogurt and hip fracture.

Our current analysis expands upon earlier NHS I work by including women aged 40 years and older and fragility fractures at three sites, instead of just using hip fracture. Our findings were in agreement with the 2018 NHS I study, and further supported the beneficial effects of milk and total dairy intakes on risk of fracture in women aged 40 years or over. The null effects of milk consumption on hip fracture in the first two NHS I research might be due to the shorter follow-up time and small number of cases. Because 2004 was the last year asking about wrist fracture, we stopped the follow-up in 2004, which different from the 2018 NHS I paper which stopped follow-up in 2012. Still, the follow-up time in the current analysis (24 years) was still longer than that of the 1997 (12 years) and

2003 (18 years) NHS I analysis. Moreover, the total number of cases in the current analysis was much higher (5348 cases) than any of the three previous NHS I research (the highest one had 2138 cases), which gave us greater statistical power.

Another important difference between the current analysis and the 2018 NHS I hip fracture research was the inclusion of younger women who were still premenopausal. At baseline, approximately 50% of the women in the current analysis were still premenopausal. Studies have suggested that postmenopausal women have much larger risk of osteoporosis and fragility fracture than premenopausal women.[65] Therefore, most studies have focused solely on fracture risk in women after menopause. However, the incidence of fracture begins to rise in younger middle-aged women as early as in their 40s.[11] Although the absolute BMD value in premenopausal women was higher than that of postmenopausal women, research has shown that there is a significant loss of BMD by 0.25 % to 1 % per year in premenopausal women, leading to osteopenia.[127] Osteopenia is an earlier stage osteoporosis, which is defined as BMD between 1 to 2.5 standard deviation below the mean at the femoral neck or total femur. Osteopenia is commonly seen in an early middle-aged population and contributes to fragility fracture. A cross-sectional study reported that in people between 35 to 50 years, 28% of men and 26% women had osteopenia.[128] Another study found that in women between 40 and 49 years of age, the prevalence of osteopenia was as high as that for women in older age groups.[61]

Osteopenia contributes to a considerable number of fractures in women. Real-world data indicates that osteopenia contributes to 34.9% of wrist fractures and 27% of hip fractures in women.[61] Moreover, premenopausal fractures are associated with a 1.5 to 3.0-fold increase in the risk of postmenopausal fractures.[127] Therefore, identification of early interventions in younger middle-aged women presents a unique opportunity to prevent irreversible bone loss and minimize future fracture risk. As expected, our analysis indicated that premenopausal women had a considerably lower absolute risk of fracture than postmenopausal women. In postmenopausal women, consuming at least 1 s/d of total dairy intake was associated with an 11% (95% CI: 0.84 - 0.95) lower risk of fracture. Further, dairy intake in premenopausal women was also associated with a lower fracture risk, a finding that extended the results of the 2018 NHS I research, where the effects of total dairy were examined only for the hip fracture outcome and only in postmenopausal women. Therefore, these current analyses support that higher total dairy intake not only helps to prevent fracture risk in postmenopausal women, but also provides protection for premenopausal women. In addition, these results suggest that cheese intake may also lower fracture risk in premenopausal women.

