The effect of plyometric exercise on bone turnover markers and osteokines in younger and older women

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Abstract

The effect of a single-bout of plyometric exercise on markers of bone turnover and Wnt signalling-related osteokines was studied in 20 younger, pre-menopausal women (23.14±2.33 years) and 20 older, post-menopausal women (57.90±4.35 years of age). Blood samples were obtained at rest (i.e., pre-exercise) and 5 min, 1h, and 24h post-exercise and were analyzed for C-terminal crosslinking telopeptides of type I collagen (CTX), osteoprotegerin (OPG), sclerostin and dickkopf-1 (DKK-1), and estradiol. Resting levels of CTX, OPG, and sclerostin were significantly higher while DKK-1 and estradiol were significantly lower in older compared to younger women. CTX was higher at 5 min post-exercise compared to pre-exercise in younger women (326.0±27.0 vs. 292.0±29.0 pg/mL; p=0.049); however, no response was seen in older women. Sclerostin significantly decreased from 5 min (319.9±34.6 pg/mL) to 1h post-exercise (245.3±29.5 pg/mL) but increased between 1h and 24h post-exercise (368.3±33.9 pg/mL) only in younger women. DKK-1 decreased in both groups. In younger women, the decrease was continuous from 5 min (2560.20±120.65 pg/mL) to 24h post-exercise (2176.60±115.29 pg/mL, p=0.006). In post-menopausal women, the decrease was between pre-exercise (1949.69±177.95 pg/mL) and 1h post-exercise (1549.82±187.11 pg/mL, p=0.001) but returning to near pre-exercise levels 24h post-exercise. In the older women, OPG also decreased from pre-exercise (535.8 ± 36.8 pg/mL) to 5 min post-exercise (475.1± 39.0 pg/mL; p=0.048) and remained lower than baseline for up to 24h post-exercise (505.0±32.4 pg/mL; p=0.046). No changes were seen in the younger women. These results suggest that in women, one session of plyometric exercise is sufficient to induce

significant changes in bone turnover and Wnt signalling related osteokines, however, the

timing of the response varies significantly between age groups.

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ANOVA	Analysis of Variance
ALP	Alkaline Phosphatase
BIA	Bioelectrical Impedance Analysis
BMD	Bone Mineral Density
BMI	Body Mass Index
CTX	C-Terminal Crosslinking Telopeptides of Type I Collagen
DEI	Dietary Energy Intake
Dsh	Dishevelled receptor
EA	Energy Availability
EE	Energy Expenditure
E2	Estradiol
FFM	Fat Free Mass
Fzd	Frizzled receptor
GSK-3β	Glycogen synthase kinase-3β
LRP	Low density lipoprotein
LRP5	Low density lipoprotein receptor 5
LRP6	Low density lipoprotein receptor 6
NTX	N-telopeptides of type I collagen
OC	Osteocalcin
OPG	Osteoprotegerin
P1CP	Procollagen type I C-terminal peptide
P1NP	Procollagen I Intact N-Terminal

RANK	Receptor activator of nuclear factor kappa-β
RANKL	Receptor activator of nuclear factor kappa-β ligand
RM ANOVA	Repeated Measures Analysis of Variance
TRAP5b	Plasma tartrate-resistant acid phosphatase
WA_{eq}	Weekly physical activity metabolic equivalent

CHAPTER 1: LITERATURE REVIEW

1.1   General Bone Physiology and Aging

1.1.1   Bone Anatomy and Remodelling

There are two types of bone found in the body, cortical and trabecular bone (1,2). Cortical bone is considered structural bone that determines the structure of the skeleton and is a heavily calcified bone (1). Cortical bone makes up 80 percent of the skeletal composition (1). Trabecular bone, 'spongy bone,' is less dense and makes up 20 percent of the skeletal composition (1,2). Trabecular bone is considered a more active bone that is found in the vertebrae, pelvis, and ends of long bones where more movement occurs, usually referring to the joint cavities. (2).

The bone matrix includes the organic matrix, a collection of type I collagen, and the inorganic matrix, composed of calcium and phosphate (1,3). The inorganic matrix is largely associated with bone strength (3). Remodelling occurs through a tightly regulated sequence of bone breakdown, microdamage, and bone formation (1,2,4). Continuous mechanical loading causing microdamage to the bone structure initiates the constant remodelling of bone (2,5). Osteocytes, found within the bone matrix, differentiate precursor cells that play a large role in bone metabolism (6). One of the functions of osteocytes is to mediate the response to mechanical bone stress through the production of proteins, which influence the activation of the bone formation cells, the osteoblasts, and the activation of the bone resorption cells, the osteoclasts (4,5,7). Osteocytes, osteoblasts and osteoclasts are important in the regulation of the remodelling cycle (2,4,6,7).

Osteoclastic resorption followed by osteoblastic bone formation determines the remodelling cycle (2,4,7). During the remodelling process, the osteoclasts, found on the surface of the bone, begin to resorb the bone matrix. The secretion of hydrogen ions and enzymes (cathepsin K and matrix metalloproteases) begin resorption of the bone matrix, breaking the bone structure down. This resorption creates a deficit within the matrix that will be replaced by the osteoblastic cells The osteoblastic cells, which are found lining the surfaces of bone, regenerate the bone matrix, initiating mineralization and increasing structural strength (1,2).

1.1.2 Bone Changes in Adulthood

After physical growth has completed, bone mineral density is maintained until approximately 50 years of age in women (8). The maintenance phase of bone remodelling indicates a balance between bone formation and bone resorption (8,9). However, as the body ages demineralization of bone occurs, which eventually leads to a decrease in bone mass. Loss of bone mass starts in early adulthood, approximately 50 years of age, and continues throughout adulthood (10–12). Bone loss is attributed to the uncoupling of bone resorption and bone formation (5,10,11).

This decline in bone mass is amplified as women reach menopause (10,13). The increase in bone resorption occurs due to the decrease in the secretion or production of sex steroid hormones, and other endocrine changes as the body ages (1,5). These hormones also regulate the rate at which bone formation occurs, and therefore a decrease in bone formation is observed in the absence of sex steroids and endocrine factors (5). Moreover, the decline in bone formation rate can be indirectly attributed to the reduced production of osteocytes within the bone matrix, which promote bone formation by

responding to mechanical loading (5). Due to the factor of age, post-menopausal women are most likely to experience this imbalance in their remodelling ratio (5).

1.1.2a Osteoporosis

Osteoporosis is a disease defined by low bone mass and diminishment of the skeletal microarchitecture (14,15). Osteoporosis is a result of extreme low bone mineral density (BMD), which leads to the deterioration of bone strength and stability, increasing the rate of fragility fractures and injury (14,15). A variation in genetic composition and interactions with environmental factors cause osteoporosis (15). Osteoporosis is defined by a BMD score 2.5 standard deviations below the mean value for a young adult (14). The deteriorating bone disease is more common among the elderly population, and even more so in women, specifically post-menopausal women (14,15). Primary osteoporosis, having an unknown cause, and secondary osteoporosis, having a traceable etiology, can be either localized or generalized within the skeleton (14,16). The underlying mechanism is an imbalance in bone remodelling, hindering the accumulation of bone mass which results in insufficient bone mass and strength (14). Osteoporosis is a debilitating disease that affects quality of life significantly, requiring treatments to improve overall bone health.

1.2.2b Estrogen and Menopause

Estrogen, is a sex hormone that plays a crucial role in the development and regulation of the female reproductive system (17,18). Estrogen, more specifically estradiol, is important for bone development and maintenance of bone mineral density throughout various stages in life (18,19). Estrogen elicits a protective effect over the development and maintenance of bone through the inhibition of bone resorption (19).

Estrogen deficiency is correlated with longer osteoclast life and shorter osteoblast life (20,21). Thus, a deficiency in circulating estrogen in post-menopausal women may cause an increase in bone resorption through the promotion of prolonging osteoclastic activity (20). Estrogen interacts with and affects multiple mechanisms with respect to bone turnover and development (19). Estrogen represses osteoclastic cytokine production from immune cells (19,22), increases osteoblast proliferation while decreasing osteoblast and osteocyte apoptosis (23), and induces osteoclast apoptosis (21).

Menopause is a natural process that occurs in women. Menopause occurs when a woman's menstrual cycle has ceased, leading to a loss of ovarian follicular function (8,24,25). Menopause tends to occur around 50 years of age; however, the timing is individual (8). Menopause stems from the reduction or lack of circulating estrogen (26). Estrogen, a sex steroid, increases the sensitivity of bone to mechanical loading during reproductive years, i.e., before menopause, through its influence on osteoblastic cells (3). During menopause, estrogen deficiency occurs, which affects bone turnover rate (3,27). Post-menopausal women experience an increase in bone resorption due to the deficit in circulating estrogen, and this increase in bone resorption increases bone loss compared to bone replenishment, and therefore, compromises the skeletal structure (3,8,27). This, in turn, causes an overall reduction in bone mass and mineralization (3,8,26,27). The compromise to the skeletal system in post-menopausal women causes an increased risk for low bone mineral density, and an increased fragility and fracture rate (8,27). In short, a decrease in estrogen lessens bone formation and increases the risk of deterioration of bone, leading to osteoporosis (8,27). This effect indicates that women have a high risk of developing osteoporosis after menopause (8).

1.2 Markers of Bone Turnover

Markers of bone turnover include those that reflect formation and those that reflect resorption. Formation markers of bone turnover include total alkaline phosphatase (ALP), bone ALP, osteocalcin (OC), procollagen type I C-terminal peptide (P1CP), and procollagen I intact N-terminal (P1NP) (28). These markers play a role in different stages of maturation and differentiation of osteoblasts (28). Plasma tartrate-resistant acid phosphatase (TRAP 5b), N-telopeptides of type I collagen (NTX), and C-terminal crosslinking telopeptides of type I collagen (CTX) are bone resorption markers that reflect osteoclast activity (28). These markers are all considered bone turnover markers, as they demonstrate the activity of the osteoblasts and the osteoclasts during bone metabolism (28). Although all these markers are good indicators of the activity of both the osteoblasts and osteoclasts, CTX and P1NP are well accepted bone turnover markers that are recognized by the International Osteoporosis Foundation (IOF) as they are by-products of osteoblastic or osteoclastic cell activation (28,29). P1NP concentrations indicate the synthesis of protein within bone tissue, the activation of osteoblasts, allowing the concentrations of P1NP serum to be a direct indicator of bone formation (29). CTX, is also a direct indication of osteoclast activity, indicating the activity of bone resorption, as CTX is secreted from osteoclasts when they are activated. (30–33). More importantly, as strong indicators of bone turnover (28,29), both P1NP and CTX are the most sensitive markers in the post-menopausal population as indicators of bone metabolism and osteoporotic tendencies (28).

1.2.1 C-terminal Crosslinking Telopeptides of Type I Collagen (CTX)

The organic matrix found within bone is primarily made up of collagen, which provides the skeleton with its biochemical structure (31). Collagen crosslinks are peptides that are crucial biochemical markers of bone turnover, more specifically bone resorption, that have been created through the process of collagen degradation (30–33). CTX, is a specific type of collagen crosslink that is an indicator of bone resorption and can be measured in both urine and blood (30–33). Increased levels of CTX are highly correlated with a higher fracture rate due to the increase in bone resorption, weakening the overall bone structure (32). Further correlations have been observed between levels of CTX and consequential bone loss within post-menopausal women (31)., CTX is indicative of bone resorption (30–33).

1.2.2 Procollagen Type I N-Terminal Peptide (P1NP)

P1NP is a type I collagen, which is the main component of bone (34). P1NP is the most specific bone formation marker, as it is a predictor of various phases of osteoblastic activity, and is an indicator of bone metabolism (29,35,36). P1NP serum concentrations are indicative of the synthesis of the most abundant protein of bone tissue, type I collagen (29). During bone formation, the osteoblasts secrete the precursor procollagen molecule, P1NP, indicating a direct correlation between serum P1NP and bone turnover activity (29). Levels of P1NP are highly reliable and are not influenced by food intake or renal function but may be influenced by circadian rhythm (29,36). Furthermore, P1NP is a good predictor of bone turnover rates as well as a monitoring tool in forecasting the presence of osteoporosis in post-menopausal women (36).
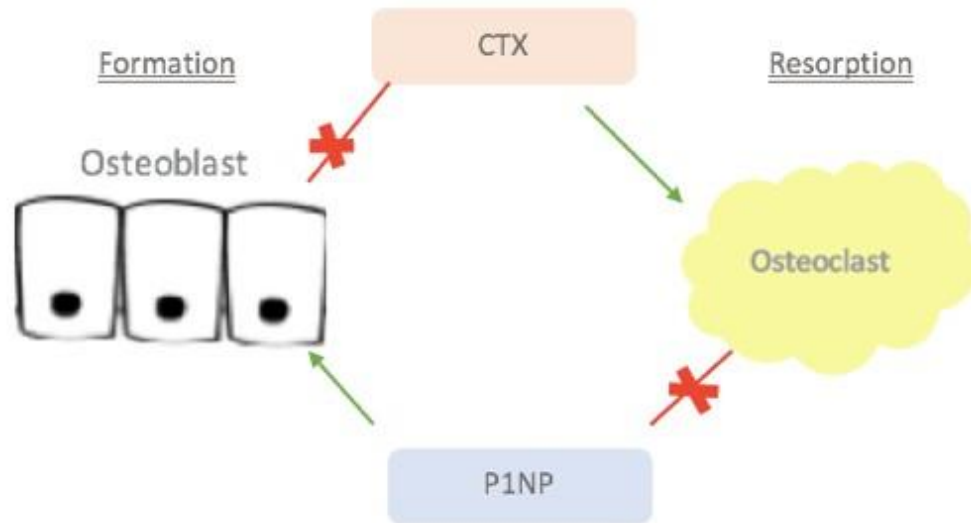
Figure 1: Bone Turnover Marker Associations (adapted from (1)).

1.3 Bone Specific Signalling Pathways

1.3.1 Wnt Signalling Pathway

The Wnt/β-catenin signalling pathway is involved in the development and maintenance of various organs and tissues including bone (37–39). In bone, the Wnt pathway is largely associated with osteoblastogenesis, which determines bone formation (37,40). The Wnt pathway encompasses 19 glycoproteins that are involved in gene transcription leading to bone formation (37,39). The canonical Wnt signalling pathway can be defined as a pathway dependent on the stabilization of β-catenin protein (37–40). As shown in Figure 2, downstream activation of the Wnt pathway begins with the Wnt ligand molecule binding to the low-density lipoprotein-related receptor 5 and low-density lipoprotein-related receptor 6 (LRP5 or LRP6) or a frizzled transmembrane receptor (37–40). The binding of the Wnt molecule activates the protein dishevelled (Dsh) triggering downstream phosphorylation of glycogen synthase kinase-3β (GSK-3β), which inhibits

GSK-3β from phosphorylating β-catenin (39,40). The inhibition of GSK-3β facilitates β-catenin to initiate nuclear translocation, which targets gene transcription leading to bone formation, (38–40). This occurs as β-catenin is building up within the cytoplasm, able to reach its threshold, allowing β-catenin to enter the nucleus of the cell. The Wnt pathway stimulates the process of cell proliferation, renewal of stem cells, stimulation of preosteoblast replication, and enhancement of osteoblast activity, increasing bone mass and functionality of bone cells (37,40). Thus, the Wnt/β-catenin signalling pathway is a complex anabolic mechanism that is involved in a diverse process to develop bone mass (37).
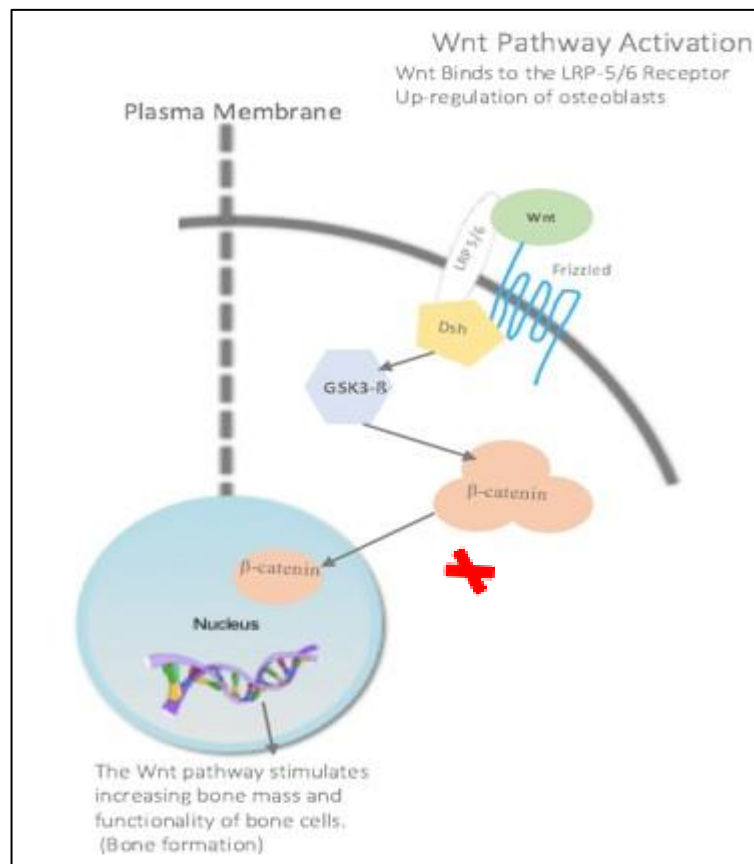
Figure 2: Wnt/β-catenin Signalling Pathway (adapted from (41)).

There are various bone specific cytokines, i.e., osteokines, which influence the Wnt/β-catenin signalling pathway. Sclerostin is a glycoprotein that is coded by the SOST gene; it is part of a family of proteins that have the shared ability to provoke bone morphogenetic protein (BMP) activity (1,5,6,42–44,41). The promotion of BMP activity allows for changes in bone mineral density and structure of bone (6). The expression of the SOST gene occurs within the matrix, allowing the secretion of sclerostin from the osteocytes (45). Sclerostin is synthesized by the osteocytes within the bone matrix in response to a reduction in mechanical loading and age-related hormonal (eg. estrogen) changes (1,42–44,41,45,46). Sclerostin inhibits osteoblast differentiation, thus decreasing bone formation (1,5,42–44,41). Specifically, sclerostin acts as a Wnt signalling antagonist through binding to the LRP-5/6 progenitors, inhibiting the Wnt molecule from binding to the LRP-5/6 receptor (5,42–44,41,45,46). Inhibition of the Wnt pathway (Figure 3) results in decreased osteoblastogenesis and bone formation (6,42,44,45). A reduction in bone formation induces the uncoupling of remodelling (1,2). Serum concentrations of sclerostin are shown to be significantly higher in post-menopausal women compared to pre-menopausal women (42,47,48). Women experience a 2.4 fold increase in resting serum sclerostin levels as they age, indicating that sclerostin levels are positively associated with age (48). However research on this topic is still limited. Future studies are needed to fully understand the role of sclerostin on bone adaptation during aging, particularly in women due to the decreased estrogen post-menopause, which has been associated with higher secretion of sclerostin (47).
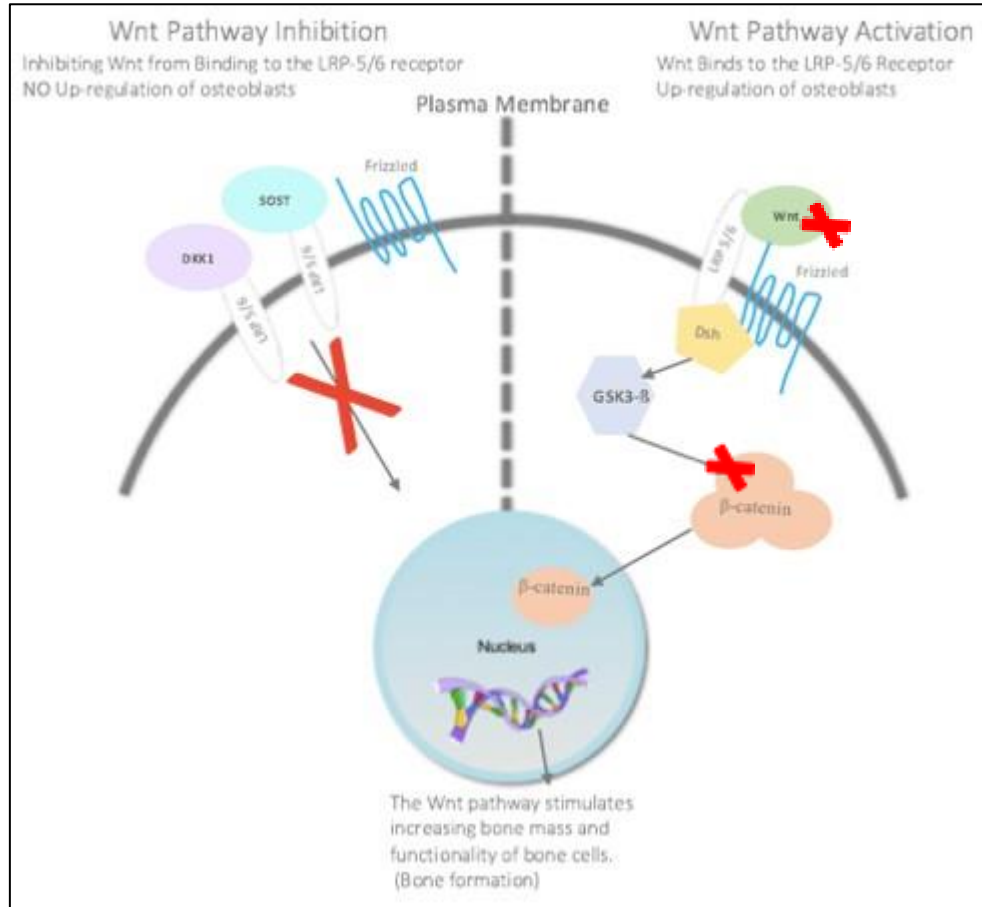
Figure 3: Sclerostin and DKK-1 inhibiting the Wnt/β-catenin signalling pathway (adopted from (41)).

Dickkopf-related protein 1 (DKK-1) is another antagonist of Wnt, which plays a role in the inhibition of bone formation similar to that of sclerostin (7,49,50). DKK-1 is a central regulator of osteoblastic activity and is expressed by the osteoblasts and osteocytes (7,51). Similar to sclerostin, DKK-1 causes a blockage of the LRP5/6 receptor sites and does not allow the Wnt molecule to bind (7). This blockage results in the inhibition of bone formation (Figure 3), influencing the balance of resorption and formation involved in bone remodeling (1,2,7). Increased concentrations of DKK-1 have been known to enhance osteoclastogenesis (52). Mechanical loading has been observed

to reduce the concentration of DKK-1 and therefore reduces the effect of the protein's inhibition on the Wnt pathway (49). Furthermore, serum concentrations have been observed to be higher in post-menopausal woman compared to women of the same age who have not experienced menopause (53). Similarly, concentrations of DKK-1 have been linked to estrogen deficiency-medicated osteoporosis, an endocrine factor associated with menopause (53,54). However, the expression of DKK-1 and its relation with aging and bone mineral density is not well established, and therefore further exploration to determine the influence is necessary (7).