Milk is the most frequently consumed dairy products in the US and it is a major source of bone-beneficial nutrients. Although the effect of milk consumption alone on fracture has been extensively studied, no consistent conclusions have been drawn. Several prior meta-analyses tended to suggest a null effect between milk intake and fracture risk.[129,130] A more recent meta-analysis hypothesized that the effect of milk consumption on fracture risk might depend on vitamin D fortification policies.[131] Hidayat et al. conducted a comprehensive meta-analysis of 14 study cohorts and found that even though the pooled analysis of all studies did not show a reduction in fracture risk associated with milk intake, the pooled analysis for those studies conducted in US cohorts found a statistically significant (RR: 0.93; 95% CI: 0.88 - 0.98) lower risk of hip fracture associated with one additional daily serving of milk.[131] In contrast, the pooled analysis in Scandinavian populations failed to find any beneficial effects of milk intake, a finding that the authors hypothesized could be due to the higher prevalence of vitamin D fortification in the US compared with Scandinavian countries. Vitamin D is an important nutrient for bone health and maintenance, as it promotes calcium absorption in the intestine and helps to maintain an adequate level of serum calcium and phosphate to ensure proper bone mineralization.[72] Vitamin D deficiency can lead to impaired calcium absorption, causing osteoporosis and fractures. Unfortunately, it is reported that around 42% of the US population is affected by Vitamin D deficiency.[73] Although Vitamin D can be synthesized endogenously by sunlight exposure, people currently spend much less time outdoors due to our modern lifestyle. Because vitamin D is rarely present in natural food sources, the most common source of Vitamin D is fortified foods, especially fortified milk. Since the 1930s, almost all the milk in the US has been fortified with about 3 ug/cup of vitamin D. Evidence from a meta-analysis with 47 randomized clinical trials supported the finding that vitamin D plus calcium supplement significantly reduced fracture risk by 16%.[74] Previous NHS I research also found that high calcium alone did not reduce fracture risk, whilst high vitamin D combined with high calcium was statistically significantly inversely associated with hip fracture.[75] Since milk in the US is mostly fortified with vitamin D, this may explain why it could lower fracture risk in the US but not in countries without vitamin D fortification. In our secondary analysis, we found that women with higher intakes of milk had a reduced risk of fracture regardless of the level of non-dairy vitamin D or calcium. We hypothesized that is because milk in the US already contains both vitamin D and calcium. The 2018 NHS I also found that calcium, vitamin D, and protein from non-dairy sources did not alter the benefits of milk consumption on hip fracture.[78] In our analysis, we did not find any significant protective effect against fracture of non-dairy calcium alone.

While calcium supplementation or dietary calcium has been previously thought to improve bone health and prevent fracture, results from longitudinal studies have failed to support this claim,[68–70] including prior NHS I studies.[75,126] A 2007 meta-analysis found no association between total calcium intake and hip fracture in women or men based on the pooled-results from 8 prospective studies, it even found an adverse effect of calcium supplementation on hip fracture risk based on the pooled analysis of randomized clinical trials.[68] A more recent meta-analysis which summarized the findings from 44 cohort studies and

26 randomized clinical trials, also concluded that neither dietary calcium intake nor calcium supplements had protective effects against fracture.[132] There are several possible explanations. First, the absorption of calcium depends on adequate level of vitamin D. If the vitamin D level is insufficient, higher calcium may not exert its benefits. In fact, a meta-analysis has confirmed a lower risk of hip fracture when calcium is combined with vitamin D.[71] Another reason might be the impaired phosphate absorption following high calcium intake may lead to an unbalanced ratio of calcium to phosphate and thereby cause bone loss. Furthermore, baseline calcium levels of the participants may also affect the results as it has been shown that women with low calcium level at baseline have a reduced risk of fracture associated with the use of calcium supplements.[75,133]

We also observed no benefit of non-dairy protein in this study. Evidence from other research has also suggested that there was no association between dietary protein intake and relative risk of frailty fractures,[134] a finding that is in concordance with our study. Fung et al. found that women who had an AHEI score in the top quintile had a statistically significant 27% lower risk of hip fracture than women in the bottom quintile of AHEI, but this benefit was only observed in women less than 75 years of age.[135] In the study of Fung et al., women with higher AHEI scores were also had higher milk intakes, which could contribute to the lower fracture risk. Our analysis showed that there was no reduction in fracture risk among women who had higher AHEI alone (combined with lower total dairy or milk intakes), which may suggest that it is important to incorporate total dairy or milk into an overall healthy diet in order to lower fracture risk.

Physical activity was an important modifiable factor associated with fracture. In the Women's Health Initiative prospective cohort, investigators found that higher physical activity levels were associated with lower total and hip fracture risk in postmenopausal women.[136] Another large national cohort of Korean adults aged ≥ 40 years, reported that compared with individuals who continuously lacked physical activity, those with higher physical activity levels had 11% (RR: 0.89; 95% CI: 0.88 - 0.90) lower risks of developing fracture.[137] However, women who had higher activity but not consume higher total dairy or milk failed to demonstrate any reduction in fracture risk in the current analysis, and physical activity did not seem to modify the effects of total dairy or milk on fracture risk. This may suggest that consuming adequate total dairy (or milk) may be superior to physical activity alone as a strategy for preventing fracture risk among women.

Both our study and the 2018 NHS I paper on hip fracture failed to find a statistically significant protective effect of yogurt or cheese consumption against fracture. However, both of these analyses tended to support a non-statistically significant inverse association between cheese and fracture risk. Due to the presence of probiotics, fermented dairy products were considered to have additional bone-friendly benefits. The probiotics were reported to promote bone health by improving the intestinal barrier function, reducing a variety of pro-inflammatory biomarkers, and modulating gut microbiota.[81,82] In the Swedish Mammography Cohort study, Michaëlsson et al. reported that women who consumed more than 400 g of yogurt per day showed a 30% (RR: 0.70; 95% CI: 0.57 - 0.86) lower risk of hip fracture than women who rarely consumed yogurt. However, but no statistically significant reduction was found for total fracture risk (RR: 0.93; 95% CI: 0.84 - 1.03). This study also reported that consumption of cheese more than 60 g daily was associated with 11% (RR: 0.89; 95% CI: 0.83 - 0.94) less risk of any fracture, and 36% (RR: 0.64; 95% CI: 0.55 - 0.74) less risk of hip fracture.[80] In the FHS study, Sahni et al. reported that yogurt intake was positively associated with trochanter-BMD, but it had no protective effect against hip fracture. However, that study did not observe any association between cheese intake and BMD or hip fracture.[124] Given the limited number of studies that investigated effects of cheese and yogurt intake on fracture risk, it is hard to draw firm conclusions. However, there were no detrimental effects of either yogurt or cheese on fracture risk.

There are several strengths of the present study. First, it expanded the previous NHS I fracture study by including women as young as 40 years and examining not only hip fracture but fractures at the hip, wrist, and vertebrae together. Secondly, our secondary analyses demonstrated the combined effects of dairy foods and other potential modifiable risk factors for fracture, which provided in-depth information on the cross-talk between dairy food and other factors on fracture risk. Moreover, we examined the individual impact of total dairy, milk, yogurt, and cheese on fracture risk separately, while most research only focused on milk intake. Furthermore, we used the mean intake from the 2 most recent FFQs for the dairy exposure in this study and we continued to update that exposure as well as other exam information over time. Finally, we excluded subjects who had diagnosis of osteoporosis in the analysis to minimize potential reverse causality, since subjects with known osteoporosis may increase their dairy intake to try to prevent fracture.

The findings of this study must also be assessed in light of the limitations of the data. First, the dietary data were collected from FFQs, which may be subject to measurement error. However, a previous validation study in the NHS I has shown high validity of self-reported dairy food consumption.[91] Secondly, the consumption of yogurt was low in this cohort, which may prevent us observing any potential benefits on fracture associated with higher yogurt consumption. Thirdly, NHS I participants were nurses that are more health conscious, so it might be possible that they could take more preventive measures to improve their bone health than the general population. This may limit the generalizability of the findings in this study. In conclusion, this study suggests that both total dairy and milk intakes protect against fracture risk in women aged 40 years and older. This finding may provide middle-aged and older women with an easy and acceptable method for preventing future fractures.

CHAPTER FIVE: GENERAL DISCUSSION

Menopause and pregnancy are two major triggers of excessive weight gain in women as a result of the major physical and psychological changes that occur during these two critical stages. By the time of menopause, approximately 27% of women are overweight and 13% are obese.[1] Perimenopausal women have been found to gain an average of 2.25 kg over the three years surrounding menopause, with approximately 20% of perimenopausal women gaining more than 4.5 kg.[24]

In terms of pregnancy-related weight gain, it has been reported that about 70% women experience excessive weight gain during pregnancy and most of them fail to return to their pre-pregnancy weight.[42] Endres et al. reported a mean of 11 lb of weight retention one year postpartum, with 47% of women gaining more than 10 lb and 24% gaining more than 20 lbs.[5] The study showed that even for women who had normal pre-pregnancy BMIs, one-third of them became overweight or obese at one year postpartum. Excessive weight retention has been identified as a strong predictor of obesity risk in later life. Women who successfully lost their pregnancy weight have been shown to have gained an average of 2.4 kg one decade later while those who failed to lose their pregnancy weight gained an additional 8.3 kg over the next decade.[104]

Both perimenopausal and postpartum weight gains are associated with a preferential deposition of abdominal fat.[2,3,105] Abdominal fat accumulation is particularly dangerous, because it has been linked to a variety of health problems such as hypertension, diabetes, and CVD.[138] Emerging evidence demonstrates that excessive weight gain contributes to a significant health burden for women during the menopausal transition and postpartum stages.[29,106] Therefore, it is imperative that we find effective strategies to support weight management for both perimenopausal and pregnant and postpartum women.