1.3.2 OPG-RANKL Pathway

The interaction between Osteoprotegerin (OPG) and the receptor activator of nuclear factor kappa-β ligand (RANKL) is a key pathway that regulates the outcome of bone remodelling (1,55,56). The OPG-RANKL pathway dictates a large portion of bone turnover, specifically regulating bone resorption (1,55,56).

RANKL plays a role in the process of differentiation of osteoclast precursor cells into mature osteoclasts (4,57,55,58). RANKL is expressed by osteoblasts on the extracellular surface of their plasma membrane, as well as by bone marrow stromal cells and T cells within the bone matrix (4,57,56). In response to mechanical strain, the osteocytes recruit osteoclasts to induce bone resorption, activating the secretion of RANKL from the osteoblastic cells (4). RANKL's expression is up-regulated to activate bone resorption (58). Once secreted, RANKL is bound to one of two receptors: the receptor activator of nuclear factor kappa-β (RANK) or OPG (4,57,55,56,58). As shown in Figure 4, the role of bone turnover is determined by the interaction of RANKL to

either the OPG receptor, inhibiting the activation of osteoclastogenesis, or the RANK receptor, activating the osteoclast to resorb the bone matrix (4,57,55,56,58).

RANK is a protein expressed on the surface of the osteoclasts (4). The interaction between RANKL and RANK is responsible for the differentiation, survival, and activation of osteoclasts, causing bone resorption (4). The binding of RANKL to RANK activates the down-stream process of bone resorption within the bone matrix (4,57,55,56,58).
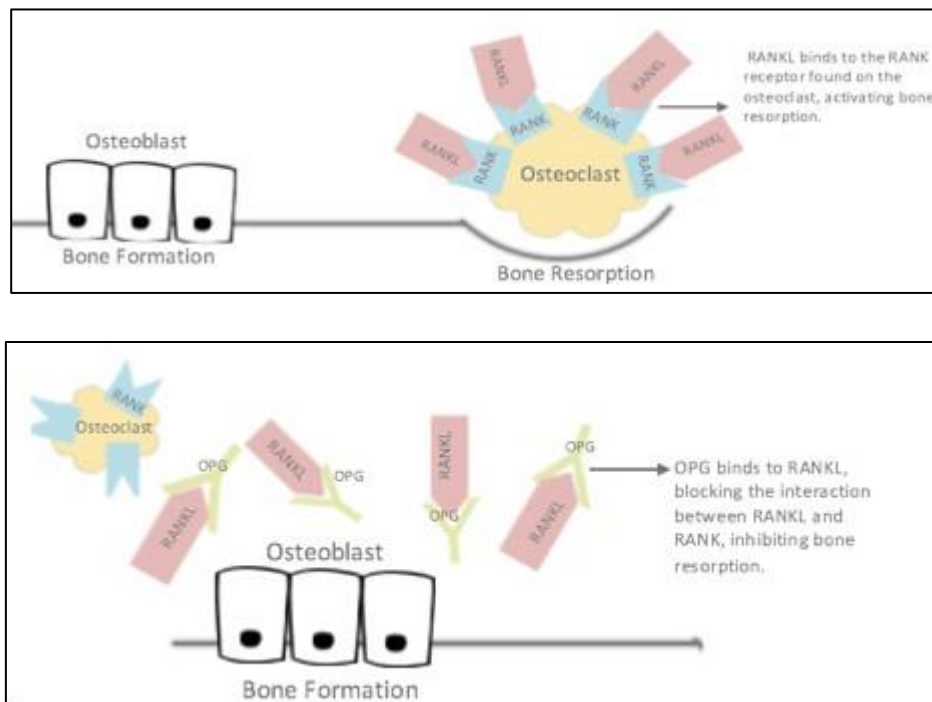
Figure 4: The OPG-RANKL interaction (adopted from (4)).

OPG is considered a bone protector, as it acts as a decoy receptor for RANKL (4,57,55,56,58). OPG essentially blocks the RANKL protein from binding to RANK by attaching itself to the RANKL molecule, and therefore indirectly inhibiting bone resorption from occurring (4,57,55,56,58). OPG is down-regulated and primarily

expressed through bone marrow stromal cells as well as osteoblasts (56,58). Estrogen stimulates the secretion of OPG from the osteoblasts and inhibits the production of RANKL (4). This interaction affects the post-menopausal female population because the estrogen production is reduced, allowing the RANKL-RANK complex to occur due to the decreased amount of OPG, increasing bone resorption mechanisms (4).

The Wnt/β-catenin signalling pathway influences RANKL and OPG (37). When the pathway is activated by the Wnt molecule, expression of OPG is stimulated through up-regulation of OPG from the osteoblasts, which results in indirect suppression of bone resorption (37). As well, the activation of the Wnt/β-catenin signalling pathway causes a cascade of down-regulation of RANKL expression in the osteoblasts, ultimately leading to an indirect suppression of bone resorption (37).

1.4 Factors Impacting Bone Integrity

1.4.1 Uncontrollable Factors

Many factors contribute to the accumulation or deterioration of bone mass; some are controllable, and some are not. Uncontrollable factors are those that cannot be changed or influenced by outside sources. Sex is an uncontrollable factor that can influence bone mass (59). Men have wider bones with a greater cortical mass compared to women (59). Women tend to have more slender skeletal frames that have lower bone strength and less accumulation of bone mass, indicating that fracture risk is 2.5 times great in women compared to men (59). Less bone mass accumulation among women can lead to higher likelihood of fragility fractures in elderly women compared to elderly men (60). Ethnicity can also play a role in the skeletal structure: fracture rates among black

women are 50% less than in white women (59). However, fracture rates among men of both ethnicities are comparable (59). Skeletal age is an uncontrollable factor associated with bone structure (60). There are three age-related processes that can cause deterioration of bone mass (60). Trabecular bone decreases with age by about 50% over the lifespan through the thinning of the trabeculae (60). Furthermore, net resorption is increased at a rate of 0.5% /year as the skeletal structure ages (60). As well, cortical BMD is reduced contributing to bone loss with aging (60). Age is a large uncontrollable contributing factor that causes an increase in bone turnover rate in favour of bone resorption (60). Uncontrollable factors are critical to bone integrity; however, they are just that: uncontrollable.

1.4.2 Controllable Factors

Controllable factors associated with osteokines can influence bone accrual through active management. Exercise, which will be discussed in detail later, is a large controllable factor that greatly influences bone turnover (61). Exercise activates osteocytes in favour of bone formation, acting as a preventative measure or a proactive measure to promote more accumulation or maintenance of bone mass through influencing bone turnover (61). Dietary consumption plays a crucial role in the bone remodelling process (62). A balanced energy state, where energy expenditure and energy intake are equal is when bone metabolism is able to thrive (62). Furthermore, the intake of specific vitamins and nutrients that promote bone turnover are essential to bone health and are controllable dietary factors (63). Vitamin D and calcium have an interdependent role in terms of bone metabolism (63). Vitamin D is found in oily fish, egg yolk, and meat; however, its greatest source is in the skin, where is activated through exposure to the

sun's ultraviolet B rays (63). The recommended daily dietary allowance is 600 IU for individuals who are 1-70 years of age, and 800 IU for individuals over the age of 70 (63). Vitamin D promotes the intestinal absorption of calcium (63). Calcium, a controllable factor of bone, is a large component of bone accrual (64). Calcium is essential to bone development and the increase or maintenance of bone integrity (64). The daily requirement of calcium is 1000mg for adults; however, many individuals are not meeting the recommended daily allowance (64). Increasing calcium consumption to the daily recommended allowance has been demonstrated to increase BMD upwards of 16% compared to individuals who are severely under-consuming calcium (64).

Estrogen therapy is a controllable factor that elicits a large bone turnover response (65). Positive effects of estrogen therapy in women approaching menopause or post-menopausal women have been observed to increase BMD and reduce fragility fracture risks (65). This controllable factor is able to maintain BMD and in some cases increases BMD up to 2.4% through the equalizing effect of bone remodelling, creating a more balanced ratio between resorption and formation of bone (65).

Oral contraception has been observed to have a neutral or possible beneficial effect on bone mass in women after 30 years of age (66). Oral contraceptives are seen to be more influential in later years of pre-menopause as they prevent the activation of bone turnover though increasing estrogen within the body, causing an imbalance of resorption to formation, ultimately increasing BMD (66). However, oral contraceptives have been known to increase the risk of fracture in women who have been taking them for longer durations (66). Likewise, medication such as glucocorticoids, thiazolidinediones, and unfractionated heparin have been known to cause a decrease in bone formation (67).

Others such as medroxyprogesterone acetate and aromatase inhibitors have been determined to reduce estrogen levels and production (67). Older women should consider the impact on the skeletal integrity of a medication before consuming it, and furthermore should consider choosing medication that does not impact bone (67).

1.5 Exercise and Bone

1.5.1 Exercise and Bone Mineral Density

Exercise is considered the most influential non-pharmacological method for improving or maintaining bone mass (68,69). Exercise is both a preventive and a therapeutic strategy to improve bone mineral density and work against the weakening of bone due to natural aging processes (70,71). During exercise, the various stresses on the body induce mechanical loading (jumping, running, resistance training etc.), which allows bone to initiate the bone remodelling process. However, not all exercise modalities and intensities are equally efficient in increasing bone mass (61,72,73). Furthermore, there are uncertainties with respect to the intensity, duration, and frequency of exercise that elicit an optimal osteogenic exercise response (74). Mechanical strain as a result of physical activity causes bone metabolism to react and initiate bone remodelling in favour of bone formation (73). Bone structure and strength must be able to endure the mechanical forces of everyday life to avoid fracture or deterioration of bone mass (72), allowing for bone tissue to experience higher mechanical forces that increase bone mass during physical activity (75).

It is well established that individuals of all ages and sexes who participate in sport or physical exercise have higher bone mass, bone strength, and a greater osteogenic

potential compared to individuals who are not physically active (73,76–78). In particular, individuals of all ages and sexes who participate in high-impact dynamic sports, which apply various directional impacts, have a higher osteogenic response compared to other individuals participating in less impactful exercise (73,76). Although it has been observed that mechanical strain stimulates proliferation of osteoblasts in order for bone adaptation to occur, further research is needed to obtain a comprehensive understanding of the mechanisms involved (77).

1.5.2 Plyometric Exercise

Plyometric exercise encompasses explosive jumping and mechanical force that can generate force up to seven times an individual's body weight (73, 75). Due to the high mechanical load on bone, which activates bone remodelling, plyometric exercise is considered the most valued modality of exercise for improving bone mass (73). Previously, one study has shown positive changes in bone mass and reduction in deterioration of bone mineral density in a population of post-menopausal women as a result of a plyometric exercise intervention (79). However, there is limited data in older populations, including post-menopausal women, on the bone response to an acute plyometric exercise (73). More investigation is needed to better understand the direction and magnitude of the bone response to acute plyometric exercise (73).

1.5.3 Exercise and Bone Turnover

Many observations have been made in terms of acute exercise and its impact on bone turnover and bone health in numerous intensities and modalities (80–83). These observations have shown that response may vary with respect to type, duration, and

intensity, of exercise (80–83). Therefore, the influence that an acute exercise bout has on markers of bone turnover is not well understood.

Despite the variation among the studies, some collective conclusions can be made (34). The magnitude of stimulation for both osteoclasts and osteoblasts are also mode, intensity as well as duration dependent, demonstrating differences in immediate and delayed impact of exercise on bone turnover markers (34,84–91). Furthermore, a sex and age difference is indicated in various studies that show that exercise does not influence markers of bone turnover in the same magnitude in varying populations (80,81,83,92). Both males and females exhibit a varied response to an exercise stimulus while there seems to be an age variation within the same sex in response to the same exercise protocol (80,81,83,92). Generally speaking, not all exercise protocols and durations will elicit the same response in all populations, with potential differences between sexes and between age groups.

CTX in particular has demonstrated some varying results in response to exercise. However, in an acute training session CTX has demonstrated no change in serum levels post-exercise in a group of male and female young (20 years of age) adult athletes (85). As well, a decrease in CTX was seen in young women 30 minutes post-exercise in a resistance whole body vibration study (91). Lastly, an increase in CTX serum levels were seen 30 minutes post- 1h cycle ergometer at 80% VO2max in young male triathletes (93). Seeing as these results are widely different, some conclusions can be speculated. CTX can be influenced by an acute exercise protocol, however the magnitude and direction of that response is determined by population as well as exercise modality and intensity variation.

1.5.4 Exercise and Osteokines Related to Wnt Signalling Pathway

1.5.4a Sclerostin

Osteocytes regulate inhibition of bone formation via secretion of sclerostin (43,94,95), indicating that mechanical strain influences the osteocyte expression of sclerostin (45). A linear relationship between mechanical strain and concentration of sclerostin has been observed. In animal studies, sclerostin has been found to evoke the regulation of mechanotransduction within bone cells (95,96). However, human studies using exercise to increase the mechanical loading on bone are scarce. A cross-sectional study in adults indicates a correlation between physical activity and lower levels of sclerostin (97). However, in a young female population, pre- and post-menarche, an acute bout of plyometric exercise did not induce significant changes in serum sclerostin neither immediately or up to 24h post-exercise (80). Similar to the girls, conducting the same protocol, young boys saw no significant response in serum sclerostin after the plyometric exercise protocol but an immediate increase post-exercise was observed in young men (81). As there are no published studies examining the response of sclerostin to an acute bout of exercise in post-menopausal women, the acute response to high-impact exercise in this population is unknown. However, no significant changes in sclerostin were found following a 12 month physical training program in post-menopausal women (45-65 years) (98). As there were varying results from these previous studies, the association between exercise and sclerostin is not clear, and that there may be age and sex related variations in the sclerostin response to exercise. It would be expected that a decrease in sclerostin post-exercise should be seen.

1.5.4b DKK-1

DKK-1 is another protein that inhibits the Wnt pathway in a similar way to sclerostin (50). Therefore, it is postulated that DKK-1 would react to mechanical strain to a comparable magnitude (94). There is one human study on the response of DKK-1 to a plyometric exercise protocol. Specifically in girls, DKK-1 concentrations were significantly higher at rest in the pre-menarcheal compared to the post-menarcheal groups (80). As well, a decrease from baseline to 1h post-exercise was demonstrated in both groups and remained decreased even 24h post-exercise (80). However, little is known about the role of DKK-1 in exercise, and thus, more research is needed to understand the response of DKK-1 to mechanical loading (99).

1.5.3c OPG

OPG serum concentrations have been examined within varying exercise modalities, populations, and duration of intervention with inconsistent results (71,80–82,98). It is thought that increasing mechanical strain and stress on bone compared to a resting level would result in a response in OPG serum levels (82). An increase in OPG concentrations, leading to a reduction in osteoclastic activity, lessening bone resorption, and increasing overall bone mass and strength, would be observed post-exercise intervention (82). In pre- and post-menarcheal girls, no response occurred after a single bout of plyometric exercise (80). In contrast, both adult women and men experienced an increase in OPG after a marathon run (82). The variability found in these studies suggests that OPG's response to mechanical strain elicited by an exercise intervention is not the same in terms of magnitude or direction in all populations and for all modalities and durations of exercise.

1.5.5 Summary

Markers of bone turnover are often used to examine the dynamic course of bone turnover during acute exercise (100). Thus, many observations have been made in terms of acute exercise of various intensities and modalities, and its impact on markers of bone turnover. For example, strenuous weight-bearing exercise is capable of stimulating a response, increasing bone resorption activity over bone formation in adults (100). An acute plyometric exercise trial was performed in boys, girls, adolescent girls, and men to determine the influence that this exercise had on bone biomarkers (80,81). It was determined however, that only men experienced an increase in sclerostin concentrations 5 min post-exercise while no other groups had a significant time-effect (80,81). In response to the same protocol, no significant time effect was determined in OPG and RANKL concentrations in girls and adolescent girls (80). Table 1 presents the relevant studies on the post-exercise responses of the markers and osteokines of interest.

Despite the variability among the studies, some collective conclusions can be made (34). The magnitude of stimulation for both osteoclasts and osteoblasts are also exercise-dependent, demonstrating a difference in immediate and delayed impact of exercise on bone turnover (34). However, there are still many unanswered questions in regards to the bone response to an acute bout of exercise, in general and in association with endocrine and other age related changes across the lifespan.

Table 1: Response to Acute Exercise in Bone Turnover and Osteokines.

Study Reference	Population	Exercise	Results
Dekker et al. 2017 (80)	14 Pre-menarcheal (10.5 years) 12 Post-menarcheal girls (15.0 years)	Acute Plyometric Exercise over 24 hours	No group effect or time main effect in girls or adolescence for sclerostin and OPG. DKK-1 was higher in the pre-menarcheal girls compared to the post-menarcheal girls. In both groups, DKK-1 decreased from baseline to 1h post-exercise and remained lower than baseline 24h post-exercise.
Falk et al. 2016 (81)	12 Boys (10.2 years) 17 young men (22.7 years)	Acute Plyometric Exercise over 24 hours	Resting levels of sclerostin higher in boys than men. Sclerostin ↑ 5 min post-exercise in men.
Ziegler et al. 2005 (82)	11 women and 20 men, recreational runners	Pre- and post-either a marathon or a short distance run (42.195 km vs. 15.8 km)	OPG increased only in the marathon group.
Herrmann et al. 2007 (85)	32 male and female athletes and controls	Incremental anaerobic cycle ergometer	No changes in CTX serum levels post-exercise.
Sherk et al. 2013 (91)	10 recreationally active women (20.7 years)	Resistance training and whole-body vibration	CTX concentrations post vibration and 30 min post resistance in the combination group.

CHAPTER 2: INTRODUCTION TO RESEARCH PAPER

Osteoporosis is a chronic disease characterised by diminishment of the bone quantity and quality leading to an increase in fracture rate (100). Unfortunately, 1 in 3 women and 1 in 5 men experience an osteoporotic fracture, and 1.4 million Canadians have been diagnosed with osteoporosis (100). The bone quality and quantity are the contributing factors to the overall bone health and provide better understanding of the tissue involved in bone metabolism and strength (100). Bone quality is referring to the mineralization, materials or geometry, of the bone. Bone quantity refers to the mass of the bone, the bone mineral density, which makes up about 74% of bone strength in adults. Thus, the geometry, volume and cortical thickness of bone as well as the bone mass are determinants of bone strength and can attribute to the overall variability of bone strength (100).

Accumulation and loss of bone mass occur in various stages in a woman's lifespan (1,13). Once physical growth has been reached, bone mass is maintained throughout adulthood through bone remodelling until women begin menopause (10,13). Remodelling occurs when osteoblasts, the bone-forming cells, replace the deficit that osteoclasts, the bone resorption cells, have produced (1,2,4,7). Bone mass is, therefore, maintained through a delicate balance/coupling between bone resorption and bone formation (1,2,4). As a result of menopause, there is an accelerated decline in bone mass caused by the uncoupling of bone resorption and formation, when the rate of replenishment of bone by the osteoblasts does not match the loss of bone by the osteoclasts (1,5,10,11,13). This uncoupling is due to the lack of circulating estrogen,

which influences the balance of remodelling (3,8,26,27). Specifically, estrogen elicits a protective effect over bone by indirectly inhibiting bone resorption (19) and inducing osteoclast apoptosis (21). In addition, estrogen has been found to repress osteoclasticogenic cytokine production from immune cells (19,22) and to increase osteoblast proliferation while decreasing osteoblast and osteocyte apoptosis (23). These changes in bone turnover that occur with menopause are reflected in increasing bone resorption markers like CTX (87,90–93).

The Wnt signalling pathway is a multifaceted anabolic system that is involved in various organs and tissues including bone (37–39). In bone, the Wnt pathway is largely connected to the activation of osteoblastogenesis, causing bone formation (37,40). The Wnt ligand molecule activates the pathway through binding to the low-density lipoprotein-related receptor 5/6 (LRP5 or LRP6) or a frizzled transmembrane receptor (37–40). Once bound, activation of the protein dishevelled (Dsh) triggers the downstream phosphorylation of glycogen synthase kinase-3β (GSK-3β) (39,40). GSK-3β is inhibited but Dsh and does not activate the phosphoalation of (39,40). The build up of β-catenin in the cytoplasm initiates nuclear translocation in to the nucleus, which targets gene transcription in favour of bone formation (38–40). The activation of gene transcription stimulates cell proliferation, renewal of stem cells, stimulation of osteoblastic replication, and enhancement of osteoblast activity, which leads to increased bone mass and functionality of bone cells (37,40).

Osteocytes play an important role in sensing variations in mechanical loading, and coordinating signals of resorption and formation (40). Sclerostin has been suggested as one way that osteocytes regulate this osteogenic response to loading, by acting as a

negative inhibitor of the Wnt/ß-catenin pathway, reducing osteoblast activity and therefore bone formation (101). Previous research conducted with animal models has shown that mechanical loading of the bone results in decreased sclerostin levels, whereas mechanical unloading results in increased levels (102). This decrease in sclerostin after loading is a crucial step to promote osteogenesis. Failure to downregulate sclerostin after loading impedes the activation of the Wnt pathway and therefore osteoblast activity is not increased (95). Dickkopf-related protein 1 (DKK-1) is another Wnt-inhibiting protein that plays a crucial role in the inhibition of bone formation (7,49,50). DKK-1 is expressed by both the osteocytes and the osteoblasts as it regulates the activity of the osteoblastic cells (7,51). DKK-1 binds to the LRP-5/6 receptor inhibiting the Wnt molecule from binding (7). Elevated serum DKK-1 concentrations have been observed to enhance osteoclastogenesis, increasing activity of the osteoclastic cells (52). In contrast, osteoprotegerin (OPG) is an anabolic downstream product of the Wnt-signalling pathway. OPG is considered a bone protector and acts as a decoy receptor to the receptor activator of nuclear factor-kappaβ ligand (RANKL) (4,57,55,56,58). Specifically, OPG blocks RANKL from binding to the RANK receptor, indirectly inhibiting bone resorption (4,57,55,56,58).

Limited research has focused on comparing resting levels of DKK-1 in young and older adults, specifically pre- and post-menopausal women. On the other hand, it is well established that sclerostin resting levels are significantly higher in post-menopausal women in comparison to their younger counterparts (42,47,102). One explanation for the higher sclerostin levels post-menopause is the inverse relationship between estrogen and sclerostin, in which decreased estrogen results in higher secretion of sclerostin (47). In

contrast, higher daily physical activity levels are associated with lower serum sclerostin levels, probably due to the increased mechanical loading on the bones (97).

Despite the above cross-sectional studies suggesting that exercise influences bone through an increased mechanical loading, very few studies have examined the sclerostin and DKK-1 response to an acute bout of exercise, in general, and in association with changes in bone turnover markers. Falk et al (81) were the first group to examine the sclerostin response to an acute bout of plyometric exercise in boys and men, pre-pubertal and young men (20 years). A significant increase in sclerostin levels immediately after plyometric exercise was present, however only in men. The same plyometric protocol led to higher OPG from pre- to 24h post-exercise in pre-and early pubertal boys (103) On the other hand, in pre- and post-menarcheal girls, ages 8-16 year, the plyometric exercise protocol resulted in no change in OPG, while DKK-1 decreased up to 24h post-exercise (47). Sclerostin did not change following plyometric exercise in either girls (47) or boys (103).