Dairy foods play a critical role in the typical diet of the US population. Dairy foods are nutrient-dense and contain a variety of fatty acids, proteins, minerals, vitamin D, and other bioactive components. The *2020-2025 Dietary Guidelines for Americans* recommend 3 servings of dairy per day as part of a healthy and balanced diet. [13] Due to the high fat content of dairy, it is commonly believed that dairy intake, especially non-reduced fat dairy foods, may lead to weight gain. However, both short-term and long-term studies that looked at the effects of dairy food consumption on weight change have had conflicting results, and a growing body of evidence finds that not all forms of dairy are the same in terms of effects on weight change. One systematic review concluded that yogurt consumption in particular is inversely associated with weight change,[15] with each additional serving of yogurt per day leading to approximately 40 grams of weight loss per year. In fact, most observational studies tend to support a favorable effect of yogurt on various measures of adiposity.[139]

Given that there are likely to be different effects of individual types of dairy products on weight change, and that yogurt may provide particular benefits to weight management, we decided to explore how different dairy foods impacted weight change in women during perimenopause and the postpartum period. Therefore, the first two aims of this dissertation examined the effects of different dairy foods intakes on weight gain and obesity risk in perimenopausal and postpartum women, respectively.

In the first aim, we investigated the associations between total dairy, yogurt, milk, and cheese on weight change and risk of obesity among NHS II women during the 12 years surrounding menopause. We found that women with higher yogurt intakes (≥ 2 s/w) consistently had less weight gain than women with the lowest yogurt intakes (< 1 s/m) over 12 years. Moreover, higher yogurt intakes were associated with a 42% (95% CI: 0.54 - 0.62) reduction in obesity risk, while those with the highest intakes of total dairy (≥ 2 s/d vs. < 1 s/d) had only a 9% (95% CI: 0.84 - 0.97) reduction in obesity risk. Physical activity and AHEI scores alone were linked with lower obesity risks, but higher consumption of yogurt further reduced the obesity risk in women with higher activity or AHEI scores.

To the best of our knowledge, this is the first study that examined the effects of individual types of dairy foods on weight change during a period centered around menopause. Overall, the findings from the first aim suggest that yogurt intakes may be particularly helpful in reducing weight gain and obesity risk in perimenopausal women, and the favorable effects of yogurt were additive to that of a generally healthy diet or active lifestyle. Incorporating yogurt intake as a part of healthy lifestyle may be an effective way for women to optimize their weight status in this critical stage.

There are a number of factors that may make it difficult to assess the effects of dairy food intakes on weight change. First of all, large studies of necessity must rely on imperfect assessment tools, such as the FFQ to assess dietary intake. It is also very possible that people may change their dairy consumption during the follow-up time in a study and most studies have insufficient power to evaluate change over time. Yogurt intake in particular has changed over time which may contribute to the problem of misclassification in the categories of yogurt intake. Another problem is the yogurt intake has been historically relatively low, thus future long-term studies or clinical trials are needed to explore the effects of a higher level (e.g., > 1 s/d) intake of yogurt on weight change.