In the above studies, the osteokine response to exercise was studied in children in relation to older age groups (males and females separately). However, an examination of these osteokines in response to plyometric exercise, and in relation to changes in bone turnover markers, between younger, pre-menopausal women as compared to older, post-menopausal women is yet to be performed. Insight into this response has important implications; a better understanding of the bone's response to mechanical loading can contribute to preventative strategies against bone diseases and age-related osteopenia. Therefore, the purpose of this study was to examine the acute response of CTX, and osteokines related to Wnt signalling pathway such as sclerostin to a single bout of high

impact exercise in younger versus older women. Based on previous findings, and the theoretical assumptions on the potential effects of mechanical loading applied by acute exercise on CTX and Wnt related osteokines outlined in Table 2, it is expected that CTX, sclerostin and DKK-1 will decrease, while OPG will increase, in the hours following the exercise in both the younger and older women.

Table 2: Response to Acute Exercise; CTX and Wnt Related Osteokines.

Bone Turnover and Osteokines	Influence of Exercise	Bone Response
CTX	↑Mechanical Loading ↓ CTX	↓ Osteoclast Activation
Sclerostin	↑Mechanical Loading ↓ Sclerostin	Wnt Signalling uninhibited
DKK-1	↑Mechanical Loading ↓ DKK-1	Wnt Signalling uninhibited
OPG	↑Mechanical Loading ↑ OPG	↑ Inhibition of Bone Resorption

CHAPTER 3: METHODS

3.1 Participants

This study and its procedures were approved by the Brock University Biosciences Research Ethics Board (REB-14-267 KLENTROU). A total sample size of 40 healthy, normal weight, recreationally active women were recruited: 20 younger (pre-menopausal) women (23.1±2.3 years) and 20 older (post-menopausal) women (57.9±4.3 years). Participants were recruited through circulating poster advertisements and by word of mouth based on the following inclusion criteria: (1) had a body mass index <30; (2) were non users of pharmaceutical agents that directly affect bone; and (3) had no facture within the last six months. In addition, all post-menopausal women self-reposted being at least 2 years post-menopause, and were asked to obtain medical clearance from their physician. All participants in the pre-menopausal group self-reposted being eumenorrheic, and were tested during their follicular phase. The majority of younger women (17 of 20) were also on birth control. The hormonal profile of the birth control method used was noted in the medical questionnaire, but was neither an inclusion or exclusion criterion.

3.2 Study Design and Procedures

The two-day study protocol involved two visits to the Applied Physiology Laboratory at Brock University. Participants were asked to refrain from caffeine and alcohol for 8 hours prior to visiting the laboratory and to not perform any vigorous activity for 24 hours prior to their first visit and during the three-day protocol. All visits

to the laboratory were scheduled in the morning and in fasted state. This was to account for circadian rhythm and fasted to have less influence on the biochemical markers.

Upon their first arrival to the laboratory, the participants were informed of the research protocol and asked to sign an informed consent and complete a medical history questionnaire. Following this, a baseline, resting blood draw was taken using venipuncture of the median cubital vein. After the first blood sample, participants were provided with a standardized breakfast, a granola bar, a juice box, and a banana, followed by anthropometric measurements: height (cm), weight (kg), waist and hip circumference (cm), jump height (cm), and body composition. Maximal Jump height, an average of 3 jumps, was measured at 33.6±1.2 cm for the younger women and 22.7± 0.9 cm for the older women; therefore, making the box height 35.0±0.0 cm for the younger women and 26.7±0.7 cm for the older women. This was calculated using the participants jump height, measured by a vertical jump height measuring device, and having the box height be equal to or greater than 100 percent of their jump height (closes to measurement as possible). This was to ensure that the ground reaction forces produced from the box jump were sufficient for each participant to elicit a measureable response without compromising the safety of older participants. The women then performed a plyometric exercise trial followed by 2 post-exercise blood samples: 5 min, 1h, and returned the next day for a 24h post-exercise. The participants then completed a series of questionnaires including the Godin-Sheppard Leisure Time Exercise Questionnaire, and a Food Frequency Questionnaire between the various blood draws during the first visit as visually illustrated in Figure 5.
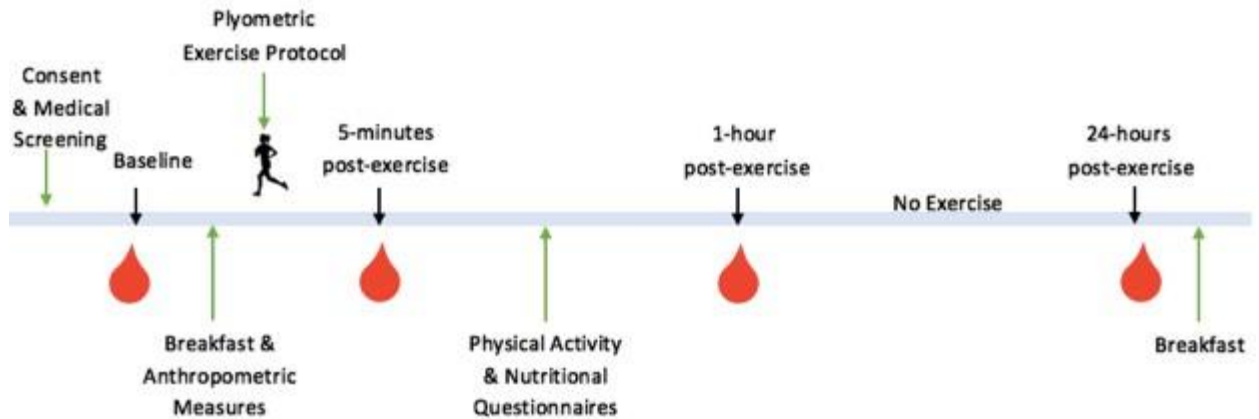
Figure 5: Study Protocol.

3.3 Exercise Trial

The acute exercise plyometric trial was designed to elicit an impact on bone through various exercises performed in a circuit as previously used in our laboratory (80,81). It began with 5-min warm-up on the cycle ergometer and dynamic stretching. The participants were familiarized with the exercises through a detailed explanation and a demonstration of each exercise. The exercise protocol consisted of a total of 128 jumps organized into five circuit exercise stations with three minutes of rest between each station. The circuit contained box jumps, lunge jumps, tuck jumps, single-leg hops, and jumping jacks. Each participant was instructed to rotate through each of the five stations 3 times for 3 sets of 8 repetitions of these jumps, with the exception of the single-leg hops, which were performed as 2 sets of 8 repetitions on each leg. The box jump height was adjusted to the individual's jump height measured; therefore, allowing the participant to jump at least 100% percent of their jump height to elicit a ground reaction force within the bone. The participants were also encouraged to go at their own pace to ensure comfort

and reduce injury during the protocol. A five-minute cool down using static stretching completed the exercise protocol.

3.4 Measurements

3.4.1 Anthropometry

Measurements of body mass, body mass index (BMI), and percent body fat (%BF) were taken using the InBody520 bioelectrical impedance analysis (BIA) system (Biospace.228). Participants were asked to be well hydrated before the BIA measures, and were asked to void all liquids before the measurement. Height was measured using a stadiometer to the nearest 0.1 cm. Jump height was measured using a Vertec Jump Height measurement device to the nearest 1.0 cm. Waist and hip circumference were measured using a measuring tape (under clothing for the waist circumference and over clothing for the hip circumference) to the nearest 0.1 cm. These measures were taken with no shoes and in exercise clothing. To maintain measurement consistency, the same investigator performed all anthropometric measures.

3.4.2 Physical Activity and Dietary Intake

Physical activity was assessed by the Godin-Shepard Leisure Time Exercise Questionnaire using intensity categories of light, moderate or strenuous exercise (104). This questionnaire can be used in male and female populations of all ages. The Godin-Shepard Leisure Time Exercise Questionnaire estimates the weekly physical activity metabolic equivalent (METs/week) based on the number of 15 min blocks at each intensity level. The number of blocks at each intensity level is then multiplied by known energy consumption values to determine metabolic equivalents (104). This questionnaire

is simple to follow, accurate, and easy to analyse, and it has been demonstrated to be valid and reliable in male and female paediatric, adolescent, and adult populations (104).

Participants' dietary intake was determined using the Block Questionnaire 2014, which is designed to determine nutritional habits over the last 6 months. Portion sizes were provided in a pictorial display to maintain consistent portion size between participants. The questionnaires were analysed by Nutrition Quest (Nutrition Quest, USA) to provide estimations of total caloric intake (kcal/day) and energy balance (kcal/day). These values were used to determine energy balance for the participants.

3.5 Serum and Plasma Analysis

Four venous blood samples were taken: at rest, and at 5 min, 1h, and 24h following the exercise. At the first-time point, (i.e., resting sample) 18 ml of blood was collected, and 10 ml of blood was collected at all post-exercise blood draws. The serum for the biochemical markers was collected in vacutainers with an SST serum separator and the plasma samples for the endocrine markers were collected in vacutainers that contain K_2EDTA.

At all blood draw instances, the hematocrit was measured to determine changes in plasma volume. This was to ensure the alteration in the markers measured were true measures due to the exercise intervention and not influenced by plasma volume changes. Both serum and plasma samples were allowed to clot in the laboratory for 30 minutes at room temperature before centrifuging. The vacutainers were centrifuged at 3000 x gravity for 15 minutes to separate the serum and plasma from the red blood cells. The serum was

stored at -80°C until all samples were collected. Plasma samples were stored in cryotubes and frozen at -80°C until all samples were collected and were ready for analysis.

CTX was assayed using a Human ELISA kit (E-EL-H0835, Elabscience, Bethesda, MD, USA). The intra-assay coefficient of variation average was 6.44% for all 5 plates. The average inter-assay coefficient for 5 plates was 6.54%. Sclerostin was assayed using four Human SOST/Sclerostin Quantikine ELISA kits (DSST00, R&D Systems, Minneapolis, MN, USA). The intra-assay coefficient of variation average was 7.68% between the 6 plates, and the inter-assay coefficient of variance between the 6 plates was 7.10%. The results of the bone marker assays were assessed in duplicate using a Synergy HT Biotek spectrometer and Gen 5 Software at a wavelength of 450 nm with a correction wavelength of 570 nm. All samples from an individual participant were analyzed on a single plate. DKK-1 and OPG were analyzed using Magnetic Luminex Assays (LXSAHM, R&D Systems, Minneapolis, MN, USA). The inter-assay coefficient of variation average was 6.32% and 8.23%, respectively for all 5 assays. The intra-assay was 5.09% and 5.71%, respectively for all 5 assays. All assays were analyzed using the Luminex MAGPIX reader as well as the MAGPIX software. Estrogen was measured using am ELISA kit (KGE014, R&D Systems, Minneapolis, MN, USA), with an inter-assay coefficient of variation was 11.67%.

3.6 Statistical Analysis

Normal distribution was screened and verified using the Kolmogorov-Smirnov test of normality, as well as for skewness and kurtosis. Group differences for anthropometric measures, dietary intake, physical activity, and resting biochemical

concentrations were tested using an independent t-test. For other normally distributed variables, a series of two-way repeated measures (group X time) ANOVAs were used to assess group differences over time in the biochemical markers. When significant main effects for group were found, pairwise comparisons for each time point were made using the Tukey post-hoc test. In the event of a significant time effect, further pairwise comparisons were made using paired t-tests to determine significant differences between time points within each group. In the case of CTX, which was not normally distributed based on the normality criteria, non-parametric tests were used to examine the difference between groups (Mann-Whitney tests), as well as the time effects within groups (Friedman tests with Wilcoxon tests for post hoc pairwise comparisons).

Note that from a total of 160 blood samples (40 participants x 4 time points) there were 16 missed samples. Missing values were imputed using the group's mean value for that particular time point. In addition, if the assumption of sphericity for a particular case was not met, the Greenhouse-Geisser test of significance was used. If the assumption of equality of covariance was not met, a Pillia's Trace test of significance was used. Significance was accepted at an alpha level of <0.05 for all analyses. Statistical Analysis was performed using SPSS version 22 for Windows.

CHAPTER 4: RESULTS

4.1 Baseline measurements

The physical characteristics of the participants are presented in Table 3. Post-menopausal women were significantly older and had significantly higher BMI, waist, and hip circumference. There was no other significant difference between the groups.

Table 3: Physical Characteristics of participants in both age groups.

	Pre-Menopausal (n=20)	Post-Menopausal (n=20)	*P*-value
Age (years)	23.14 ± 0.51	57.90 ± 0.97	<0.0001*
Weight (kg)	62.63 ± 1.83	65.63 ± 2.03	0.280
Height (cm)	165.70 ± 1.88	163.90 ± 1.58	0.468
Body Fat Percentage (%)	24.71 ± 1.24	30.99 ± 1.90	0.008*
BMI	22.74 ± 0.54	24.70 ± 0.79	0.046*
Waist Circumference (cm)	71.62 ± 1.35	79.21 ± 1.81	0.002*
Hip Circumference (cm)	90.31 ± 2.46	99.78 ± 1.93	0.005*
Waist-Hip Ratio	0.79 ± 0.01	0.78 ± 0.01	0.729

All Values are Expressed as Mean ± Standard Error of Mean; *indicates a significant group difference

Energy intake and expenditure for both the pre- and post-menopausal women are depicted in Table 4. There were no significant differences between the groups in terms of their physical activity energy expenditure, total energy intake, fat, protein, or carbohydrate intake.

Table 4: Energy intake and energy expenditure of participants in both age groups.

	Pre-Menopausal (n=20)	Post-Menopausal (n=20	*P*-value
Total Energy intake (kcal/day)	1777.75 ± 135.11	1509.70 ± 126.31	0.156
Fat (g/day)	65.81 ± 5.66	63.51 ± 6.40	0.788
Protein (g/day)	68.64 ± 5.77	61.10 ± 4.46	0.311
Carbohydrates (g/day)	197.18 ± 17.98	171.31 ± 17.10	0.304
Physical Activity Energy Expenditure Metabolic Equivalent (METs/week)	75.52 ± 9.13	70.75 ± 9.02	0.712

All Values are Expressed as Mean ± Standard Error of Mean; *indicates a significant group difference

Morning, resting baseline concentrations of all markers, osteokines and hormones for both the pre- and post-menopausal women are shown in Table 5. First, estradiol was lower in older women, confirming their post-menopausal status. Post-menopausal women have significantly higher levels of CTX, sclerostin and OPG compared to the pre-menopausal women. Moreover, post-menopausal women demonstrated a significantly lower level of DKK-1 at baseline compared to their pre-menopausal counterparts.

Table 5: Resting concentrations of Bone Turnover Markers and Osteokines.

	Pre-Menopausal (n=20)	Post-Menopausal (n=20)	*P*-value
CTX (pg/ml)	292.0 ± 29.0	431.0 ± 33.0	0.05*
Sclerostin (pg/mL)	264.56 ± 26.61	542.69 ± 64.58	<0.0001*
DKK-1 (pg/mL)	2404.74 ± 136.73	1949.69 ± 177.95	0.049*
OPG (pg/mL)	372.45 ±15.14	535.79 ± 36.85	<0.0001*
Estradiol pg/mL	97.23 ± 12.41	67.36 ± 4.75	0.028*

All Values are Expressed as Mean ± Standard Error of Mean; *indicates a significant group difference; CTX= C-terminal crosslinking telopeptides of type I collagen; DKK-1= dickkopf-1; OPG=Osteoprotegerin.

4.2 Exercise response

At each time interval, plasma volume was determined to support the significance of a time-alteration for the bone turnover markers and osteokines measured. It was determined that no significant plasma change was observed in any of the participants, indicating that values of the markers measured are that of true magnitude and not influenced by plasma volume changes (Table 4).

Table 6: Plasma volume as a percent of total blood volume for each time point.

Baseline (%)	5-min post-exercise (%)	1 hour post-exercise (%)	24 hours post-exercise (%)
0.58 ± 0.03	0.58 ± 0.03	0.58 ± 0.03	0.59 ± 0.03

A significant time effect was observed for CTX using the Friedman test for repeated measures with the Wilcoxon post hoc comparisons indicating a significant increase between baseline and 5 min post-exercise in the young women. Unfortunately, the Friedman non-parametric analysis does not test the time-by-group interaction. However, no significant changes were found in the older women (Figure 6a). Furthermore, for ease of interpretation, Figure 6b summarizes the percent change in the median concentrations of CTX from pre- to post-exercise in each group.
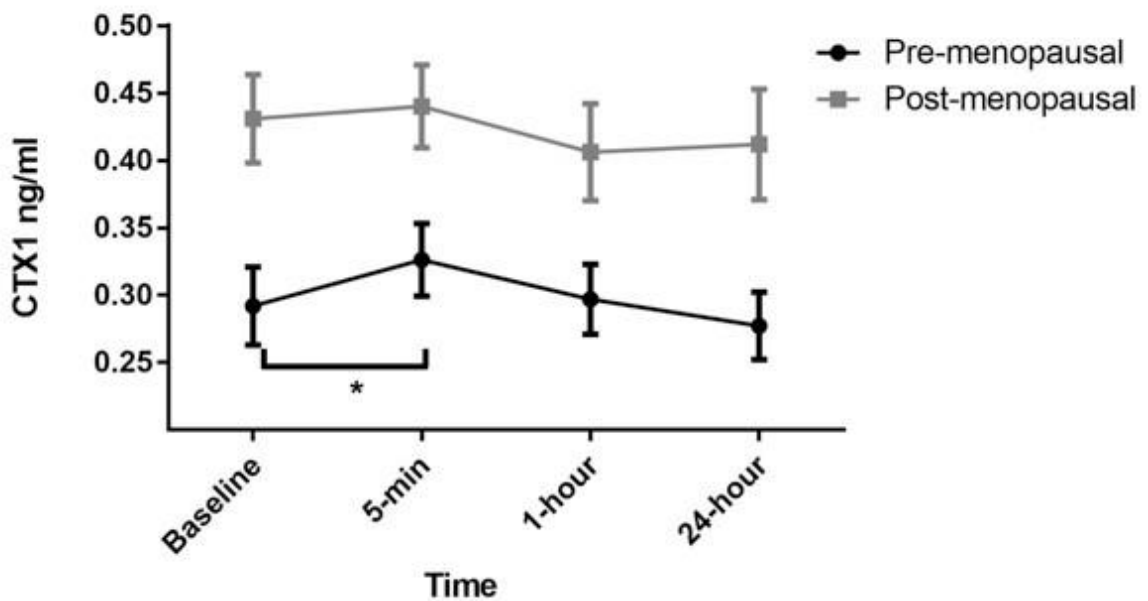
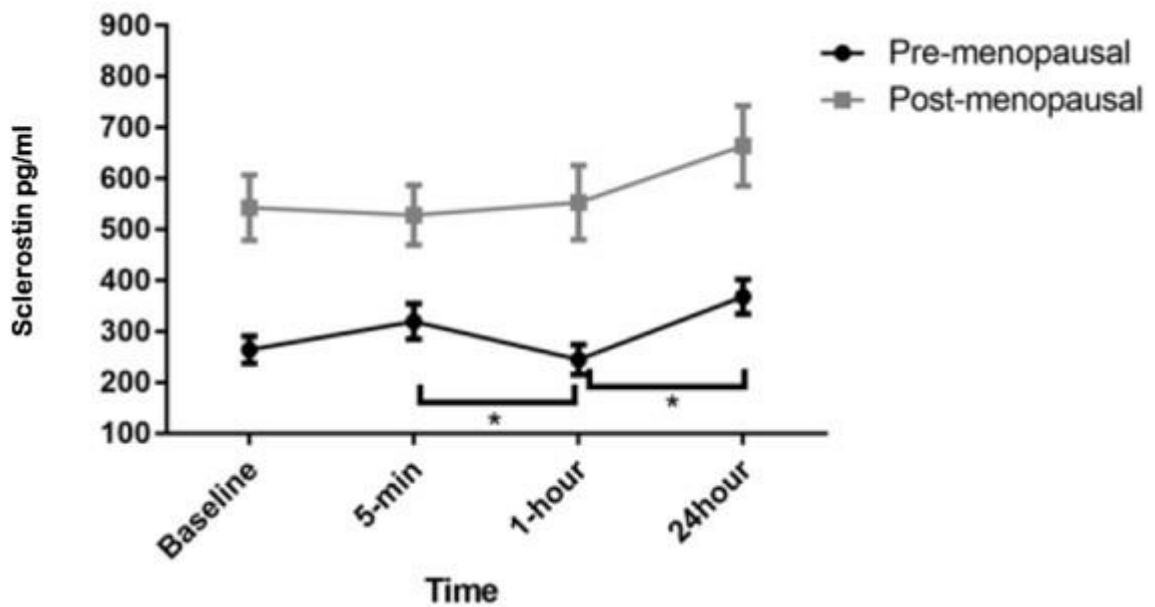**Figure 6a:** C-terminal crosslinking telopeptides of type I collagen (CTX) concentration from pre- to 24h post-exercise in younger (N=20) and older (N=20) women. Values are expressed as mean ± standard error of mean; *indicates significance in the Wilcoxon post-hoc pairwise comparisons.
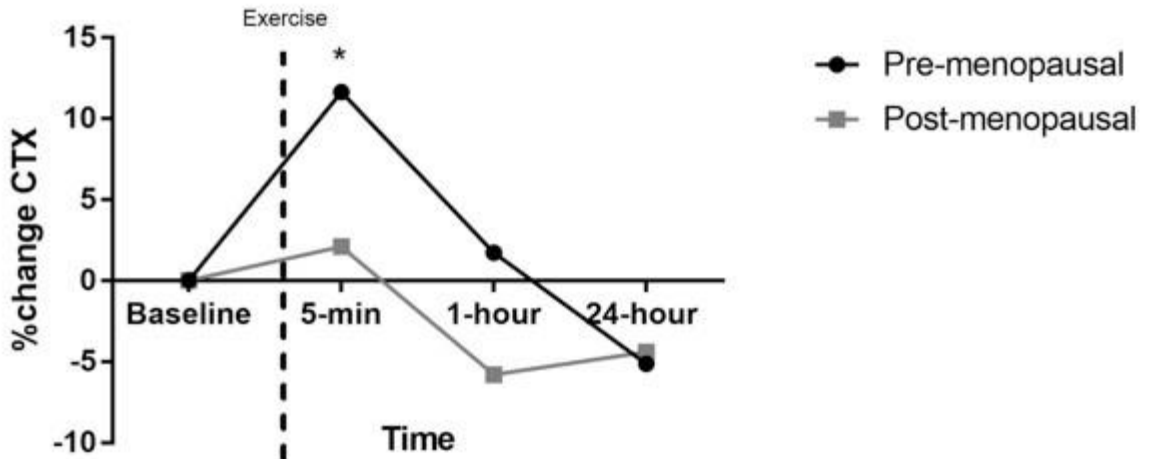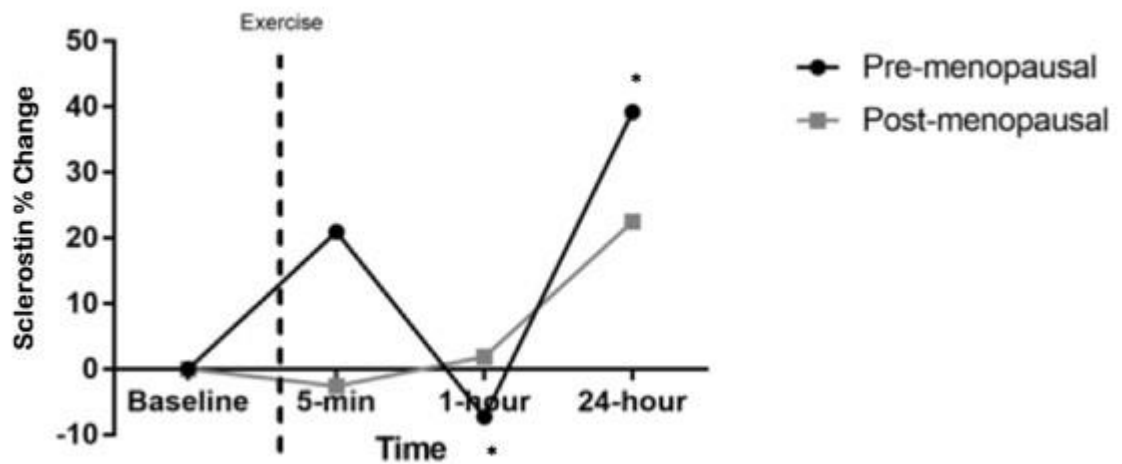
Figure 6b: Percent changes in C-terminal crosslinking telopeptides of type I collagen (CTX) from pre- to 24h post-exercise in younger (N=20) and older (N=20) women. Values are expressed as mean ± standard error of mean (note that when the standard error is <2% the error bars are not shown on the graph). *indicates significance in the Wilcoxon post-hoc pairwise comparisons.

In addition to a group-effect observed in the resting levels of sclerostin, there was a significant time effect and a significant group-by-time interaction. In pre-menopausal women, sclerostin significantly decreased from 5 min to 1h post-exercise, then significantly increased from 1h to 24h post-exercise (Figures 7a and 7b). There were no significant time-related changes in the post-menopausal group.
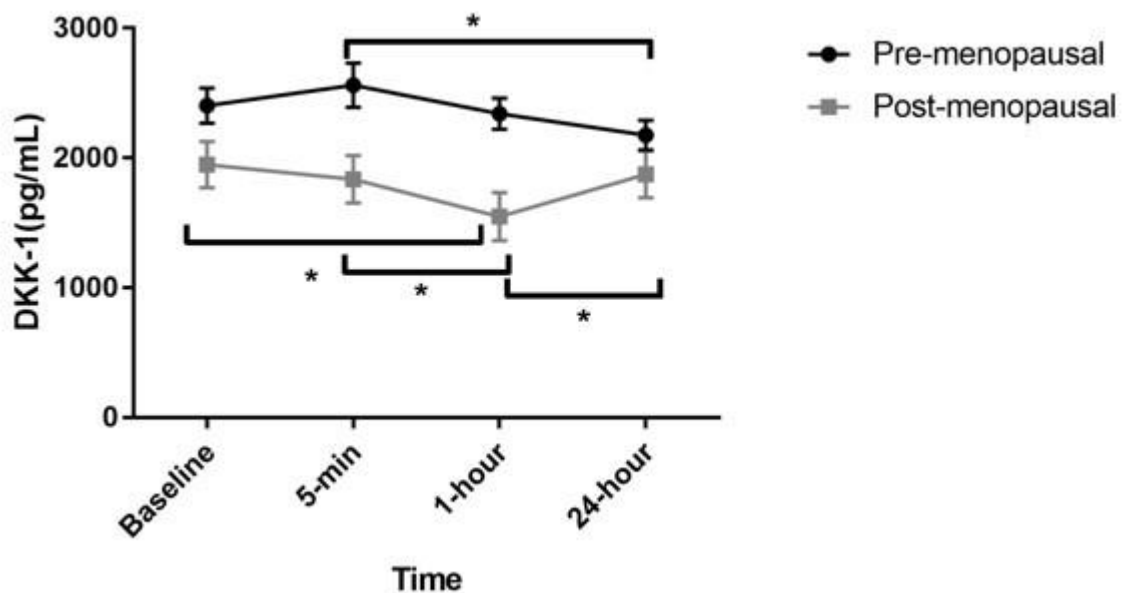
Figure 7a: Sclerostin concentration from pre- to 24h post-exercise in younger (N=20) and older (N=20) women. Values are expressed as mean ± standard error of mean; *indicates significance in the Tukey post-hoc pairwise comparisons.
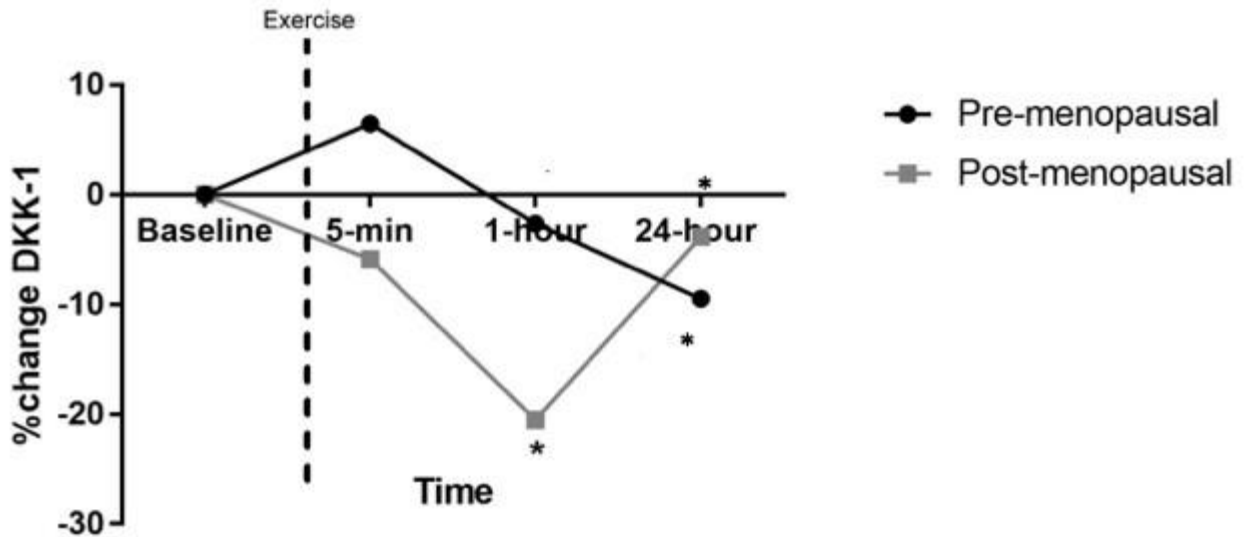

Figure 7b: Percent changes in Sclerostin from pre- to 24h post-exercise in younger (N=20) and older (N=20) women. Values are expressed as mean ± standard error of mean (note that when the standard error is <2% the error bars are not shown on the graph). *indicates significance in the Tukey post-hoc pairwise comparisons.

A significant time effect and a significant group-by-time interaction were observed in DKK-1. In the pre-menopausal women, there was a significant decrease from 5 min to 24h post-exercise (Figures 7a and 7b). In the post-menopausal women, a significant decrease in DKK-1 concentration presented from baseline to 1h post-exercise and from 5 min to 1h post-exercise In addition, a significant increase from 1h to 24h post-exercise was observed in the post-menopausal women (Figures 8a and 8b).
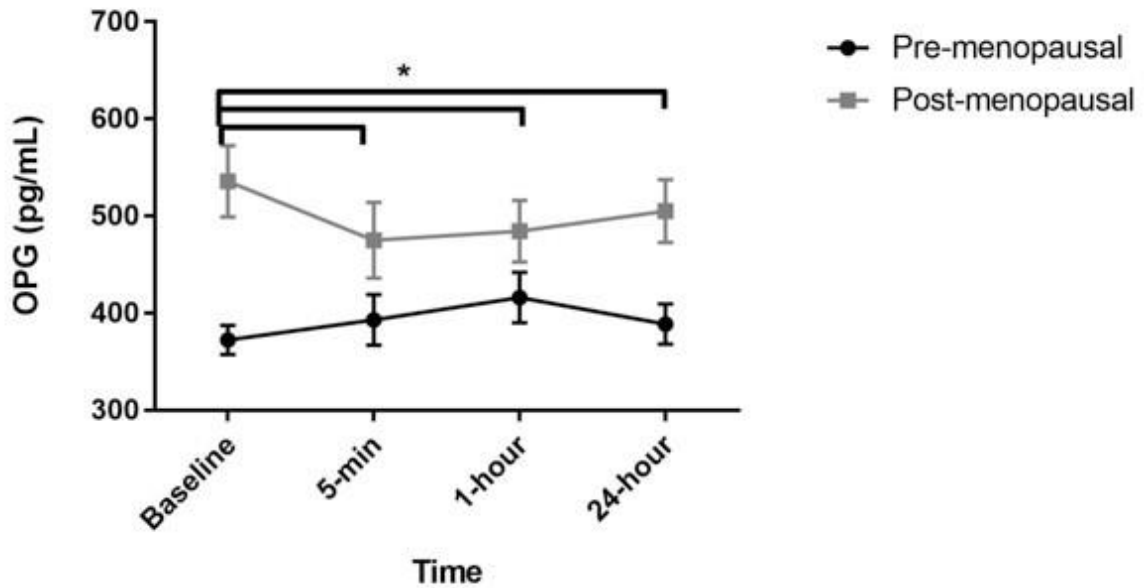

Figure 8a: Dickkopf-1 (DKK-1) concentration from pre- to 24h post-exercise in younger (N=20) and older (N=20) women. Values are expressed as mean ± standard error of mean; *indicates significance in the Tukey post-hoc pairwise comparisons.
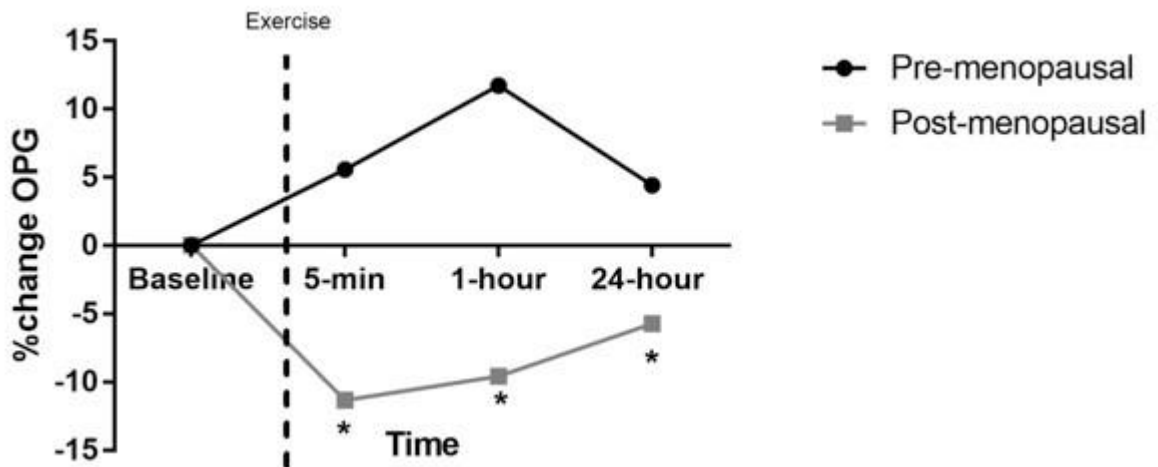
Figure 8b: Percent changes in Dickkopf-1 (DKK-1) from pre- to 24h post-exercise in younger (N=20) and older (N=20) women. Values are expressed as mean ± standard error of mean (note that when the standard error is <2% the error bars are not shown on the graph). *indicates significance in the Tukey post-hoc pairwise comparisons.

There was a significant time effect and a group-by-time interaction for OPG. Specifically, in post-menopausal women OPG significantly decreased from baseline to 5 min and continued to be lower than baseline both 1h and 24h (Figures 9a and 9b).

Figure 8a: Osteoprotegerin (OPG) concentration from pre- to 24h post-exercise in younger (N=20) and older (N=20) women. Values are expressed as mean ± standard error of mean; *indicates significance in the Tukey post-hoc pairwise comparisons.

Figure 9b: Percent changes in Osteoprotegerin (OPG) from pre- to 24h post-exercise in younger (N=20) and older (N=20) women. Values are expressed as mean ± standard error of mean (note that when the standard error is <2% the error bars are not shown on the graph). *indicates significance in the Tukey post-hoc pairwise comparisons.

CHAPTER 5: DISCUSSION

To our knowledge, this is the first study to compare bone markers and Wnt signalling related osteokines at rest and in response to an acute bout of plyometric exercise over a 24-hour duration between younger, ages 20-30 in their follicular phase, and older women, 50-65 post-menopause. Compared to the younger women (pre-menopausal), at rest CTX, sclerostin and OPG were significantly higher in the older women, and DKK-1 and estrogen were significantly lower in the older women (post-menopausal). Second, there was a significant time effect for all markers and osteokines but the responses varied between groups. CTX increased post-exercise, but only in the younger women, sclerostin significantly decreased 1h post-exercise and then increased 24h post-exercise only in young women, while DKK-1 continued to decrease in both groups up to 24h following plyometric exercise, and OPG also decreased post-exercise but only in the older women.

5.1 Bone Markers and Osteokines at rest

At rest, levels of serum CTX were higher in the older (post-menopausal) women compared to the younger (pre-menopausal) women. This is indicative of a group difference in CTX that may reflect differences in the rate of bone turnover (30–33). More specifically, post-menopausal women are known to have higher serum concentrations of CTX compared to pre-menopausal women (30–33). This increased serum level may be reflection of the increase in bone resorption rates seen in post-menopausal women, which

influences the rate of bone turnover, leading to consequential bone loss in older women (30–33).

Resting sclerostin was significantly higher in post-menopausal women compared to the younger women, and this difference is quite possibly associated with the significantly lower resting estrogen levels also found in the post-menopausal women when compared to the younger women. As women age and enter menopause, a significant decrease in circulating estrogen is observed, which in turn has been associated with higher serum sclerostin concentrations (47,105–107). The lower estrogen found in our group of post-menopausal women confirms the association of estrogen with sclerostin, as these results are in agreement with previous studies that indicate a negative correlation between estradiol and sclerostin serum levels (5,43,47,105–107). This is important because the younger women were in the follicular phase of their menstrual cycle when estrogen are lower, thus at the closest possible level to that of their older, post-menopausal, counter parts. In addition, testing all younger women during their follicular phase allows for consistency in the levels of estrogen within the younger group because of the possible differences in the bone response to exercise at the different phases of the menstrual cycle.

In contrast, DKK-1 concentrations at rest (i.e., pre-exercise) were significantly higher in the younger (pre-menopausal) women compared to the older (post-menopausal) women. These findings contradict a previous study by Coulson et al., who found resting DKK-1 concentrations to be lower in younger women compared to older women (108). Furthermore, at baseline, pre-menarcheal girls, who have lower estrogen levels, demonstrated higher DKK-1 serum concentrations compared to older post-menarcheal

girls (80). Based on this previous literature, one would predict that the post-menopausal women would have a significantly higher level of DKK-1 at rest. This difference between the studies may be due to the fact that DKK-1 is not bone specific like sclerostin, and so free DKK-1 concentrations may be indicative of other functions such as muscle damage occurring during the protocol (109). This makes it difficult to make sensible conclusions based on these contradicting studies. However, due to DKK-1's relation to bone metabolism, this is a sensible supporting measure to other more concrete markers. This data in combination with CTX, sclerostin and OPG allows for a large picture of what may be occurring at a cellular level during plyometric exercise.

Resting OPG was significantly lower in the younger (pre-menopausal) women compared to the older (post-menopausal) women. This finding contradicts expectations of this osteokine, as OPG is a bone protector that acts as deterrent receptor to the bone resorption osteokine RANKL (4,57,55,56,58). As well, estrogen simulates the secretion of OPG, which has been demonstrated to be lower in the post-menopausal women, and the reduction in estrogen increases the likelihood for the RANKL-RANK interaction to occur, increasing bone resorption mechanisms (4). Therefore, OPG was anticipated to be higher in the younger women compared to the older women (4,57,55,56,58). However, using the same plyometric acute protocol, Dekker et al. found no significant differences in resting OPG between the pre- and post-menarcheal girls regardless their differences in estrogen (80). It is postulated that the higher levels of OPG found in post-menopausal women were a possible protective measure of bone formation that may be influenced by outside measures that were not taken in to consideration in this project. As such, more observation is needed to determine a definitive pattern between the age groups.

5.2 Bone Markers and Osteokines in response to exercise

The response of CTX to a single bout of plyometric exercise was attenuated and not significant over 24h post-exercise in the older women. In contrast, our results show that a single bout of plyometric exercise, altered serum CTX concentrations in younger, pre-menopausal women over 24 hours. In comparison to baseline measures of serum CTX, a significant increase was observed at 5 min post-exercise in the younger group. However, in a group of males and females, no changes were seen post-exercise when performing progressive cycling bouts (85). This can be explained possibly by the difference in of modality and the lower intensity level of exercise. The influence an exercise protocol has on a specific bone marker is impacted by intensity, modality, and duration of the intervention. More intense, impactful exercise is predicted to have a greater response compared to a lighter form of exercise. When exercise induces increased level of mechanical strain on bone, a larger magnitude of response occurs. For instance, weighted, increased repetitions, and more singular exercises may have caused a larger response compared to what was seen post-exercise in the women in our study. As such, more research is needed to determine concrete conclusions and what threshold needs to be reached in order to produce the desired response in osteokines and markers of bon turnover (85,88–91). Furthermore, a catabolic response leading to higher resorption, marked by an increase in CTX post-exercise, may complement the process of remodelling, as an increased breakdown is followed by higher bone formation (1,2,4). Therefore, the effect that plyometric exercise is having on CTX in younger women is indicative of an increase in bone turnover rate (1,2,4). The CTX increase 5 min post-exercise, was then followed by a return to baseline. It is postulated that although there

was no post-exercise decrease in CTX in acute exercise, a long-term training intervention could lead to an overall decrease of CTX (85,88–91). However, more research is needed to understand the timing of such responses.

A significant time effect and a significant group-by-time interaction in sclerostin concentrations were found, suggesting that sclerostin responds to the acute plyometric exercise differently between these two groups of women. Sclerostin had no significant changes post-exercise in older women. This has been previously demonstrated in post-menopausal women during a 12-month resistance and aerobic exercise training intervention, and therefore could be validated, as no response was shown post-exercise (98). However, in younger women, sclerostin significantly decreased from 5 min to 1h post-exercise, then significantly increased to near resting levels 24h post-exercise. This decrease in post-exercise levels of sclerostin indicates an exercise-induced upregulation of Wnt-signalling, favouring bone formation in young women. A similar, but not significant, decrease was previously found in pre- and post-menarcheal girls in response to a similar plyometric exercise protocol (80). However, in young men, sclerostin concentrations have been found to increase from pre- to 5 min following plyometric exercise (81). This indicates a sex difference in the osteogenic response to plyometric exercise between young men and women of the same age range and physical activity levels. It is also interesting that not only was the direction of the response different but the timing as well. Specifically, men had higher sclerostin 5 min post-exercise whereas women had no change in sclerostin 5 min post-exercise but saw a decrease from 5 min to 1h post-exercise (81).

In younger (pre-menopausal) women, DKK-1 did not change 5 min post-exercise but then significantly decreased from 5 min to 1h and 24h post-exercise, indicating a late post-exercise response. In the older (post-menopausal) women DKK-1 significantly decreased from pre- to 5 min and 1h post-exercise, then increased back to near resting levels 24h post-exercise, indicating an immediate post-exercise response. Overall, an exercise-induced decrease in DKK-1 seems to be consistent across age groups in females, as an immediate decreasing post-exercise trend was also observed in pre- and post-menarcheal girls (80). We speculate that these differences in the timing of the response may be related to estrogen. Our pre-menopausal women, who have a later post-exercise decrease, have a higher level of estrogen compared to all other age groups, i.e., the pre- and post-menarcheal girls, and the post-menopausal women, who have a similar immediate response in DKK-1. These observations could be indicative of an age-related timing of the DKK-1 response to high impact exercise influenced by circulating levels of estrogen. This could be due to the higher estrogen levels in the pre-menopausal women creating a protective measure, not allowing DKK-1 to inhibit the Wnt pathway, allowing for more potential formation to occur compared to the other groups which would decrease the serum levels of DKK-1. However, due to the nature of DKK-1, these results could be influenced by the breakdown of muscle, a by-product of muscle damage, occurring in all populations during the exercise protocol and this influence should be taken in to consideration. No other study to date has assessed the DKK-1 response to exercise and a more cellular explanation is needed in order to make definitive conclusions.

Younger women had no OPG response post-exercise, whereas older women had a significant continuous decrease from baseline to up to 24-hours post-exercise. The

difference in post-exercise response between the groups was reflected in by the significant group-by-time interaction. As OPG is considered a protective osteokine (4,57,55,56,58), a higher level of OPG in post-menopausal women may be reflective of either an age response to bone metabolism or a result to a decrease in RANKL concentrations. Indeed, RANKL decreased between 1h and 24h post-exercise in both pre- and post-menarcheal girls using a similar protocol leading to an increase in OPG/RANKL ratio (80). It can be speculated that a decrease in OPG levels may be indicative of a larger decrease in RANKL, and therefore, leading to an overall anabolic increase in their ratio (4,57,55,56,58). However, RANKL was not measured in our study. Moreover, the post-exercise response was only found in the older group, and not in the pre-menopausal women, which indicates an age-related response to an acute bout of plyometric exercise.

5.3 Strengths and Limitations

This study had several strengths. Having the visits scheduled in the morning allowed us to control for circadian rhythm variability. In addition, the measurement of jump height (more specifically in the older women) resulted in a safe box jump height adjusted to each participant's jumping ability so not to cause over exhaustion or increased chance of falling. A significant strength was the measurement of post-exercise plasma changes. We found no significant changes in plasma volume across time; thus, the observed differences in the concentrations of osteokines were not influenced by changes in plasma volume.

The study also has some limitations. First, the sample of 20 women per group was a limiting factor. Most of the markers were normally distributed, however CTX was not and the use of non-parametric statistics prevented the direct comparison between groups for the repeated measures. Second, physical activity status was self-reported, and we found our groups included a range of less active individuals and more active individuals, which could skew the effect of the exercise protocol. As well, due to a defect in the ELISA kits for P1NP we were unable to measure P1NP, which is a direct marker of bone formation, and this limits our ability to have a complete and clear interpretation on the effect of sclerostin and DKK-1 on bone turnover.

CHAPTER 6: CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

This study is the first to determine significant differences in bone markers and Wnt-influencing osteokines between younger and older women both at rest and in response to an acute bout of plyometric exercise. At rest, post-menopausal women had significantly higher CTX, sclerostin, and OPG concentrations, and lower DKK-1 serum concentrations compared to the younger (pre-menopausal) women. Plyometric exercise induced an osteokine response and a bone turnover response over a 24-hour period in both groups, but with differences in the timing of this response. CTX significantly increased immediately post-exercise in younger women; however, no response was seen in older women. Sclerostin significantly decreased post-exercise in the younger (pre-menopausal) women, but not in the post-menopausal group. DKK-1 significantly decreased post-exercise in both groups, but this response was delayed and prolonged for up to 24h in the younger women. OPG significantly decreased post-exercise but only for the older women. Overall, these results suggest that age, especially menopause, is an important factor of bone's response to a plyometric exercise protocol in women.