In the second aim of this dissertation, we shifted our focus to women during the postpartum stage. We investigated effects of total dairy, yogurt, milk, and cheese on postpartum weight retention and the risk of postpartum obesity among women from the NHS II. The analysis focused on a 4-year period from the before pregnancy up to two years postpartum. Results suggested that both yogurt (p - trend = 0.022) and cheese (p - trend = 0.002) were inversely associated with less postpartum weight retention. Women who consumed moderate and higher amounts of yogurt or cheese lost more of their pregnancy-associated weight gain than those with the lowest intakes. In addition, those women with moderate and higher yogurt intakes had lower risks of postpartum obesity. The combination of healthy lifestyle factors such as higher levels of activity when combined with higher intakes of yogurt or cheese was linked with lower risks of becoming obese postpartum. To our knowledge, this is the first study to investigate the impact of different types of dairy products on postpartum weight retention and obesity risk. The findings of the second aim confirm that neither total dairy nor individual dairy foods led to increased postpartum weight retention or obesity. Instead, incorporating yogurt or cheese into a healthy maternal diet could have favorable effects on postpartum weight retention.

Although a number of observational studies and clinical trials have examined the effects of dairy food intakes on weight change and obesity, none have targeted the women during the postpartum stage.[15,53] However, other studies have examined effects of dairy consumption or dairy-related dietary patterns on gestational weight gain, which is an important predictor of postpartum weight retention.[58] While a few studies have found dairy consumption to be positively associated with weight gain in later pregnancy,[55] others have found only beneficial effects,[57,54] Our study with more than 15,000 pregnancies was much larger than most previous studies and found no detrimental effects of total dairy or milk intakes on postpartum weight retention and obesity risk. Since we did not have specific data on weight gain during the 9-month gestational period in the current study, we were unable to directly assess gestational weight gain in association with dairy food intakes. Future studies are needed to track women from pre-pregnancy, through the gestational period, and on to one-year post-partum. Another important consideration is that dairy intake is likely to change during these three distinct periods surrounding pregnancy.

The dairy intake in the current study reflected the average intake from pre-pregnancy to the postpartum stages, a period in which diet is likely to be highly variable (for example, due to morning sickness). As a result, we may not be assessing diet during the most relevant time period in order to assess the effects of different dairy foods on post-partum weight retention.

It has been suggested the time around pregnancy is an excellent opportunity to for women to optimize their weight control, because of the increased exposure to healthcare providers and improved awareness of their own health status.[48] In addition, the concern about the health of their offspring could be used to promote weight loss prior to pregnancy in women of childbearing age. The results of this current study taken together with previous research may add particular value for women wishing to optimize their weight status at this important life-stage.

In both perimenopausal and postpartum women, yogurt intakes were consistently associated with reduced weight gain and inversely associated with obesity risk. There are several potential mechanisms may account for this benefit including previously-demonstrated anti-inflammatory effects of yogurt,[140] the role of calcium in fat metabolism[97,98] and weight loss,[34,38] enhancement of the bioavailability of calcium due to the acidity of yogurt,[99] and the role of probiotics (from fermentation) in enhancing the integrity of the intestinal barrier and regulating gut microbiota.[39] Gut microbiota plays an important role in the obesity development, and the disorders of the gut microbiota is believed to promote obesity by increasing chronic inflammation. Finally, the semi-solid matrix of yogurt promotes satiety and lowers feelings of hunger.[100] Data from a clinical trial has shown that consumption of yogurt effectively reduced hunger feeling, increased fullness, and delayed the onset time for the next meal.[141] Lastly, yogurt intake has been associated with an overall healthier lifestyle, including more exercise, less smoking, and a healthier general diet,[40] which could explain why yogurt consumers have lower weight gain and reduced obesity risks. Despite all these proposed explanations, the exact mechanisms remain unclear, indicating that more studies are warranted.

The third aim of this dissertation was to evaluate the risk of fragility-related fractures associated with dairy intake. Women are more prone to osteoporosis and related fractures than men. In fact, women have twice the risk of osteoporosis, 4 times the occurrence of forearm fractures, more than twice as many hip fractures, and 1.6 times more vertebral fractures than men.[9,10] The rate of fragility fractures begins to rise for women in their 40s and continues to increase to the end of life.[11] Due to the drop in estrogen levels, fracture rates in women rise even more rapidly after menopause.[63] Fractures in middle-aged and older women are an important cause of premature death and disability. It has been reported that women with a fracture have twice the one-year mortality risk of those without fractures.[12] Dairy foods provide a variety of bone-beneficial nutrients, such as calcium, vitamin D, phosphorus, and potassium.[76] Although many studies underscore the finding that dairy intakes can improve BMD, whether dairy foods can reduce fracture risk in the long-run is still debated.[16–18]