These findings are significant and should be used to further knowledge about bone turnover and osteokines in response to exercise. Further research should be done to understand the mechanisms involved and if the changes in markers are physiologically meaningful in terms of changes in bone structure, density or remodelling. As well, an

exercise training intervention is needed to examine whether the responses found in this acute exercise study can be influential long-term.

6.2 Future Directions

The current study examined the effect of a single bout of plyometric exercise in younger and older women. A previous study observed the effect of the same exercise in young men and boys. An age difference was seen between the younger and older women, specifically in sclerostin concentrations, and in the boys and men, while there were also differences observed in the osteokine response to exercise. Therefore, a proposed future study should examine the osteokine response in older men to complete all age groups. It would be interesting to see if indeed the bone response of serum markers of bone turnover and osteokines to exercise is age-dependent also in men as seen in women.

In addition, a plyometric exercise intervention, either short-term or long-term, should be considered. It would be fascinating to determine if there could be either a training effect, a lasting effect, or an overall impact on the Wnt signalling related osteokines as a direct result of the short-term or long-term plyometric training. Furthermore, detecting variations in osteokine alterations would be interesting and informative. Do duration, intensity and type of exercise matter? Comparing different training modalities, durations, and intensities would be very interesting: is the response of a different magnitude? These are questions that can be examined by a short-term or long-term plyometric training protocol.

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APPENDICES

Young Women Recruitment Poster

Participants needed for
Exercise & Bone Research

Who: Women over the age 18 years

Why: To determine the effect of exercise on bone turnover

What is involved: Two visits to the lab (90 min + 15 min)

An exercise session and blood samples before and after exercise.

$20 honorarium for participating.

Contact Kate Nelson

905 688-5550 ext. 5623 OR kn10ko@brocku.ca

This study has been reviewed and received clearance from the Brock University Research Ethics Board (file #14-267) – reb@brocku.ca, 905-688-5550 ext 3035.
Principal Investigator, Dr. Nota Klentrou, Department of Kinesiology, can be contacted at 905 688-5550 (ext. 4538) OR nklentrou@brocku.ca

EXERCISE & BONE RESEARCH Contact Kate Nelson at 905-688-5550 ext.5623 kn10ko@brocku.ca

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EXERCISE & BONE RESEARCH Contact Kate Nelson at 905-688-5550 ext.5623 kn10ko@brocku.ca

Participants needed for
Exercise & Bone Research

Who: Women age 50-65 years

Why: To determine the effect of exercise on bone turnover

What is involved: Three visits to the lab (90 min + 15 min + 15 min)

An exercise session and blood samples before and after exercise.

$20 honorarium for participating.

Contact Kate Nelson

905 688-5550 ext. 5623 OR kn10ko@brocku.ca

This study has been reviewed and received clearance from the Brock University Research Ethics Board (file #14-267) – reb@brocku.ca, 905-688-5550 ext 3035.
Principal Investigator, Dr. Nota Klentrou, Department of Kinesiology, can be contacted at 905 688-5550 (ext. 4538) OR nklentrou@brocku.ca

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Invitation Letter

Effects of plyometric exercise on markers of bone turnover and inflammatory cytokines in women

Principal Investigator: Dr. Nota Klentrou, Department of Kinesiology, Brock University

Co-investigators: Dr. Bareket Falk, Dr. Peter Tiidus, Katlynne Nelson, and Zack Root

We would like to invite you to participate in our current study, which investigates bone turnover after an acute bout of exercise.

The purpose of this research project is to investigate the immediate response and subsequent recovery of bone metabolism and inflammation induced by a variety of jumping exercises over a period of 48 hours in women.

Tests and measurements will require 90 minutes on one day and 15 minutes on the post-exercise days. In short, measurements will include filling out several questionnaires, completing a 30 min exercise routine and measurements of bone turnover using blood samples.

Participation in this project will provide information such as height, weight and percent body fat. Participants will receive a $20 compensation for their time and travel expenses. Parking is also provided.

This research is being performed by researchers in the Applied Physiology Laboratory of Brock University.

This study has been reviewed and received ethics clearance through the Brock University Research Ethics Board (REB #14-267). If you have any pertinent questions about your rights as a research participant, please contact the Brock University Research Ethics Officer (905 688 5550 ext 3035 or reb@brocku.ca).

If you are interested in finding more about this study, please contact us by email (kn10ko@brocku.ca or nklentrou@brocku.ca) or by phone (905 668 5500 ext 5623 or 4538)

Thank you,

Dr. Nota Klentrou
Department Kinesiology
Faculty of Applied Health Science
Brock University
Tel: 905-688-5550 ext: 4538
Email: nklentrou@brocku.ca

Appendix 2: Consent Forms

INFORMATION AND CONSENT TO PARTICIPATE IN RESEARCH

Effects of plyometric exercise on markers of bone turnover and inflammatory cytokines in girls and women

You are being invited to participate in a research study being conducted by the investigators listed below. Prior to participating in this study please read this form to find out the purpose and tests of this study. For the tests, you will have to visit the Applied Physiology Laboratory at Brock University. This study is a part of the Faculty of Applied Health Sciences (FAHS) of Brock University.

PRINCIPAL INVESTIGATOR:	DEPARTMENT	CONTACT
Dr. Nota Klentrou	FAHS, Brock University	905-688-5550 ex. 4538
CO-INVESTIGATORS:	**DEPARTMENT**	**CONTACT**
Dr. Bareket Falk	FAHS, Brock University	905-688-5550 ex. 4979
Dr. Andrea Josse-Obar	FAHS, Brock University	905-688-5550 ex. 3502
STUDENT CO-INVESTIGATORS:	**DEPARTMENT**	**CONTACT**
Rozalia Kouvelioti	FAHS, Brock University	905 688-5550 ex. 5623
Katlynne Nelson	FAHS, Brock University	905 688-5550 ex. 5623
Celina Clark	FAHS, Brock University	905 688-5550 ex. 5623
Zach Root	FAHS, Brock University	905 688-5550 ex. 5623

PURPOSE

The purpose of this study is to investigate the immediate response and subsequent recovery of bone metabolism and inflammation induced by jumping exercise over a period of 48 hours in children and adults.

DESCRIPTION OF TESTING PROCEDURES

If you agree to participate in this study, you will visit our laboratory for one 90 minute session of testing and two other sessions 24 and 48 hours later, lasting 15 minutes. At the end of the study you will be given a summary of the findings upon request. Shorts, a short sleeved shirt and running shoes are recommended for the measurements.

You will undergo the measurements and procedures listed below; please note that you may choose not to answer a question in any questionnaire.

Pre-exercise Assessments

1.) Participants will be asked to complete several questionnaires, outlining their general health, physical activity, nutritional habits and pubertal status. The health questionnaire screens if participants can safely participate in all of the required measurements, and offers us a better understanding of each participant's general health that helps us properly interpret the data. Please note that this questionnaire includes questions about smoking, alcohol consumption and drug use. In all questionnaires, you may choose not to answer any question.

2.) Body Composition: we will measure height, weight, hip circumference, waist circumference and percent body fat. Percent body fat will be estimated using skinfold thicknesses and bioelectrical impedance analysis (BIA). The BIA assessment requires participants to stand on a weight scale and grasp handles. A mild electrical current (50kHz, 800µA) will pass through hands to feet. This current cannot be felt and causes no harm. Valid measurements require abstinence from consuming caffeine and alcohol for 6 hours prior as well as refrain from vigorous or high impact exercise for a minimum of 24 hours prior to exercise testing.

3.) Vertical jump height: we will measure how high you can jump from a standstill. In brief, you will first reach up against a flat wall and mark off the highest point you can reach flat-footed (i.e, "standing reach"). Then, you will take a jump from a standstill, marking off the highest point you can reach. The distance between the two marks is your standing vertical jump height.

4.) A total of five (5) blood samples will be collected to assess biochemical markers of bone turnover and inflammatory cytokines: pre-exercise, 5 min post exercise, 60 min, 24 and 48 hours post exercise. The blood samples will be drawn using a standard technique by a certified phlebotomist hired from a licensed laboratory (e.g., Life Labs) or a registered nurse. Up to 20 ml of blood will be withdrawn. It should be noted that the venous blood drawing procedure is a routine procedure performed by a certified technician and offers minimal risk to participants. In rare instances, participants may experience slight pain and/or tingling in the area and/or a minor bruise from the needle. However, with the use of anaesthetic creams (e.g., Emla), which we use in the laboratory, any sensation of pain is minimal. Once samples are collected, we will slit them into air-tight smaller tubes for later analysis and storage. These tubes will be stored in a freezer until ready for analysis. The freezer is located in a locked laboratory, which is only accessible to qualified personnel. We will hold onto these tubes for 5 years at which time they will be disposed by qualified, trained personnel and according to biosafety and University protocols.

Exercise Protocol

The exercise session begins with a warm-up that includes 5 min of low intensity jogging and a series of dynamic stretches. The exercise protocol has been designed to provide high-impact, weight bearing loads in the form of circuit training stations. Each participant will be instructed to rotate through each of the five stations (Jumping jacks, lunge jumps, hopping, jumping over obstacles such as a bench and drop jumps) three times for a total of three sets. Jump height will be tailored to each participant based on body size. The participant will be at each station for approximately 2 minutes and the participant will then be instructed to move to the next station. A recovery period of 2 minutes will be given between each set. All exercise testing will be done in the presence of 2 study personnel, a tester/demonstrator and a spotter.

CONFIDENTIALITY

All data collected though this study will remain confidential and will be stored in locked offices and secured computers to which only the principal and co-investigators has access. You should be aware that the results of this study will be made available to other scientists though publication in a

scientific journal, but your name and personal data will not appear in the compilation or publication of these results. A master list will be kept to link participants' names with codes. This list and all data will be kept for 5 years after the date of publication, at which time all information will be confidentially destroyed. Additionally, you will have access to your own data, as well as group data when it becomes available and if you are interested. This can easily be provided to you by contacting the principal investigators.

SECONDARY USE OF DATA

Some of this data may be of use in the future for comparative purposes by colleagues, students or other researchers. The data used by these future researchers will remain anonymized, as all personal identifiers will have been removed. You may refuse to allow their information to be used in the future and still remain a subject in this study. In this case, your data will be confidentially destroyed 5 years after the date of publication.

Do you want your data to be used anonymized (i.e., de-identified) **in a future study?**

Please check one box: □ Yes, I want my data to be kept anonymized for future studies.

                          □ No, I do not want my data to be kept for future studies.

PARTICIPATION AND WITHDRAWL

You can choose whether or not to participate in this study and may remove your data from the study if you wish. You may do so at any time by contacting the principal investigator in writing (email or mail). Participants will receive pro-rated compensation in the event they withdraw. You may also refuse to answer any questions posed to them during the study and will still remain a subject in the study.

RISKS AND BENEFITS

Participation in this study will allow you to become exposed to a research protocol, contribute to the advancement of science and gain personal and general knowledge about their body. All the results will be provided to you upon request.

The only foreseeable risks involved in participation include:

a) Possible muscle soreness, muscle fatigue and/or joint pain within 48 hours of the exercise tests. This is likely to occur since you are not a usual exerciser. If this does occur, it is only temporary and will dissipate within 2-3 days. Plyometric exercise involves high impact forces so a low risk for minor injury from the jumping exercise also exists. To minimize the risk, a spotter will be present at all time. Also, for any reason, you can request to not do a particular exercise if you don't want to. Our goal is to complete all the exercises, but it is also to maintain a positive and encouraging environment during testing. We will always ask you how you are doing following each exercise.

b) In rare instances, participants may experience slight pain and/or tingling in the area and/or a minor bruise from the venous blood draw. Participants who are not allergic to medications may choose to use an aesthetic cream that usually has no side effects. However, there is a small risk for minor effects such as burning, swelling, itching, or skin rash at application site.

c) Brock students must know that their decision to participate, not participate, or withdraw will in no way affect their academic standing at Brock.

RIGHTS OF RESEARCH PARTICIPANTS

You wiU receive a sigoed copy of this conseot 01m. You may withdraw your conseot to participate in this shidy at any time, and you may also discontinue participation at aoy time without peoalty. In signifl.$ this consent form 01in pa rlic-ipa ting: in this St'l.l dy you are not waiving any legal claims or remedies. This study has b-eeo reviewed aod received clearance from *the* Brock University Research Ethic-s Board (lite u RE8 14-2671.11 v ou *have* any pertinent questions abou t yOu t ri$h ts as a research participaot, please cootact *the* 8roc-k Uo iv ersity Research Eth ics Ollicet (90S 688 5550   JOJS , reb @ b rock u .).

INFORMATION

Please contact *Ot*. N ota   at   906 688 5550   4538, ok?.eo trou @ brocku.ca 01 Kate Nelsoo at 90S 688   5550   S621, kn lOko @ brocku.ca if you have any questions abou t this St'l.l dy.

I HAVE READ AND UNDERSTAND THE ABOVE EXPLANATION OF THE PU RPOSE ANO PROCEDURES OF THE PROJECT. I HAVE ALSO RECEIVED A SIGNED COPY OF THE INFORMATION ANO C-ONSENT FORM. MY QUESTIONS HAVE BEEN AN SWERED TO MY SATISFACTION AND I AGREE TO PAR TICIPA TE lN THIS STUDY.

_____   _____
SIGN ATURE OF PARTICIPANT   DATE

_____
PRINTED NAME Of PARTICIPANT

_____   _____
WITN ESS   DATE

In my judement *the* participan t is voluntarily and know in gly giving i nform ed consent and possesses *the* legal capacity to give informed consen t and participate in this research stud'y.

_____   _____
SIGN ATURE Of INVESTIGATOR   DATE

Physician Medical Consent Form

Your patient _____ has expressed interest in participating in our research study in the Department of Health Sciences at Brock University (see attached Letter of Information and review paper for details). We ask that you sign this letter to acknowledge your approval of your patient's participation in our study and more specifically that your patient is able to participate in the exercise aspect of the study. Please see attached the consent form for the study that outlines the expectations of the study and the plyometric exercise protocol. We appreciate your support. Please do not hesitate to contact us if you have any questions, comments or concerns.

Sincerely,

Dr. Nota Klentrou and Ms. Kate Nelson
Department of Health Sciences, Brock University
905-668-5550 ext. 4538
905-668-5550 ext. 5623

_____

2016-11-09

Dear Dr. Klentrou and Ms. Nelson

This is to acknowledge that my patient _____ has expressed interest in participating in your study and that I approve of her participation.

_____   _____
Health Care Provider Signature   Date

Appendix 3: Questionnaires

SUBJECT SCREENING AND MEDICAL HISTORY QUESTIONNAIRE

Name: _____   Date: _____

Date of Birth: _____

Dominant Hand: _____   Dominant Leg: _____

Your responses to this questionnaire are confidential. If you answer "YES" to any of the following questions, please give additional details in the space provided and discuss the matter with one of the investigators. You may refuse to answer any of the following questions.

1. Have you ever had any major joint instability or ongoing chronic pain such as in the knee, back or elbow?   **YES**   **NO**

2. Are you currently taking any medication (including aspirin) or have you taken any medication in the last two days?   **YES**   **NO**

3. Have you taken any medication in the past six months?   **YES**   **NO**

4. Is there any medical condition with which you have been diagnosed and are under the care of a physician (e.g. asthma, diabetes, anorexia)?   **YES**   **NO**

5. Do you, or have you in the past, consumed any alcohol on a regular basis?   **YES**   **NO**

6. Do you, or have you in the past, smoked on a regular basis?   **YES**   **NO**

7. Are you, or have you in the past, engaged in any extreme diet?   **YES**   **NO**

8. Do you, or have you in the past, engaged in physical activity on a regular basis?   **YES**   **NO**

9. Have you had any fractures?   **YES**   **NO**

10. **FEMALES ONLY:** Have you had your period?   **YES**   **NO**

GODIN-SHEPHARD LEISURE-TIME EXERCISE QUESTIONNAIRE

1. Considering a **7-day period** (a week), how many times on the average do you do the following kinds of exercise for **more than 15 minutes** during your **free-time** (write on each line the appropriate number)?

Times Per Week

(a) STRENUOUS EXERCISE
    (HEART BEATS RAPIDLY)

    ──────────
    (i.e. running, jogging, hockey, football, soccer, squash, basketball,
    cross country skiing, judo, roller skating, vigorous swimming,
    vigorous long distance bicycling)

(b) MODERATE EXERCISE
    (NOT EXHAUSTING)

    ──────────
    (i.e. fast walking, baseball, tennis, easy bicycling, volleyball,
    badminton, easy swimming, alpine skiing, popular and folk dancing)

(c) MILD EXERCISE
    (MINIMAL EFFORT)

    ──────────
    (i.e. yoga, archery, fishing from river bank, bowling, horseshoes,
    golf, snow-mobiling, easy walking)

2. Considering a **7-day period** (a week), during your leisure-time, how often do you engage in any regular activity long enough to work up a sweat (heart beats rapidly)?

1. OFTEN   **2. SOMETIMES**   **3. NEVER/RARELY**

    ☐   ☐   ☐

FOOD AND ACTIVITY QUESTIONNAIRE

RESPONDENT ID #

TODAY'S DATE

Jan | DAY | YEAR
May
Jun
Aug — 2015
Sep — 2016
Oct — 2017
Dlo — 2018
2019
2020
... 2021
2022
2023

ABOUT YOU

SEX   AOE   WEIGHT   HE,IOHT
                     pounds

If female, are you
pregnant or
breast feeding?
○ No
○ Yes
○ Not female

ABOUT THIS SURVEY

Please answer each question a• best you can.
Eltimat. if you aron't tur'9.

- DETACH THE LAST PAGE OF THIS 80011!.ET.
  These """yovt portion plcnns.
- USE ONLY A NUMBER 2 PENCIL
  FILL IN THE CRCLES COMPi.ETELY and
- erase completely if you make B"'f ch

INSTRUCTIONS

Thi•torm 11 about the food• Vo" 1ua V 11t. nk about your htlQ ovtr &est 6 months Tha lne:ludff all mNtl

1. HOW OFTEN, on average, did you eat the toad?

or snack•,et homo, In •reataul'llnil or carry-out.

Pleaae tefl u•.. 00 NOT SKIP any foods. Mal1<"Novo lt you dm1Olliany al the IOod.

2. HOW MUCH ol the tood did you usualty eat on tM days)'OU ate it?
   Some llm..Wl MI< 1 -rruci>" ..A, 8,Cótll LOOK AT THE PORTION FICT\JRES.
   Pick tho plctu.,ll>ot lool<s tho most lko tho sorvlng llleyou usually oot.
   (It)'OU don't have pictures: Aa1/4 cup, B a l/2 Q,,1p. C•1 cup, 0=2 cups.)

3. WHAT TYPE? For eome foods we ask lhe type (k:Jw fet, k:lw sugeir•) near the end of the UrY9'/.

EX.AMPLE: This person drank orange juice twice a 'Bek.., and had one glass each time.
   Once a week 1hit naw a "C" sl:zld Hf'V lng COidc&real(lbOut 1). lIiilNCMB

HOW OFTEN ? HOW MUCH oo#>pM diva?

1Mge]uloe

Coldcoroal

SERIAL/

EGGS and DAIRY FOODS	NEVER	A FEW TIMES PER 6 MONTHS	ONCE per MONTH	2-3 TIMES per MONTH	ONCE per WEEK	2 TIMES per WEEK	3-4 TIMES per WEEK	5-6 TIMES per WEEK	EVERY DAY		HOW MUCH on those days? SEE PORTION SIZE PICTURES FOR A-B-C-D				
Breakfast sandwiches or breakfast burritos with eggs or meat	○	○	○	○	○	○	○	○	○	▷	How many sandwiches in a day	○ 1	○ 2		
Other eggs like scrambled or boiled, or quiche (not egg substitutes)	○	○	○	○	○	○	○	○	○	▷	How many eggs a day	○ 1	○ 2	○ 3	○ 4
Yogurt (not frozen yogurt)	○	○	○	○	○	○	○	○	○	▷	Which bowl or glass		○ B	○ C	○ D
Cottage cheese, ricotta cheese	○	○	○	○	○	○	○	○	○	▷	How much	○ A	○ B	○ C	○ D
Cream cheese, sour cream, dips	○	○	○	○	○	○	○	○	○	▷	How many tablespoons	○ 1	○ 2	○ 3	○ 4
Cheese, sliced cheese, cheese spread, including in sandwiches and quesadillas	○	○	○	○	○	○	○	○	○	▷	How many slices	○ 1	○ 2	○ 3	○ 4

CEREALS, GRAINS, BREADS

	NEVER	A FEW TIMES PER 6 MONTHS	ONCE per MONTH	2-3 TIMES per MONTH	ONCE per WEEK	2 TIMES per WEEK	3-4 TIMES per WEEK	5-6 TIMES per WEEK	EVERY DAY						
Cold cereals, ANY KIND, like corn flakes, fiber cereals, sweetened cereals	○	○	○	○	○	○	○	○	○	▷	Which bowl		○ B	○ C	○ D
Oatmeal, or whole grain cereal like Wheatena or Ralston	○	○	○	○	○	○	○	○	○	▷	Which bowl	○ A	○ B	○ C	○ D
Grits, cream of wheat, cornmeal mush	○	○	○	○	○	○	○	○	○	▷	Which bowl	○ A	○ B	○ C	○ D
Milk or milk substitutes on cereal	○	○	○	○	○	○	○	○	○						
Brown rice, or dishes made with brown rice	○	○	○	○	○	○	○	○	○	▷	How much in a day		○ B	○ C	○ D
White rice, or dishes made with rice, like rice and beans	○	○	○	○	○	○	○	○	○	▷	How much in a day		○ B	○ C	○ D
Pancakes, waffles, French toast, crepes	○	○	○	○	○	○	○	○	○	▷	How many	○ 1	○ 2	○ 3	○ 4
Breakfast pastries, like muffins, scones, sweet rolls, Danish, Pop Tarts, pan dulce	○	○	○	○	○	○	○	○	○	▷	How many pieces	○ 1 sm	○ 1 med	○ 2	○ 3
Biscuits, not counting breakfast sandwiches	○	○	○	○	○	○	○	○	○	▷	How many	○ 1 sm	○ 1 med	○ 2	○ 3
Corn bread, corn muffins, hush puppies	○	○	○	○	○	○	○	○	○	▷	How many pieces in a day	○ 1/2	○ 1	○ 2	○ 3
Hamburger buns, hotdog buns, submarine or hoagie buns	○	○	○	○	○	○	○	○	○	▷	How many buns in a day	○ 1/2	○ 1	○ 2	○ 3
Bagels or English muffins, dinner rolls, pita, naan	○	○	○	○	○	○	○	○	○	▷	How many	○ 1/2	○ 1	○ 2	○ 3
Tortillas (not counting in tacos or burritos)	○	○	○	○	○	○	○	○	○	▷	How many in a day	○ 1	○ 2	○ 3	○ 4
Any other bread or toast, including white, dark, whole wheat, and what you have in sandwiches	○	○	○	○	○	○	○	○	○	▷	How many slices in a day	○ 1	○ 2	○ 3	○ 4

VEGETABLES

	NEVER	A FEW TIMES PER 6 MONTHS	ONCE per MONTH	2-3 TIMES per MONTH	ONCE per WEEK	2 TIMES per WEEK	3-4 TIMES per WEEK	5-6 TIMES per WEEK	EVERY DAY						
Broccoli, Chinese broccoli, or Brussels sprouts	○	○	○	○	○	○	○	○	○	▷	How much	○ A	○ B	○ C	○ D
Carrots and mixed vegetables containing carrots	○	○	○	○	○	○	○	○	○	▷	How much	○ A	○ B	○ C	○ D
Corn	○	○	○	○	○	○	○	○	○	▷	How much	○ A	○ B	○ C	○ D
Green beans, string beans, green peas	○	○	○	○	○	○	○	○	○	▷	How much	○ A	○ B	○ C	○ D
Cooked greens like spinach, collards, turnip greens, kale, mustard greens	○	○	○	○	○	○	○	○	○	▷	How much	○ A	○ B	○ C	○ D

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	NEVER	A FEW TIMES PER 6 MONTHS	ONCE per MONTH	2-3 TIMES per MONTH	ONCE per WEEK	2 TIMES per WEEK	3-4 TIMES per WEEK	5-6 TIMES per WEEK	EVERY DAY		HOW MUCH on those days? SEE PORTION SIZE PICTURES FOR A-B-C-D
Cabbage, cole slaw, Chinese cabbage	○	○	○	○	○	○	○	○	○	▶	How much ○ A ○ B ○ C ○ D
Green salad with lettuce or raw spinach	○	○	○	○	○	○	○	○	○	▶	How much ○ 1/2 cup ○ 1 cup ○ 2 cups ○ 3+ cups
Raw tomatoes	○	○	○	○	○	○	○	○	○	▶	How much ○ 1/4 ○ 1/2 ○ 1 ○ 2
Salad dressing	○	○	○	○	○	○	○	○	○	▶	How many tablespoons ○ 1 ○ 2 ○ 3 ○ 4
Avocado, guacamole	○	○	○	○	○	○	○	○	○	▶	How many tablespoons ○ 1 ○ 2 ○ 3 ○ 4
Sweet potatoes, yams	○	○	○	○	○	○	○	○	○	▶	How much ○ A ○ B ○ C ○ D
French fries, home fries, hash browns, tater tots	○	○	○	○	○	○	○	○	○	▶	How much ○ A ○ B ○ C ○ D
Potatoes not fried, like baked, boiled, mashed, or in stew or potato salad	○	○	○	○	○	○	○	○	○	▶	How much ○ A ○ B ○ C ○ D
Any other vegetable, like squash, cauliflower, peppers, okra, nopales	○	○	○	○	○	○	○	○	○	▶	How much ○ A ○ B ○ C ○ D

FRUITS

How often do you eat the following fruits? Include fresh or frozen fruits. Only include canned or dried fruit when mentioned.