In this research, we examined the effects of total dairy, yogurt, milk, and cheese on fragility fractures of the hip, wrist, and vertebrae in women ages 40 years and older in NHS I. Results from the fracture analyses showed that both total dairy and milk intakes can effectively prevent fragility fractures in women. Those who consumed more than 2 s/d of total dairy had about a 20% lower fracture risk than those with very low intakes of dairy (<1 s/w). Similarly, more than two servings of milk per day was associated with a 14% reduction in fracture risk compared with those who had very low intakes. Neither cheese nor yogurt intakes had any effect on fracture risk. In this study, women who were overweight experienced a lower fracture risk, a finding that has been shown previously in a number of studies. Moreover, we also evaluated whether non-dairy sources of calcium, vitamin D, and protein might prevent fragility fractures in women of 40 years of age and older. None of these nutrients from non-dairy sources reduced the fracture risk, and none modified the inverse association between total dairy or milk intakes and fracture risk.

Milk is the dominant dairy products in the US diet, and it has been promoted as bone-beneficial food because of its high calcium content and fortification of vitamin D. Calcium is an essential component of the bone that provides the skeletal structure and strength, while vitamin D regulates calcium absorption in the intestine and helps to maintain an adequate level of serum calcium and phosphate to ensure proper bone mineralization.[72]

Since milk contributes the most to total dairy intake, this could explain why the effects of milk and total dairy are similar. Our results are in agreement with an earlier analysis from the NHS I cohort that found benefits of total dairy and milk on hip fracture risk.[78] Our current analyses expand upon this earlier NHS I work in postmenopausal women by including women aged 40 years and older and adds fragility fractures at three sites (hip, wrist, vertebrae), instead of just hip fracture.

Contrary to the common belief that calcium alone is essential to bone health, meta-analyses have failed to support this belief.[68–70] In fact, pooled analyses of clinical trials even revealed an increased fracture risk associated with calcium supplementation.[68] More recent meta-analysis summarized the findings from 44 cohort studies and 26 randomized clinical trials, and concluded that neither dietary calcium intake nor calcium supplements had protective effects against fracture.[132] Vitamin D is present in few natural food sources in the American diet and even though it can be synthesized endogenously by sunlight exposure, our modern lifestyle largely limits the outdoor time for adults. Approximately 42% of the US population has inadequate levels of vitamin D.[73] A previous NHS I study found that the women with high intakes of calcium but low vitamin D levels had no reduction in hip fracture risk (RR: 1.27; 95% CI: 0.77 -

2.11), while women with higher calcium and higher vitamin D had a lower hip fracture risk.[126] Other clinical trial with co-administration of calcium and vitamin D have also shown reductions in fracture in older adults [133]

The fact that milk in the US market is largely fortified by vitamin D could explain the particular benefits of milk in reducing fracture risk. This hypothesis is supported by a meta-analysis in which studies from the US, where milk is widely fortified with vitamin D, had a beneficial effect on fracture risk.[57] Given the weak, non-statistically significant reduction in fracture risk that we observed and that found in other studies,[58] it is essential that future studies examine the specific impact of fermented dairy foods on bone health.

It is particularly interesting to note that the first two papers in this dissertation found that yogurt and cheese, but not milk, were the dairy foods that were associated with weight-related related outcomes. In contrast, milk (and total dairy) but not yogurt was associated with lowering of fracture risk. It is likely that these dairy food specific results may be a consequence of the different mechanisms involved in weight regulation vs. fracture risk. For example, effects on the gut microbiota may be important to the regulation of weight during critical life stages but may have no effect on bone health. In contrast, the combined vitamin D and calcium contents of milk may act synergistically to help maintain bone density in older women. Since yogurt is not fortified with vitamin D, it is logical that milk may preferentially benefit bone health.

In summary, the findings of this dissertation suggested that regular consumption of yogurt may help to suppress weight gain in perimenopausal women and promote weight loss in the postpartum period. In contrast, the intakes of total dairy and milk may help to support bone health and lower the risk of fragility fractures among middle-aged and older women.

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CURRICULUM VITAE

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