	NEVER	A FEW TIMES PER 6 MONTHS	ONCE per MONTH	2-3 TIMES per MONTH	ONCE per WEEK	2 TIMES per WEEK	3-4 TIMES per WEEK	5-6 TIMES per WEEK	EVERY DAY		HOW MUCH
Watermelon, cantaloupe, honeydew, other melons	○	○	○	○	○	○	○	○	○	▶	How much ○ A ○ B ○ C ○ D
Strawberries or other berries	○	○	○	○	○	○	○	○	○	▶	How much ○ A ○ B ○ C ○ D
Bananas	○	○	○	○	○	○	○	○	○	▶	How many in a day ○ 1/2 ○ 1 ○ 2
Apples or pears	○	○	○	○	○	○	○	○	○	▶	How many in a day ○ 1/2 ○ 1 ○ 2
Oranges, tangerines, grapefruit	○	○	○	○	○	○	○	○	○	▶	How many ○ 1/2 ○ 1 ○ 2
Peaches and nectarines	○	○	○	○	○	○	○	○	○	▶	How many ○ 1/2 ○ 1 ○ 2
Any other fresh fruit, like grapes, plums, mango, fruit salad	○	○	○	○	○	○	○	○	○	▶	How much ○ A ○ B ○ C ○ D
Raisins, dates, other dried fruit	○	○	○	○	○	○	○	○	○	▶	How much ○ A ○ B ○ C
Canned fruit, like applesauce, fruit cocktail, canned peaches or pineapple	○	○	○	○	○	○	○	○	○	▶	How much ○ A ○ B ○ C ○ D

BEANS, TOFU, and MEAT SUBSTITUTES
Include those eaten alone, or in mixed dishes like burritos, chili, stir-fry, salad

	NEVER	A FEW TIMES PER 6 MONTHS	ONCE per MONTH	2-3 TIMES per MONTH	ONCE per WEEK	2 TIMES per WEEK	3-4 TIMES per WEEK	5-6 TIMES per WEEK	EVERY DAY		HOW MUCH
Refried beans, bean burritos, or hummus	○	○	○	○	○	○	○	○	○	▶	How much ○ A ○ B ○ C ○ D
Pinto beans, black beans, kidney beans, baked beans, lentils	○	○	○	○	○	○	○	○	○	▶	How much ○ A ○ B ○ C ○ D
Tofu or tempeh	○	○	○	○	○	○	○	○	○	▶	How much ○ A ○ B ○ C ○ D
Meat substitutes, like veggie burgers, veggie chicken, vegetarian hot dogs or vegetarian lunch meats	○	○	○	○	○	○	○	○	○	▶	How much ○ A ○ B 1 patty or slice ○ C ○ D

PROOF
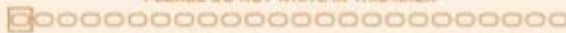
SOUPS, MIXED DISHES, and NOODLES

	NEVER	A FEW TIMES PER 6 MONTHS	ONCE per MONTH	2-3 TIMES per MONTH	ONCE per WEEK	2 TIMES per WEEK	3-4 TIMES per WEEK	5-6 TIMES per WEEK	EVERY DAY		HOW MUCH on those days? SEE PORTION SIZE PICTURES FOR A-B-C-D
Split pea, bean, or lentil soup	○	○	○	○	○	○	○	○	○	▶	Which bowl — B C D
Vegetable soup, vegetable beef soup, or tomato soup	○	○	○	○	○	○	○	○	○	▶	Which bowl — B C D
Any other soup, including chicken noodle, cream soups, Cup-A-Soup, ramen	○	○	○	○	○	○	○	○	○	▶	Which bowl — B C D
Pizza or pizza pockets	○	○	○	○	○	○	○	○	○	▶	How many slices — 1 2 3 4
Macaroni and cheese	○	○	○	○	○	○	○	○	○	▶	How much — B C D
Spaghetti, lasagna, other pasta with tomato sauce	○	○	○	○	○	○	○	○	○	▶	How much — B C D
Other noodles like plain pasta, pasta salad, sopa seca	○	○	○	○	○	○	○	○	○	▶	How much — B C D
Egg rolls, won tons, samosas, empanadas	○	○	○	○	○	○	○	○	○	▶	How many pieces — 1 2 3 4

MEAT and CHICKEN

	NEVER	A FEW TIMES PER 6 MONTHS	ONCE per MONTH	2-3 TIMES per MONTH	ONCE per WEEK	2 TIMES per WEEK	3-4 TIMES per WEEK	5-6 TIMES per WEEK	EVERY DAY		HOW MUCH
Hamburgers, cheeseburgers, turkey burger, at home or from a restaurant	○	○	○	○	○	○	○	○	○	▶	How many — 1 sm 1 lrg 2 3
Hot dogs or dinner sausage like Polish, Italian, chicken apple	○	○	○	○	○	○	○	○	○	▶	How many — 1 2 3 4
Bacon or breakfast sausage	○	○	○	○	○	○	○	○	○	▶	How many pieces — 1 2 3 4
Lunch meats like bologna, sliced ham, sliced turkey, salami	○	○	○	○	○	○	○	○	○	▶	How many slices — 1 2 3 4
Meat loaf, meat balls	○	○	○	○	○	○	○	○	○	▶	How much — B C D
Steak, roast beef, pot roast, including in frozen dinners or sandwiches	○	○	○	○	○	○	○	○	○	▶	How much — A B C D
Tacos, burritos, enchiladas, tamales, tostadas, with meat or chicken	○	○	○	○	○	○	○	○	○	▶	How much — A B C D
Ribs, spareribs	○	○	○	○	○	○	○	○	○	▶	How much — A B C D
Pork chops, pork roast, cooked ham (including for breakfast)	○	○	○	○	○	○	○	○	○	▶	How much — A B C D
Any other beef or pork dish like stew, pot pie, corned beef hash, chili, Hamburger Helper, curry	○	○	○	○	○	○	○	○	○	▶	How much — B C D
Liver, including chicken livers or liverwurst	○	○	○	○	○	○	○	○	○	▶	How much — A B C
Pigs feet, neck bones, oxtails, tongue, chitlins	○	○	○	○	○	○	○	○	○	▶	How much — A B C
Veal, lamb, goat, deer meat, other game	○	○	○	○	○	○	○	○	○	▶	How much — A B C
Fried chicken, including chicken fingers, chicken nuggets, wings, chicken patty	○	○	○	○	○	○	○	○	○	▶	How many medium pieces — 1 / 2 pcs / 6 nuggets / 3 / 4
Roasted or broiled chicken or turkey	○	○	○	○	○	○	○	○	○	▶	How much — A / B medium piece / C / D half chicken
Any other chicken or turkey dish, like chicken stew or curry, chicken salad, stir-fry, Chinese chicken dishes	○	○	○	○	○	○	○	○	○	▶	How much — B C D

FISH, SEAFOOD	NEVER	A FEW TIMES PER 6 MONTHS	ONCE per MONTH	2-3 TIMES per MONTH	ONCE per WEEK	2 TIMES per WEEK	3-4 TIMES per WEEK	5-6 TIMES per WEEK	EVERY DAY		HOW MUCH on those days? SEE PORTION SIZE PICTURES FOR A-B-C-D				
Oysters	○	○	○	○	○	○	○	○	○	▶	How much	A	B	C	
Shellfish like shrimp, scallops, crab	○	○	○	○	○	○	○	○	○	▶	How much fish	A	B	C	D
Tuna, tuna salad, tuna casserole	○	○	○	○	○	○	○	○	○	▶	How much of the tuna	A	B	C	
Salmon, mackerel, sea bass, trout, sardines, herring, without breading	○	○	○	○	○	○	○	○	○	▶	How much	A	B	C	D
Fried fish, fish sticks, fish sandwich, breaded fillets	○	○	○	○	○	○	○	○	○	▶	How much	A	B	C	D
Any other fish	○	○	○	○	○	○	○	○	○	▶	How much fish	A	B	C	D

NUTS, SEEDS, SNACKS

	NEVER	A FEW TIMES PER 6 MONTHS	ONCE per MONTH	2-3 TIMES per MONTH	ONCE per WEEK	2 TIMES per WEEK	3-4 TIMES per WEEK	5-6 TIMES per WEEK	EVERY DAY		HOW MUCH				
Peanut butter or other nut butters	○	○	○	○	○	○	○	○	○	▶	How many tablespoons	1/2	1	2	3
Walnuts or flax seeds (don't count flaxseed oil)	○	○	○	○	○	○	○	○	○	▶	How much	1 Tbsp	2 Tbsp	1/4 cup	1/2 cup
Peanuts, sunflower seeds, other nuts or seeds	○	○	○	○	○	○	○	○	○	▶	How much	A	B	C	D
Energy or protein bars, like Power Bar, Clif, Balance, Luna, South Beach, Atkins	○	○	○	○	○	○	○	○	○	▶	How much	Small	Medium	Large	
Breakfast bars, cereal bars, granola bars (not energy or protein bars)	○	○	○	○	○	○	○	○	○	▶	How many	1	2	3	
Popcorn	○	○	○	○	○	○	○	○	○	▶	How many cups	1-2	3-6	7-9	10-12
Whole grain crackers, like Wheat Thins, RyeKrisp, Ryvita, Wasa	○	○	○	○	○	○	○	○	○	▶	How much	A	B	C	D
Any other crackers, like saltines, Ritz, Cheez-Its, cheese-filled pretzels	○	○	○	○	○	○	○	○	○	▶	How much	A	B	C	D
Tortilla chips or corn chips, like Fritos, Doritos, corn nuts	○	○	○	○	○	○	○	○	○	▶	How much	A	B	C	D
Any other snack chips, like potato chips, Cheetos, Chex mix	○	○	○	○	○	○	○	○	○	▶	How much	A	B	C	D

SWEETS AND DESSERTS

	NEVER	A FEW TIMES PER 6 MONTHS	ONCE per MONTH	2-3 TIMES per MONTH	ONCE per WEEK	2 TIMES per WEEK	3-4 TIMES per WEEK	5-6 TIMES per WEEK	EVERY DAY		HOW MUCH				
Donuts	○	○	○	○	○	○	○	○	○	▶	How many	1 mini	1 med	2	3
Cake or snack cakes like cupcakes, Twinkies, pound cake, banana bread	○	○	○	○	○	○	○	○	○	▶	How many pieces	1 sm	1 med	2	3
Cookies, brownies	○	○	○	○	○	○	○	○	○	▶	How many	1-2	3-4	5-6	7+
Pumpkin pie, sweet potato pie	○	○	○	○	○	○	○	○	○	▶	How many pieces	1/2	1	2	3
Any other pie or cobbler, including fast food pies, snack pies	○	○	○	○	○	○	○	○	○	▶	How many pieces	1/2	1	2	3
Ice cream, ice cream bars, frozen yogurt, fast food milkshakes	○	○	○	○	○	○	○	○	○	▶	How much		B	C	D
Pudding, custard, rice pudding, flan	○	○	○	○	○	○	○	○	○	▶	How much		B	C	D
Chocolate or other flavored sauces or syrup, on ice cream	○	○	○	○	○	○	○	○	○	▶	How much	1-2 Tbsp	3-4 Tbsp	1/2 cup	

■ ■ ■ ■

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| | A FEW TIMES PER 6 | ONCE per | 2-3 TIMES per | ONCE per | 2 TIMES per | 3-4 TIMES per | 5-6 TIMES per | EVERY | HOW MUCH on those days? SEE PORTION SIZE PICTURES FOR A-B-C-D |
	NEVER	MONTHS	MONTH	MONTH	WEEK	WEEK	WEEK	WEEK	DAY	
Popsicles, jello, frozen fruit bars, slushies, sherbet (don't count sugar-free)	○	○	○	○	○	○	○	○	○	How much — A B C D
Chocolate candy, candy bars like Snickers, Hershey's, M&Ms	○	○	○	○	○	○	○	○	○	How much in a day — 1 mini, 1 med, 1 lrg, 1 king
Any other candy, not chocolate, like hard candy, Lifesavers, Skittles, Starburst, breath mints, chewing gum (NOT sugar free)	○	○	○	○	○	○	○	○	○	How much in a day — 1-2 pcs, 1/2 pkg, 1 pkg, 2 pkgs

SPREADS, SAUCES, OTHER FOODS

	NEVER	A FEW TIMES PER 6 MONTHS	ONCE per MONTH	2-3 TIMES per MONTH	ONCE per WEEK	2 TIMES per WEEK	3-4 TIMES per WEEK	5-6 TIMES per WEEK	EVERY DAY	HOW MUCH
Margarine (not butter) on bread, rice, vegetables, or other foods	○	○	○	○	○	○	○	○	○	How many pats (tsps) — 1 2 3 4
Butter (not margarine) on bread, rice, vegetables, or other foods	○	○	○	○	○	○	○	○	○	How many pats (tsps) — 1 2 3 4
Mayonnaise, sandwich spreads	○	○	○	○	○	○	○	○	○	How many tablespoons — 1/2 1 2 3
Ketchup, salsa, chili sauce, chili peppers	○	○	○	○	○	○	○	○	○	How many tablespoons — 1/2 1 2 3
Mustard, barbecue sauce, soy sauce	○	○	○	○	○	○	○	○	○	How many tablespoons — 1/2 1 2 3
Gravy, or other rich sauces like Alfredo, white sauce, mole, peanut sauce	○	○	○	○	○	○	○	○	○	How many cups — 1/4 1/2 1
Jam, jelly, marmalade	○	○	○	○	○	○	○	○	○	How many tablespoons — 1/2 1 2 3
Pickles, pickled vegetables, sauerkraut, kimchi	○	○	○	○	○	○	○	○	○	How much — A B C D
Salt, added to your food at the table	○	○	○	○	○	○	○	○	○	How many shakes in a day — 1-3 4-5 6-7 8+

BEVERAGES

	NEVER	A FEW TIMES PER 6 MONTHS	ONCE per MONTH	2-3 TIMES per MONTH	ONCE per WEEK	2 TIMES per WEEK	3-4 TIMES per WEEK	5-6 TIMES per WEEK	EVERY DAY	HOW MUCH
Chocolate milk, cocoa, hot chocolate	○	○	○	○	○	○	○	○	○	How many 12 ounce servings — 1/2 1 2 3
Glasses of milk or soy milk, not counting on cereal, in coffee, or chocolate milk	○	○	○	○	○	○	○	○	○	How many 8 ounce servings — 1 2 3 4
Meal replacement drinks like Slim Fast, Ensure, or high protein drinks or powders	○	○	○	○	○	○	○	○	○	How many cans or glasses — 1 2 3 4
Tomato juice, V-8, other vegetable juice	○	○	○	○	○	○	○	○	○	How many 8 ounce servings — 1/2 1 2 3
Real 100% orange juice or grapefruit juice. Don't count orange soda or Sunny Delight.	○	○	○	○	○	○	○	○	○	How many 8 ounce servings — 1/2 1 2 3
Other 100% juices, like apple, grape, 100% fruit blends, or fruit smoothies	○	○	○	○	○	○	○	○	○	How many 8 ounce servings — 1/2 1 2 3
Hi-C, cranberry juice cocktail, Hawaiian Punch, Tang	○	○	○	○	○	○	○	○	○	How many 12 ounce servings — 1/2 1 2 3
Drinks with some juice like Sunny Delight, Knudsen	○	○	○	○	○	○	○	○	○	How many 12 ounce servings — 1/2 1 2 3
Iced tea, homemade, instant or bottled, like Nestea, Lipton, Snapple, Tazo	○	○	○	○	○	○	○	○	○	How many 16-oz. glasses or bottles — 1/2 1 2 3
Gatorade, Powerade, or other sports drinks	○	○	○	○	○	○	○	○	○	How much in a day — ○ 1 16-ounce bottle ○ 1 20-ounce bottle ○ 2 16-ounce bottles ○ 2 20-ounce bottles

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	NEVER	A FEW TIMES PER 6 MONTHS	ONCE per MONTH	2-3 TIMES per MONTH	ONCE per WEEK	2 TIMES per WEEK	3-4 TIMES per WEEK	5-6 TIMES per WEEK	EVERY DAY	HOW MUCH on those days? SEE PORTION SIZE PICTURES FOR A-B-C-D
Energy drinks like Red Bull, Rockstar, Monster	○	○	○	○	○	○	○	○	○	► How much in a day: ○ 1 8-ounce can ○ 1 12-16 ounce can ○ 1 20-ounce can ○ 24 ounces or more
Kool-Aid, lemonade, fruit flavored drinks, like Crystal light, atole, horchata (not iced tea)	○	○	○	○	○	○	○	○	○	► How much in a day: ○ 1 8-ounce glass ○ 1 12-16-ounce glass or bottle ○ 1 20-ounce bottle ○ 30 ounces or more
Soft drinks, soda, pop, like cola, 7-Up, orange soda, regular or diet	○	○	○	○	○	○	○	○	○	► How many in a day: ○ 1 can ○ 1 20-ounce bottle ○ 2 cans ○ Big Gulp or 3 cans
Beer or non-alcoholic beer	○	○	○	○	○	○	○	○	○	► How much in a day: ○ 1 can ○ 2 cans ○ 3-4 cans or small pitcher ○ 5+ cans or large pitcher
Wine or wine coolers	○	○	○	○	○	○	○	○	○	► How many glasses in a day: ○ 1/2 glass ○ 1 glass ○ 2 glasses, 1/2 bottle ○ 4+ glasses
Liquor or mixed drinks, cocktails	○	○	○	○	○	○	○	○	► How many drinks: ○ 1 ○ 2 ○ 3 ○ 4
Water, bottled or tap	○	○	○	○	○	○	○	○	► How many glasses: ○ 1 ○ 2 ○ 3-4 ○ 5+
Milky coffee drinks like latte, mocha, cappuccino, Frappuccino	○	○	○	○	○	○	○	○	► How much in a day: ○ 12 oz ○ 16 oz ○ 20 oz ○ 24+ oz
Coffee (brewed or instant), regular or decaf	○	○	○	○	○	○	○	○	► How many in a day: ○ 1 ○ 2 ○ 3 ○ 4+
Hot tea (not including herbal tea)	○	○	○	○	○	○	○	○	► How many cups in a day: ○ 1 ○ 2 ○ 3 ○ 4+

MILKY COFFEE DRINKS: What kind do you usually drink? **MARK ONLY ONE**
○ Frappuccino   ○ Mocha   ○ Latte or cappuccino   ○ Café con leche   ○ Some of each   ○ Don't drink them

What are your milky coffee drinks usually made with? **MARK ONLY ONE**
○ Whole milk   ○ Skim milk or non-fat   ○ Something else
○ 1 or 2% milk (reduced fat)   ○ Soy milk   ○ Don't drink

COFFEE: Is your coffee usually regular or decaf?   ○ Decaf   ○ Regular   ○ Both kinds   ○ Don't drink coffee

What do you usually add to your regular or decaf coffee? **MARK ONLY ONE**
○ Cream or half-n-half   ○ Condensed milk   ○ None of these
○ CoffeeMate, non-dairy creamer   ○ Any other milk

Do you usually add sugar (or honey) to coffee?   ○ No   ○ Yes   IF YES, how many teaspoons each cup? ○ 1 ○ 2 ○ 3 ○ 4

HOT TEA: Is your hot tea usually regular or decaf?   ○ Decaf   ○ Regular   ○ I drink both kinds   ○ Don't drink tea

What do you usually add to your hot tea? **MARK ONLY ONE**
○ Cream or half-n-half   ○ Condensed milk   ○ None of these
○ CoffeeMate, non-dairy creamer   ○ Any other milk

Do you usually add sugar (or honey) to hot tea?   ○ No   ○ Yes   IF YES, how many teaspoons each cup? ○ 1 ○ 2 ○ 3 ○ 4

If you eat the following foods, what type do you usually eat? MARK ONLY ONE ANSWER FOR EACH QUESTION

Milk	○ Whole milk	○ 2% milk	○ 1% milk (low-fat)	○ Skim milk, non-fat
	○ Soy milk	○ Rice milk	○ Almond milk, other	○ Don't drink

Slimfast, Ensure, or high protein drinks ○ Slimfast, Ensure, regular ○ Slimfast, Ensure, light or low-carb
○ High protein drinks, regular ○ High protein drinks, light or low-carb ○ Don't know/Don't drink

Real 100% orange or grapefruit juice ○ Calcium-fortified ○ Not calcium fortified ○ Don't know ○ Don't drink

Iced tea ○ Home-made, no sugar ○ Bottled, no-sugar ○ Don't drink
○ Home-made, with sugar ○ Bottled, pre-sweetened

Drinks like Kool-Aid, lemonade, Crystal Light ○ Low-calorie, sugar-free ○ Regular ○ Don't drink

Energy drinks like Red Bull, Monster ○ Sugar-free ○ Regular ○ Don't drink

Soft drinks, soda, pop ○ Diet, low-calorie ○ Regular ○ Don't drink
   Do they usually have caffeine? ○ Has caffeine ○ No caffeine ○ Don't drink

Beer	○ Regular	○ Light	○ Non-alcoholic	○ Don't drink
Wine or wine cooler	○ Red wine	○ White wine	○ Both red and white wine	○ Don't drink
Cheese	○ Low-fat	○ Regular-fat	○ Don't eat	
Yogurt	○ Plain (no sugar or fruit)	○ With fruit or other flavors		
Yogurt	○ Low-fat	○ Non-fat	○ Regular (whole milk)	○ Don't eat
Salad dressing	○ Low-fat, lite ○ Fat free	○ Regular	○ Oil & vinegar	○ Don't use
Spaghetti or lasagna	○ Meatless	○ With meat sauce or meatballs		○ Don't eat
Noodles, pasta	○ Rarely whole grain	○ Sometimes whole grain	○ Usually whole grain	○ Don't know/don't eat
Burgers	○ Hamburger	○ Cheeseburger	○ Turkey burger	○ Don't eat
Beef or pork	○ Avoid eating the fat	○ Sometimes eat the fat	○ Often eat the fat	○ Don't eat
Chicken or turkey	○ Avoid eating the skin	○ Sometimes eat the skin	○ Often eat the skin	○ Don't eat
Hot dogs, dinner sausage	○ Beef or pork	○ Chicken or turkey, low-fat		○ Don't eat
Lunch meats	○ Beef or pork	○ Chicken or turkey, low-fat		○ Don't eat
Cakes, snack cakes, cupcakes	○ Low-sugar, low-carb	○ Low-fat	○ Regular-fat	○ Don't eat
Cookies, brownies	○ Low-sugar, low-carb	○ Low-fat	○ Regular-fat	○ Don't eat
Ice cream, frozen yogurt	○ Low-sugar, low-carb	○ Low-fat or frozen yogurt ○ Regular		○ Don't eat
Energy or protein bars	○ High energy ○ High protein	○ Some of each	○ Don't know	○ Don't eat
Bagels, English muffins, rolls	○ White ○ Multi-grain	○ 100% whole wheat	○ Eat all kinds	○ Don't eat
Burger, hot dog, submarine buns	○ White ○ Multi-grain	○ 100% whole wheat	○ Eat all kinds	○ Don't eat
Bread	○ White (not whole grain)	○ 100% whole wheat		○ Don't eat
	○ Multi-grain, rye, or other brown bread	○ Eat some of each		
Tortillas	○ Corn tortillas	○ Flour tortillas (wheat)	○ Eat all kinds or don't know	○ Don't eat
Popcorn	○ Air popped, fat-free ○ Low-fat or Light	○ Regular ○ Caramel corn	○ Don't know	○ Don't eat
Crackers, pretzels	○ Low-fat, including RyeKrisp, rice cakes, or plain pretzels		○ Don't know	
	○ Regular-fat crackers or filled pretzels		○ Don't eat	
Mayonnaise or sandwich spreads	○ Low-fat, light	○ Regular	○ Don't eat	

If you eat cold cereals, what do you usually eat? Choose ONE or TWO that you eat most often. If you usually eat just one kind, only choose one.

○ All Bran Original	○ Cinnamon Toast Crunch	○ Grape Nuts	○ Special K, plain
○ All-Bran Complete, Complete	○ Cocoa Krispies, Pebbles, Puffs	○ Honey Bunches of Oats	○ Special K, flavors
○ Apple Jacks, Cookie Crisp	○ Corn Flakes, Corn Puffs	○ Kashi GOLEAN, Heart to Heart	○ Total
○ Bran Flakes	○ Corn Pops	○ Life	○ Wheaties
○ Cap'n Crunch	○ Fiber-One, Bran Buds	○ Lucky Charms, Fruity Pebbles	○ Other sweet cereal
○ Cheerios, plain or Multi-Grain	○ Froot Loops	○ Oatmeal Squares, Oat Bran	○ Other unsweetened cereal
○ Cheerios, Honey Nut, flavors	○ Frosted Flakes	○ Raisin Bran	○ Other whole grain cereal
○ Chex, Wheat	○ Frosted Mini-Wheats	○ Rice Krispies, puffed rice	○ Other bran or fiber cereal
○ Chex, other	○ Granola	○ Shredded Wheat	○ Don't eat cereal

Which fats or oils are used most often for cooking or frying (not baking) in your home? MARK ONLY ONE OR TWO

○ Non-stick spray or none	○ Soft tub margarine	○ Corn oil, vegetable oil and blends	○ Other oil
○ Butter or ghee	○ Low-fat margarine	○ Peanut oil	○ Don't know
○ Butter/margarine blend	○ Olive oil	○ Lard, fatback, or bacon fat	
○ Stick margarine	○ Canola oil, safflower oil	○ Vegetable shortening, Crisco	

What vitamin supplements do you take fairly regularly?

	HOW OFTEN								FOR HOW MANY YEARS?			
	DIDN'T TAKE	A FEW DAYS per MONTH	1 DAY per WEEK	2 DAYS per WEEK	3-4 DAYS per WEEK	5-6 DAYS per WEEK	EVERY DAY		LESS THAN 1 YEAR	1-4 YEARS	5-9 YEARS	10+ YEARS
Multiple Vitamins. Do you take...												
Prenatal vitamins	○	○	○	○	○	○	○	▶	○	○	○	○
Regular One-A-Day, Centrum, "senior" vitamins or house brands of multiple vitamins	○	○	○	○	○	○	○	▶	○	○	○	○
Stress-tabs or B-Complex type	○	○	○	○	○	○	○	▶	○	○	○	○
Antioxidant combination, eye formula	○	○	○	○	○	○	○	▶	○	○	○	○
Single Vitamins or Minerals, taken alone or in combination. Do not count what is in your multiple vitamins above.												
Vitamin A (not beta-carotene)	○	○	○	○	○	○	○	▶	○	○	○	○
Vitamin B-6	○	○	○	○	○	○	○	▶	○	○	○	○
Vitamin B-12	○	○	○	○	○	○	○	▶	○	○	○	○
Vitamin C	○	○	○	○	○	○	○	▶	○	○	○	○
Vitamin D	○	○	○	○	○	○	○	▶	○	○	○	○
Vitamin E	○	○	○	○	○	○	○	▶	○	○	○	○
Folic acid, folate	○	○	○	○	○	○	○	▶	○	○	○	○
Calcium or antacids with calcium, like Tums	○	○	○	○	○	○	○	▶	○	○	○	○
Iron	○	○	○	○	○	○	○	▶	○	○	○	○
Zinc	○	○	○	○	○	○	○	▶	○	○	○	○
Cod liver oil, other fish oils, omega-3, flax seed oil, algae	○	○	○	○	○	○	○	▶	○	○	○	○
Fiber supplements like Benefiber, Metamucil	○	○	○	○	○	○	○	▶	○	○	○	○

If you take One-A-Day, Centrum or other types of multiple vitamins, do you usually take types that
○ Contain minerals, iron, zinc, etc.   ○ Do not contain minerals   ○ Don't know

If you take vitamin C, how many milligrams of **vitamin C** do you usually take, on the days you take it? (Select the closest amount)
○ 100   ○ 250   ○ 500   ○ 750   ○ 1000   ○ 1500   ○ 2000   ○ 3000+   ○ Don't know

If you take vitamin E, how many IUs of **vitamin E** do you usually take, on the days you take it? (Select the closest amount)
○ 100   ○ 200   ○ 300   ○ 400   ○ 600   ○ 800   ○ 1000   ○ 2000+   ○ Don't know

If you take calcium, how many milligrams of **calcium** do you usually take, on the days you take it? (Select the closest amount)
○ 100   ○ 350   ○ 650   ○ 1250+   ○ Don't know

If you take vitamin D, how many IUs of **vitamin D** do you usually take, on the days you take it? (Select the closest amount)
○ 400   ○ 600   ○ 800   ○ 1000   ○ 2000   ○ 3000   ○ 4000   ○ 5000+   ○ Don't know

If you take omega-3 supplements, what type do you usually take? **MARK ALL THAT APPLY**
○ Fish oil   ○ Flax oil, hemp oil, other seed oil   ○ Krill oil   ○ Algae   ○ Don't know

SOME LAST QUESTIONS ABOUT YOU

	RARELY	1-2 per WEEK	3-4 per WEEK	5-6 per WEEK	1 per DAY	1 1/2 per DAY	2 per DAY	3 per DAY	4+ per DAY
About how many servings of vegetables do you eat, not counting salad or potatoes? 1 serving = 1/2 cup.	○	○	○	○	○	○	○	○	○
About how many servings of fruit do you eat, not counting juices? 1 serving = 1/2 cup or 1 medium fruit.	○	○	○	○	○	○	○	○	○
How often do you eat foods prepared at home that are cooked or fried in fat or oil?	○	○	○	○	○	○	○	○	○

During a regular day, how many **meals and snacks** do you usually eat?

Meals per day	○ 1	○ 2	○ 3	○ 4	○ 5+
Snacks per day	○ 1	○ 2	○ 3	○ 4	○ 5+

PHYSICAL ACTIVITY SURVEY

Think about the last 6 months. How often did you do the activities listed below?

	HOW OFTEN IN THE PAST 6 MONTHS							HOW MUCH TIME ON THOSE DAYS			
	RARELY OR NEVER	A FEW TIMES A MONTH	ONCE OR TWICE A WEEK	3-4 TIMES A WEEK	5-6 TIMES A WEEK	ALMOST EVERY DAY		LESS THAN 30 MINUTES	30-60 MINUTES	1-2 HOURS	3 OR MORE HOURS
Cooking, shopping, light cleaning like doing laundry or dusting, or running errands	○	○	○	○	○	○	▶	○	○	○	○
Slow walking like walking the dog, or light work around the house like watering	○	○	○	○	○	○	▶	○	○	○	○
Work on the job involving standing, like store clerk, or work involving driving (like truck driver)	○	○	○	○	○	○	▶	○	○	○	○
Taking care of children (feeding, dressing), or moderate housework like sweeping, mopping, cleaning the tub	○	○	○	○	○	○	▶	○	○	○	○
Weeding, raking, mowing the lawn, or light house repairs	○	○	○	○	○	○	▶	○	○	○	○
Brisk walking, dancing, hunting or fishing, golf (NOT using a golf cart), or 'friendly' outdoor games like softball	○	○	○	○	○	○	▶	○	○	○	○
Factory work, mechanic, restaurant work, or work involving walking, like mail carrier	○	○	○	○	○	○	▶	○	○	○	○
Construction, painting, feeding livestock, or homecare like caring for an adult family member	○	○	○	○	○	○	▶	○	○	○	○
Heavy work like moving boxes, heavy digging or shoveling snow, farm chores like baling hay, or other HARD labor	○	○	○	○	○	○	▶	○	○	○	○
Exercising at the gym or at home, aerobics, weight training, jogging, or vigorous sports like basketball, soccer, tennis	○	○	○	○	○	○	▶	○	○	○	○
Bicycling or swimming for exercise	○	○	○	○	○	○	▶	○	○	○	○

Are you   ○ Hispanic or Latino   ○ Not Hispanic or Latino   ○ Do not wish to provide this information

What race do you consider yourself to be? **MARK ALL THAT APPLY**

○ White
○ Black or African American
○ Asian
○ American Indian or Alaska Native
○ Native Hawaiian or Other Pacific Islander
○ Do not wish to provide this information

Thank you very much for filling out this questionnaire.
Please take a minute to go back and fill in anything you may have skipped.

PLEASE DO NOT WRITE IN THIS AREA

○○○○○○○○○○○○○○○○○○○○○○○○   SERIAL #

Carefully remove this page and use the
portion size pictures on the back.

Portion Size Choices

Keep this in front of you while you are filling out The Food Questionnaire. You may use either the plates or the bowls to help you choose your usual portion size.

Choose A, B, C or D: **A** = 1/4 Cup of Food   **B** = 1/2 Cup of Food   **C** = 1 Cup of Food   **D** = 2 Cups of Food

A   B

1/4 cup of food   1/2 cup of food

C

1 cup of food

D

2 cups of food

B

1/2 cup of food

C

1 cup of food

D

2 cups of food
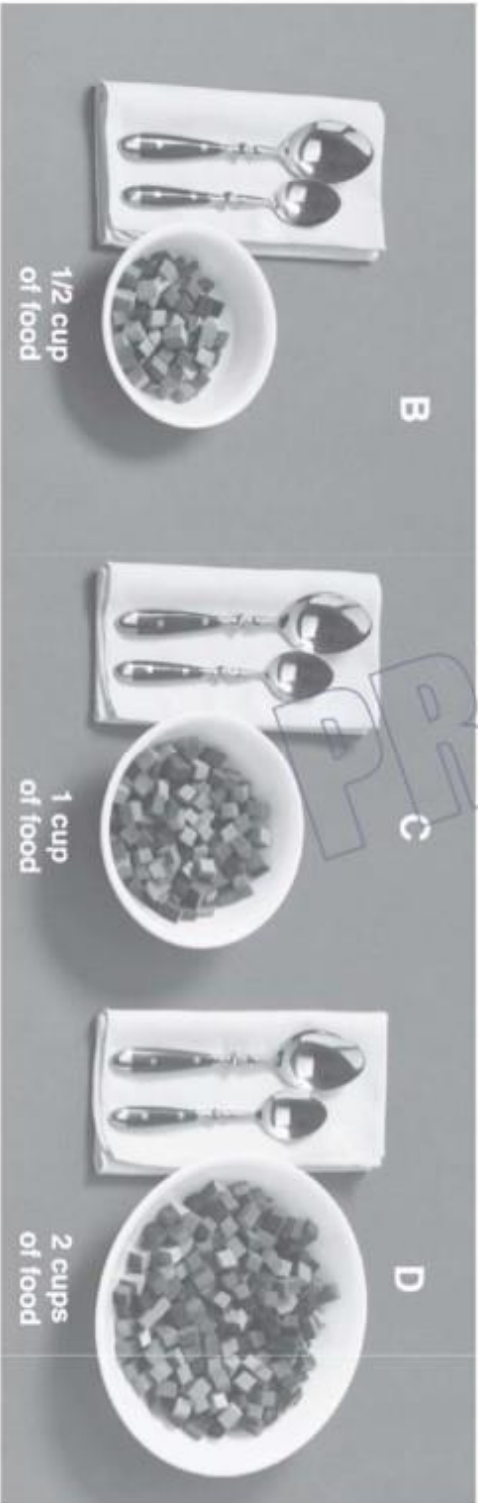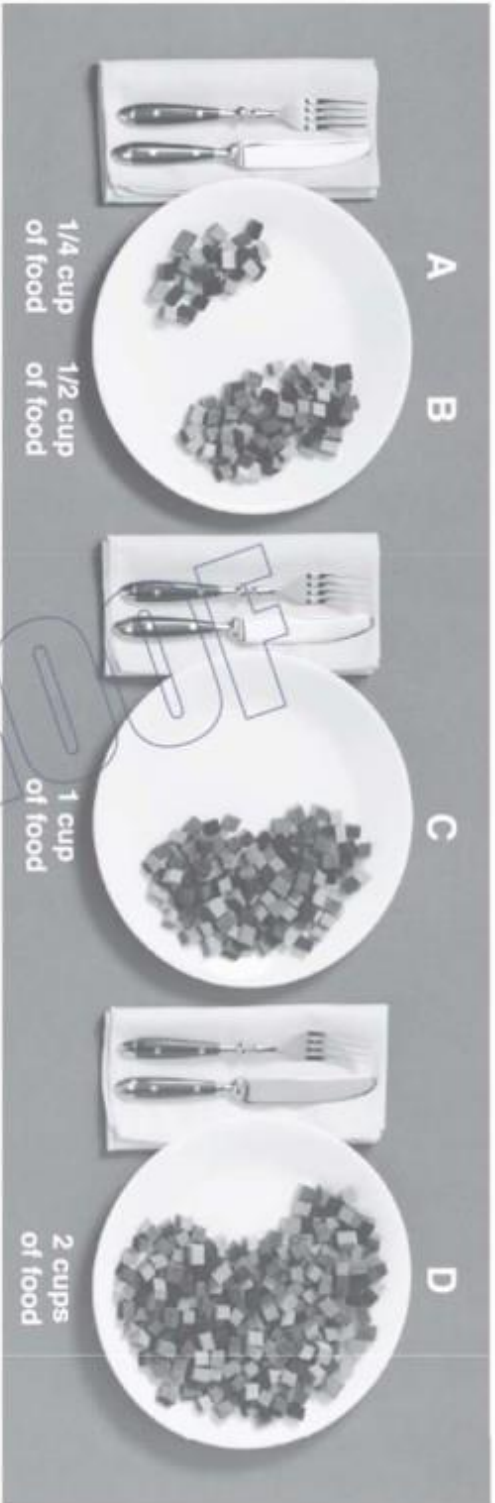
ANTHROPOMETRIC   MEASUREMENTS

Height (cm):	Jump Height (cm):
Weight (kg):	W:H Ratio:
BMI:	% Body Fat:
Hip Circumference (cm):	Waist Circumference (cm):

Hematocrit   Measurements

Draw	1	2	3	4
Percent				

Time of Blood Draw

Draw	1	2	3	4
Time				

Appendix 5: Raw Data

Anthropometric Measurements

Participant ID	Age	Pre vs. Post- menopausal	Height (cm)	Weight (kg)	% BF	BMI	WC	HC	W:H Ratio
S01 (201)	23	1.00	174.2	64.2	26.8	21.2	70.5	85.5	0.82
S02 (202)	22	1.00	161.3	62.9	22.6	24.3	72.5	74.5	0.97
S03 (203)	24	1.00	159.9	46.2	28	18	65.5	78.5	0.83
S04 (204)	24	1.00	165.9	62	25.6	22.5	69.5	90.5	0.77
S05 (205)	20	1.00	151.5	54.9	36	24.1	65.5	75.5	0.87
S06 (206)	23	1.00	163	58.1	16.9	21.9	67	81.5	0.82
S07 (207)	22	1.00	165.5	63.3	24.7	23	72.5	87.5	0.83
S08 (208)	21	1.00	151.1	50.8	24	22	64.5	77.3	0.83
S09 (209)	22	1.00	180.8	62.9	20.4	19.2	67	92.7	0.72
S10 (210)	28	1.00	178	61.6	18.2	19.4	63.5	83	0.77
S11 (211)	22	1.00	168	69.6	28.9	24.7	74.5	96.8	0.77
S12 (212)	22	1.00	168.3	54.4	16.5	19.2	65.5	78	0.84
S13 (213)	26	1.00	153	55.3	26.9	23.6	72	82	0.88
S14 (214)	27	1.00	174.1	65.2	19.6	21.5	74.7	94.7	0.79
S15 (215)	24	1.00	163.5	67	31.4	24.9	70.5	99	0.71
S17 (217)	23	1.00	161	58.4	21.6	22.6	73	92.5	0.79
S18 (218)	25	1.00	172.8	85.3	34.2	26.9	87.5	113.5	0.77
S19 (219)	24	1.00	157.9	66.2	28.9	26.5	72.4	105.5	0.69
S20 (220)	25	1.00	161.5	63.7	27	24.3	73	103	0.71
S21 (221)	18	1.00	171.4	74.4	14.9	25.8	78.5	103.5	0.76
S22(222)	21	1.00	177.1	68.9	25.8	22	84.5	101.5	0.83
W01 (301)	56	2.00	172.9	67	21.5	22.4	73	102.7	0.71
W02 (302)	58	2.00	169.1	79.1	39.2	27.7	91.5	112.5	0.81
W03 (303)	54	2.00	151.5	49.1	21.65	21.2	74	86	0.86
W04 (304)	54	2.00	172.9	67.4	22.35	22.75	72.5	93.5	0.78
W05 (305)	68	2.00	155.5	73.4	43.1	30.4	86.5	107.5	0.80
W06 (306)	65	2.00	158	53.2	28.85	21.3	73	94.5	0.77
W07 (307)	58	2.00	160.7	56	27.1	21.6	77	94.8	0.81
W08 (308)	62	2.00	158.3	71.4	39.8	28.5	84.5	112.2	0.75
W09 (309)	58	2.00	166.3	71.5	33.4	26	96	97	0.99
W10 (310)	58	2.00	153.9	78.6	50.3	33.1	93	117	0.79
W11 (311)	57	2.00	176	74.7	25.5	24.3	78	99	0.79
W12 (312)	52	2.00	162.8	60.5	22.8	26.7	78	90	0.87
W13 (313)	59	2.00	170.6	61	19.5	20.95	68	94.5	0.72
W14 (314)	55	2.00	168.8	61.7	31.4	21.6	75.5	96.2	0.78
W15 (315)	59	2.00	170.3	79.8	35.5	27.6	82.2	111.5	0.74
W16 (316)	52	2.00	157.5	63.5	39	25.8	80.3	105.5	0.76
W17 (317)	65	2.00	164.9	70.5	34.4	25.9	84	101.2	0.83
W18(318)	55	2.00	168.3	56.2	29.4	19.85	70.5	92.8	0.76
W19 (319)	54	2.00	159.4	57.1	21.2	22.5	67.5	92.3	0.73
W20 (320)	59	2.00	160.2	60.8	33.8	23.8	79.1	94.8	0.83

Activity, Jump and bone SOS data

Participant ID	Physical Activity Mets/Week	Jump Height (cm)	Box Height (cm)	Percent of Jump Height (%)	SOS Rad	SOS Tib
S01 (201)	121	31.5	35	112.8888889		
S02 (202)	109		35			
S03 (203)	55		35			
S04 (204)	27		35			
S05 (205)	116		35			
S06 (206)	66		35			
S07 (207)	82	37	35	96.10810811		
S08 (208)	20		35			
S09 (209)	59	29	35	122.6206897		
S10 (210)	56	33	35	107.7575758		
S11 (211)	46		35			
S12 (212)	39	32	35			
S13 (213)	82		35			
S14 (214)	72	37.2	35	95.59139785		
S15 (215)	167	28	35	127		
S17 (217)	54	31.2	35	113.974359	4034	3957
S18 (218)	47	28.1	35	126.5480427	4089	3838
S19 (219)	40	38.3	35	92.845953	4098	3943
S20 (220)	122	38.9	35	91.41388175	4028	3999
S21 (221)	45	38.9	35	91.41388175	4107	3928
S22(222)	161		35		4139	-3904
W01 (301)	37	30.6	35	114.379085	4032	3784
W02 (302)	57	26.4	30	113.6363636	3891	4087
W03 (303)	79	25	30	120	4007	3861
W04 (304)	95	24	25	104.1666667	3919	3870
W05 (305)	55	20	25	125	3916	3966
W06 (306)	60	18	25	138.8888889	4099	3907
W07 (307)	112	24	25	104.1666667	3756	3983
W08 (308)	76	17	25	147.0588235	4030	3878
W09 (309)	51	28.6	30	104.8951049	4075.0	3839
W10 (310)	45	16.3	25	153.3742331	4183.0	3930
W11 (311)	12	28.6	30	104.8951049	4126.0	3962
W12 (312)	11	20	25	125	4205.0	3755
W13 (313)	48	20	25	125	4026.0	3892
W14 (314)	106	27.3	30	109.8901099	3959.0	4027
W15 (315)	144	20.7	25	120.7729469	4021.0	3876
W16 (316)	28	21	25	119.047619	4385.0	4064
W17 (317)	154	24	25	104.1666667	3894.0	3903
W18(318)	45	22	25	113.6363636	4040.0	3968
W19 (319)	116	22	25	113.6363636	4110.0	3812
W20 (320)	84	19.3	25	129.5336788	4034.0	3994

Serum and Plasma Measurements

Participant ID	Plasma A	Plasma B	Plasma C	Plasma D	SOST A	SOST B	SOST C	SOST D
S01 (201)					175.6	261	161	
S02 (202)					165.2	469.3	356.6	
S03 (203)					127	78.76		
S04 (204)					203.1	243.3	218.1	
S05 (205)					328.2	418.9	235.5	
S06 (206)					170.2	220.7	135.8	134.6
S07 (207)					137.8	313.2	335.5	450.3
S08 (208)					417	737.7	335.3	
S09 (209)					529.1	473.8	290.4	272.8
S10 (210)					318.7	187.2	238.9	375
S11 (211)					261.1	110	120.7	207.1
S12 (212)					261.7	329	213.8	
S13 (213)					195.3	406.5	278.9	169.5
S14 (214)					163.6	360.6	160.9	146.3
S15 (215)						63.3	71.89	123.9
S17 (217)					92.04	189.7		156.6
S18 (218)					126.3	218.3	302.1	253
S19 (219)	0.52	0.65	0.66	0.65	314.4	388.6	501.2	435.6
S20 (220)	0.62	0.62	0.625	0.634	49.8	106	52.22	228.5
S21 (221)	0.63	0.61	0.624	0.624	335	370.8	404.6	296.5
S22(222)	0.58	0.57	0.58	0.6	62.07	81.46	69.74	127.2
W01 (301)	0.66	0.65	0.65	0.68	724.3	1004	872.3	1392
W02 (302)	0.52	0.52	0.53	0.53	1047	747	611.4	1350
W03 (303)	0.57	0.59	0.6	0.605	557.9	526	484.9	1158
W04 (304)	0.55	0.55	0.55	0.57	389.6	304.2	314.7	381.1
W05 (305)	0.575	0.55	0.57	0.58	602.5	653	780.7	816.1
W06 (306)	0.57	0.57	0.58	0.57	1033	979.3	1407	867.9
W07 (307)	0.53	0.56	0.55	0.55	160.5	353.5	245.4	547.7
W08 (308)	0.59	0.58	0.59	0.6	584.2	351.8	403.3	697.4
W09 (309)	0.57	0.56	0.57	0.55	701.9	631.7	430.9	
W10 (310)	0.58	0.57	0.58	0.57	806.6	720.4	830.5	756.3
W11 (311)	0.57	0.57	0.57	0.58	572.5	595.7	905.7	791.9
W12 (312)	0.58	0.56	0.57	0.6	623.4	799.3	689.9	827.5
W13 (313)	0.583	0.575	0.575	0.6	713.3	607.4	333.5	372.6
W14 (314)	0.6	0.59	0.595	0.592	666.3	721.6	862.1	783
W15 (315)	0.597	0.594	0.607	0.599	722.4	402.5	616.4	573.9
W16 (316)	0.607	0.59	0.58	0.6	339	404.1	338.9	515.2
W17 (317)				1	162.6	212	400.4	308.5
W18(318)	0.6	0.59	0.6	0.61	280.9	193.5	405.4	354.9
W19 (319)	0.56	0.55	0.55	0.55	162.6	188.3	391.3	133.1
W20 (320)	0.56	0.56	0.56	0.56	411.5	630.6	523	524.9

Participant ID	DKK-1 A	DKK-1 B	DKK-1 C	DKK-1 D	Leptin A	Leptin B	Leptin C	Leptin D
S01 (201)	1385.93	2419.89			4105.95	4335.88		
S02 (202)	2450.37	2724.49	2092.28		5111.24	2941.68	2662.42	
S03 (203)	1805.37	1891.11	1563.56	1805.37	3282.78	2770.3	2328.85	2519.76
S04 (204)	2208.07	1754.64	1998.84	1861.59	5903.75	3816.76	4498.15	3655.86
S05 (205)	2308.14	2642.88	2239.54	2142.59	4723.7	4225.34	3571.33	4370.17
S06 (206)	2008.33	2085.74	1768.69	1777.02	1597.02	1372.31	1143.77	1967.08
S07 (207)	2705.3	2575.6	2381.11	2539.73	4392.53	3630.2	3390.09	3449.97
S08 (208)	2874.75	3207.36	2687.86		8385.13	7520.43	7339.93	
S09 (209)	2484.56	3066.04	2701.16		1448.06	1096.46	968.67	
S10 (210)	2705.3	2935.97	2503.87	2530.07	3576.07	3109.99	2785.4	3254.95
S11 (211)	3819.27	4014.4	3479.32	3568.44	6264.64	5012.04	4169.32	5002.89
S12 (212)	3304.89	3625.43	3457.06		2476.75	2064.42	1884.09	1640.99
S13 (213)	2244.26	2455.33	2235.94	2277.37	6102.91	5309.43	4386.01	4666.85
S14 (214)	3127.59	3417.47	2767.5	2326.56	2994.56	2599.76	1898.26	1731.85
S15 (215)		2904.86	2550.46	2445.35		7651	6582.13	6115.98
S17 (217)		1102.96		1064.01		2544.74		2769.1
S18 (218)								
S19 (219)	2025.86	2348.42	2149.79	2349.5	5215.76	4648.65	4208.26	4451.84
S20 (220)	1688.86	1985.75	1894.17	2111.58	4581.13	4225.31	3928.57	4412.21
S21 (221)	1801.84	1485.43	1582.39	1424.98	11186.17	10127.91	8756.61	9287.23
S22(222)								
W01 (301)	1420.03	1513.42	854.34		4856.15	4383.66	3978.03	
W02 (302)	2494.8	2880.44	2128.98	2544.94	15160.58	15119.38	13168.41	11812.04
W03 (303)	758.74	1091.25	1004.39	1049.74	2527.18	2429.38	2559.3	3730.57
W04 (304)	1872.69	1975.14	1476.21	1541.87	4192.31	3387.55	2840.85	4305.68
W05 (305)	1166.6	1366.27	872.43	1706.7	14773.92	14734	13142.83	13613.81
W06 (306)	2466.63	2349.38	1320.59	2323.17	948.05	834.63	804.33	759.24
W07 (307)	2049.81	1928.09	1379.58	1441.24	4869.95	4292.39	4027.66	5060.81
W08 (308)	1502.77	635.54	746.8	953.26	7248.01	3273.31	4994.2	8731.33
W09 (309)								
W10 (310)	1049.26	1021.81	978.42	1009.13	21346.82	17049.56	15261.96	21771.58
W11 (311)	1675.61	1650.57	1569.77	1608.8	3145.01	2622.19	2804.22	3456.7
W12 (312)	2733.11	2724.18	2653.67	2771.31	3305.52	2863.97	2798.06	3707.2
W13 (313)	3318.5	3009.24	2792.89	3324.69	1993.49	1400.1	1377.07	1509.9
W14 (314)	2645.94	2825.82	2349.6	2607.2	3543.52	3070.37	2553.75	2909.24
W15 (315)	2951.69	2483.09	2685.79	2685.79	17928.14	13248.28	13914.88	10636.01
W16 (316)	2763.68	2442.06	2735.66	2669.1	9050.12	7528.22	7882.65	8623.09
W17 (317)	1148.12	1061.91	557.44	773.92	3607.72	3241.33	2269.35	2955.81
W18(318)	1752.63	964.63	633.6	1830.14	6947.44	2826.82	4059.47	4287.85
W19 (319)	1323.82	1110.32	1156.51	1029.2	1188.88	984.23	830.76	819.75
W20 (320)								

Participant ID	OPG A	OPG B	OPG C	OPG D	Estradiol
S01 (201)	328.77	417.23			126.1
S02 (202)	389.35	283.32	280.67		168.9
S03 (203)	255.65	251.8	262.87	281.15	
S04 (204)	339.35	281.63	341.99	340.55	67.4
S05 (205)	387.91	387.43	433.09	422.51	54.07
S06 (206)	440.76	408.86	411.8	393.51	88.27
S07 (207)	261.29	254.9	256.6	277.25	130.3
S08 (208)	725.28	668.17	616.56		230.6
S09 (209)	367.19	370.35	422.09		46.72
S10 (210)	311.41	298.26	330.45	308.86	112.7
S11 (211)	403.3	413.6	405.02	429.02	56.39
S12 (212)	533.75	573.02	632.69	443.62	
S13 (213)	430.73	471.66	477.33	485.82	52.07
S14 (214)	370.28	344.3	339.38	281.1	
S15 (215)		552.71	623.17	440.09	
S17 (217)		315.69		303.45	
S18 (218)					79.42
S19 (219)	358.62	356.87	448.92	392.47	79.68
S20 (220)	412.29	466.17	476.58	668.8	72.94
S21 (221)	364.33	353.8	351.6	356.87	67.26
S22(222)					122.8
W01 (301)	629.05	668.41	566.15		43.79
W02 (302)	453.27	476.09	452.79	467.2	77.17
W03 (303)	328.53	323.48	347.04	321.08	66.29
W04 (304)	458.57	442.86	326.22	397.93	27.89
W05 (305)	450.87	480.17	484.98	461.44	68.05
W06 (306)	722.6	619.48	625.91	625.91	59.39
W07 (307)	589.99	536.08	543.58	565.65	68.08
W08 (308)	448.93	197.37	367.4	439.5	28.91
W09 (309)					
W10 (310)	514.49	471.93	489.42	505.61	84.46
W11 (311)	468.83	435.94	514.18	500.19	73.37
W12 (312)	933.21	826.04	773.49	836.69	78.03
W13 (313)	778.43	673.47	685.92	740.25	
W14 (314)	510.11	561.26	505.31	506.72	
W15 (315)	644.92	532.63	551.16	567.98	94.02
W16 (316)	518	398.72	514.62	512.92	83.04
W17 (317)	333.63	301.27	261.18	260.31	83.03
W18(318)	410.97	175.96	319.63	367.62	84.82
W19 (319)	449.81	430.59	391.15	478.13	48.36
W20 (320)					76.35

Dietary Data

Participant ID	Carbohydrate	Carbohydrate as % of cals	Cholesterol	Dietary Fiber	Alcohol % of cals	Sweets %of cals	Vitamin A	Beta-carotene	Vit C
S01 (201)	313.82	0.5036	319.07	33.94	0.0145	0.0571	1494.09	10682.47	27
S02 (202)	144.36	0.4589	329.15	18.72	0.0332	0.2011	1459.01	9387.75	13
S03 (203)	114.21	0.4188	138.97	7.44	0.0323	0.2342	396.47	1495.94	66.
S04 (204)	130.41	0.0405	220.63	10.48	0.057	0.1028	534.93	1773.61	13
S05 (205)	128.73	0.6483	17.89	21.03	0.0279	0.0325	674.33	7797.97	11
S06 (206)	184.51	0.4866	117.8	17.01	0.00356	0.1872	813.59	2423.81	10
S07 (207)	154.55	0.4516	127.11	13.72	0.138	0.1606	689.82	5978.87	15
S08 (208)	200.95	0.0413	216.9	14.79	0.0636	0.2232	628.98	2600.86	48.
S09 (209)	300.81	0.5643	70.23	24.19	0.0373	0.2914	648.06	1332.87	12
S10 (210)	174.12	0.4721	140.65	16.74	0.1061	0.0527	526.89	2432.81	32.
S11 (211)	222.25	0.4282	338.65	23.82	0.0351	0.1564	1342.22	6548.68	16
S12 (212)	170.62	0.3937	280.37	16.99	0.0341	0.0939	729.27	4659.24	12
S13 (213)	99.85	0.2453	528.67	20.28	0.00945	0.0571	1493.64	10490.04	13
S14 (214)	191.44	0.4886	140.83	18.64	0.1495	0.0416	909.02	5448.85	84.
S15 (215)	137.56	0.4438	319.78	14.88	0.0524	0.1546	1310.86	8073.27	94.
S17 (217)	354.79	0.4905	211.3	34.9	0.0567	0.1173	859.71	3643.81	26
S18 (218)	387.91	0.7228	45.15	48.94	0.0154	0.1405	1533.7	15227.41	45
S19 (219)	209.84	0.4033	285.09	19.63	0.096	0.0852	653.69	2324.37	10
S20 (220)	128.65	0.4418	261.04	12.39	0.0548	0.1314	734.47	4555.35	57.
S21 (221)	380.73	0.4625	494.38	36.54	0.0237	0.0926	1940.68	10767	42
S22(222)	196.09	0.4978	167.58	15.68	0.0621	0.178	592.11	2551.24	12
W01 (301)	169.47	0.5005	117.82	19.79	0.00379	0.1635	746.84	4919.29	78.
W02 (302)	146.35	0.4359	254.73	17.24	0.0078	0.0904	851.72	5779.48	56.
W03 (303)	390.58	0.5465	259.73	47.42	0.00131	0.0524	1414.32	7898.84	44
W04 (304)	140.51	0.4344	144.92	15.6	0.0577	0.0284	498.56	3251.1	13
W05 (305)	132.29	0.2999	473.84	32.05	0.0268	0.00677	1232.7	12311.15	17
W06 (306)	149.93	0.0005	181.1	10.66	0.0104	0.1007	700.16	2298.75	64.
W07 (307)	262.62	0.5607	149.95	37.24	0.1015	0.0694	512.49	3011.14	10
W08 (308)	227.83	0.0448	219.37	29.87	0.0204	0.0282	1682	8390.85	17
W09 (309)	138.86	0.4227	151.72	14.23	0.103	0.0315	944.13	7992.49	10
W10 (310)	189.72	0.3575	153.76	22.54	0.0735	0.0114	1779.38	14065.06	16
W11 (311)	232.18	0.4923	162.37	27.1	0.00868	0.1174	2095.61	11021.11	34
W12 (312)	158.24	0.3939	433.55	24.84	0.00355	0.0362	1512.77	12561.51	18
W13 (313)	174.03	0.5253	148.53	13.1	0.0268	0.122	388.14	1161.91	14
W14 (314)	184.44	0.4745	194.23	22.83	0.000774	0.1563	981.54	6125.76	11
W15 (315)	106.39	0.4002	123.16	13.67	0.1285	0.1049	564.88	3818.22	65.
W16 (316)	67.56	0.3716	224.53	9.34	0.091	0.0334	395.53	2677.27	69.
W17 (317)	99.21	0.5049	81.12	7.82	0.0282	0.0959	442.61	1761.05	36.
W18(318)	149.27	0.0422	164.99	16.72	0.1104	0.0751	662.42	4123.22	10
W19 (319)	39.52	0.4577	58.47	2.99	0	0.0905	135.84	876.38	14.
W20 (320)	220.53	0.5323	283.95	35.85	0.0393	0.0633	1660.85	17746.78	26

Participant ID	Vitamin E	B1, B2	Niacin	Folate	Vitamin B6	Calcium	Zinc	Iron	Potassium
S01 (201)	10.58	2.44	29.45	682.51	2.62	2289.75	17.19	19.42	4716.49
S02 (202)	13.73	2.06	27.59	344.25	2.21	763.43	10.21	14.02	2535.19
S03 (203)	5.21	0.752	13.19	197.72	0.982	534.07	5.62	6.31	1434.26
S04 (204)	5.66	1.15	15.04	241.05	1.01	628.55	8.08	7.85	2062.33
S05 (205)	5.8	0.817	7.27	241.77	0.936	407.83	4.39	8.26	1751.83
S06 (206)	9.29	1.51	18.01	419.63	1.72	1210.58	9.66	11.9	2439.48
S07 (207)	8.28	1.19	15.01	279.86	1.19	756.13	7.18	9.02	2190.1
S08 (208)	7.58	1.52	22.67	321.92	1.49	806.18	9.33	11.1	2509.31
S09 (209)	13.58	2.29	21.91	443.32	1.95	1662.15	13.05	16.1	3001.98
S10 (210)	4.87	1.9	12.93	308.78	1.31	994.57	8.08	11.11	2417.71
S11 (211)	11.42	1.77	26.46	417.14	2.37	1617.65	12.07	14.88	3783.63
S12 (212)	7.38	1.33	22.19	324.06	1.64	709.59	14.09	13.21	2638.19
S13 (213)	12.2	1.36	18.77	396.23	1.63	742.69	10.81	11.26	2538.16
S14 (214)	6.19	1.52	16.47	343.41	1.83	1167.37	8.52	9.42	3239.41
S15 (215)	10.5	1.87	19.08	284.58	1.84	771.51	8.21	12.55	2264.35
S17 (217)	15.36	2.13	26.42	536.9	2.25	1592.12	15.83	19.27	3941.53
S18 (218)	14.28	2.98	25.95	752.13	3.34	789.35	11.38	21.8	4752.57
S19 (219)	7.33	1.68	19.42	381.99	1.66	1256.44	11.75	13.36	2749.05
S20 (220)	5.39	1.27	11.31	219.95	1.06	872.62	6.86	7.87	1955.01
S21 (221)	21.88	2.57	38.07	611.23	3.58	1791.58	18.2	22.67	5202.12
S22(222)	5.08	1.2	13.66	263.1	1.27	803.14	7.46	9.76	2117.24
W01 (301)	7.04	1.05	13.37	258.41	1.41	710.84	6.96	9.48	2080.65
W02 (302)	7.04	1.15	10.03	239.79	0.958	795.81	6.45	9.33	1946.64
W03 (303)	13.47	2.08	21.31	605.06	2.47	1803.84	13.2	19.21	5620.97
W04 (304)	6.7	1.53	13.96	276.09	1.34	949.05	8.97	9.31	2815.03
W05 (305)	16.07	1.34	22.69	447.17	2.27	496.22	11.14	12.06	3987.08
W06 (306)	3.93	1.89	13.98	260.68	1.13	1056.48	9.05	9.9	2744.28
W07 (307)	7.17	2.39	20.38	424.49	1.71	1152.37	10.64	17.23	3122.04
W08 (308)	12.2	2.57	23.01	432.35	2.48	1477.29	15.5	21.76	3391.03
W09 (309)	6.92	1.53	15.59	309.13	1.53	898.19	8.57	8.96	3096.08
W10 (310)	18.11	1.5	18.86	568.9	2.11	901.67	13.1	15.7	3185.6
W11 (311)	20.18	2.76	26.05	504.01	2.49	1547.51	13.84	19.22	4339.64
W12 (312)	12.59	0.988	15.7	413.73	1.94	787.08	8.06	11.58	2953.83
W13 (313)	6.32	1.8	12.69	304.34	0.999	739.82	7.14	10.55	2276.83
W14 (314)	7.29	1.77	17.3	341.33	1.45	918.44	9.89	12.95	2597.45
W15 (315)	5.7	0.921	12.08	252.93	1.06	368.06	6.64	8.4	1920.84
W16 (316)	3.61	0.519	8	185.99	0.739	357.37	4.47	5.17	1377.77
W17 (317)	3.17	1.1	7.23	180.54	0.837	813.05	5.77	7.43	1577.25
W18(318)	7.56	1.37	15.33	261.69	1.19	635.2	7.74	9.49	2324.74
W19 (319)	1.41	0.263	3.55	70.89	0.265	112.26	1.64	2.38	396.81
W20 (320)	11.35	1.82	20.94	646.29	2.12	935.76	9.9	16.71	3975.77

Participant ID	Sodium (salt)	Magnesium	Supl.Vit.A	Supl.Vit.C	Supl.E	Supl.Folate	Supl.Calcium	Supl.Iron	Supl.Zi
S01 (201)	4762.3	494.59	0	0	0	0	0	0	0
S02 (202)	1964.21	369.45	909.87	26	8.67	173.31	86.65	35.96	4.77
S03 (203)	1811.06	157.95	609	200	20.1	0	0	0	20
S04 (204)	2162.53	200.45	0	19.23	0	0	0	0	0
S05 (205)	1513.42	208.77	0	0	0	0	0	0	0
S06 (206)	2170.27	312.58	0	0	0	0	0	0	0
S07 (207)	2228.82	263.23	0	0	0	0	151.64	0	0
S08 (208)	3221.74	261.85	0	0	0	0	0	0	0
S09 (209)	2143.33	480.82	1539.78	43.99	14.66	293.29	146.65	13.2	8.07
S10 (210)	2033.05	278.18	0	0	0	0	46.15	47.66	0
S11 (211)	2964.36	420.33	0	0	0	0	0	0	0
S12 (212)	4458.85	264.91	0	0	0	400	0	0	0
S13 (213)	2687.23	291.82	0	767.51	0	114.29	0	0	36.66
S14 (214)	2385.67	311.6	0	0	0	0	0	0	0
S15 (215)	1900.25	300.71	0	0	0	0	0	0	0
S17 (217)	3812.55	509.97	161.54	4.62	1.54	30.77	15.38	1.38	0.846
S18 (218)	3096.66	567.32	0	0	0	0	0	0	0
S19 (219)	3428.96	310.85	2100	60	20	400	200	18	11
S20 (220)	2013.66	228.51	0	0	0	0	0	0	0
S21 (221)	5529	604.97	0	500	0	0	0	65	0
S22(222)	2433.4	227.2	0	0	0	0	0	0	0
W01 (301)	2330.42	245.74	0	0	0	0	85.71	9.29	0
W02 (302)	3435.67	223.25	0	76.92	0	0	281.63	5	0
W03 (303)	4100.69	527.23	1200	1120	96.57	800	200	28	15
W04 (304)	2229.09	251.06	0	0	0	0	0	0	0
W05 (305)	2598.12	344.56	2100	60	20	400	200	18	11
W06 (306)	1866.42	262.92	0	0	0	0	0	0	0
W07 (307)	2218.58	473.4	0	0	0	0	439.94	0	0
W08 (308)	3266.45	492.81	1986.32	1745.08	29.4	586.58	623.24	13.2	22.73
W09 (309)	2286.85	272.81	161.54	43.08	1.54	30.77	15.38	1.38	0.846
W10 (310)	3370.83	416.36	2100	60	20	800	800	18	11
W11 (311)	2899.27	525.27	3909	1500	60.1	1600	750	46	46
W12 (312)	2502.54	298.31	0	0	0	0	46.15	0	0
W13 (313)	1881.68	268.01	161.54	1004.62	39.82	61.54	275.35	6.38	7.99
W14 (314)	2876.13	299.57	0	0	0	0	0	0	0
W15 (315)	1777.05	206.36	0	225.87	0	30.77	0	0	0
W16 (316)	1309.61	132.07	909.87	64.46	85.24	173.31	258.08	7.8	4.77
W17 (317)	1224.82	164.59	0	0	0	0	0	0	0
W18(318)	2272.77	248.45	0	0	0	0	0	0	0
W19 (319)	602.92	40.4	0	0	0	0	600	65	0
W20 (320)	4436.05	412.9	0	0	0	0	0	0	0