

The Effect of a six month low-carbohydrate diet on the biomarkers of bone health in pre- and post-menopausal women: a randomised control crossover trial

Doreen Fitzmaurice

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Supervisor

Dr. Lorna Doyle

External Examiner

Fiona Lithander

Internal Examiner

Michael Harrison

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DECLARATION

I declare that I am the sole author of this thesis and that the work presented here has not previously been submitted as an exercise for a degree or other qualification at any university. It consists entirely of my own work, except where references indicate otherwise.

Doreen Fitzmaurice

25th June 2012

ABSTRACT

Thesis Title: The effect of a six month low- carbohydrate diet on the biomarkers of bone health in pre- and post-menopausal women: a randomised control crossover trial

Author: Doreen Fitzmaurice

With increasing levels of obesity, the low-carbohydrate diet has again become popular as a weight loss method. While weight loss through consumption of a low carbohydrate diet has been well researched, there is contradictory evidence regarding its effect on bone health. The effect of this diet on bone health for greater than 12 weeks and in menopausal women is relatively unknown. The aim of this study was to assess the effect of a low-carbohydrate diet consumed over 24 weeks on biomarkers of bone health in pre- and postmenopausal females.

Following ethical approval, informed consent and screening, 24 subjects (13 pre-menopausal; 11 postmenopausal) were randomly assigned to a control or low-carbohydrate diet group for 24 weeks. Subjects were then crossed over to the alternative dietary regimen for 24 weeks. Blood and urine samples taken at week 12, 24, 36 and 48 were analysed for biomarkers of bone resorption and formation using ELIZA. Urine pH was measured and a 3 day food diary analysed using CompEat™. Data was analysed for treatment and carryover effects. Where carryover effects were evident analysis was completed on 12 subjects only.

Consuming the low-carbohydrate diet resulted in a significant decrease in energy ($P = 0.017$) and carbohydrate ($P = 0.001$) but protein intake did not change ($P = 0.264$). There was a significant reduction in weight ($P = 0.000$), waist circumference ($P = 0.000$), diastolic blood pressure ($P = 0.021$), K ($P = 0.047$) and Mg ($P = 0.005$). Increased PRAL ($P = 0.004$), NTx ($P = 0.019$) and IGF-1 ($P = 0.043$) were observed in the low-carbohydrate period versus the control. Several other parameters changed over 12 weeks of the low-carbohydrate diet but not over the 24 week period. There were significant decreases in fibre, Ca and Na during the low carbohydrate diet over 12 weeks and increases in vitamin D and urinary Ca. There were no significant differences in any other parameters measured.

Weight loss due to a low-carbohydrate diet caused significant changes in nutrient intake with some aspects being conducive to bone health but other aspects perhaps detrimental to bone. Increased bone resorption observed in this study without concomitant increased bone formation suggests this diet could cause poor bone health over time.

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Abbreviations

AA	Amino acid
BMC	Bone mineral content
BMD	Bone mineral density
BMI	Body mass index
BMU	Basic multicellular unit
BSAP	Bone specific alkaline phosphatase
BTM	Bone turnover marker
Ca	Calcium
CHO	carbohydrate
Cr	Creatinine
CTx	C-terminal type 1 collagen telopeptide
D ₂	Ergocalciferol
D ₃	Cholecalciferol
DEXA	Dual energy x-ray absorptiometry
DPD	Deoxypyridinoline
DRI	Dietary recommended intake
E2	Oestradiol
ELIZA	Enzyme-linked immuno-absorbent Assay
FSAI	Food safety authority of Ireland
FSH	Follicle stimulating hormone
GFR	Globular filter rate
GI	Glycemic Index
HC	High carbohydrate
HCL	Hydrochloric acid
HRT	Hormone replacement therapy
IGF-1	Insulin-like growth factor
IGFBP-3	Insulin-like growth factor binding protein
IS	Insulin secretion
IU	International units
K	Potassium

Kcal	Kilocalorie
LBM	Lean body mass
LC	Low carbohydrate (diet)
LDL/HDL	low/high density lipoproteins
Mg	Magnesium
<i>n</i>	Number of subjects
N	Nitrogen
Na	Sodium
NANS	National adult nutritional survey
NEAP	Net endogenous acid production
NTx	N-terminal type 1 collagen telopeptide
OC	Osteocalcin
OI	Osteogenic Index
P	Phosphorous
PINP	pro-collagen N-terminal pro-peptide
PRAL	Potential renal acid load
PTH	Parathyroid hormone
pQCT	peripheral quantitative computed tomography
PUFA	Polyunsaturated fatty acids
PYD	Pyridinoline
RDA	Recommended dietary allowance
S	Serum
SD	Standard deviation
SLAN	Survey of lifestyle, attitudes and nutrition
SPSS	Statistical Package for Social Sciences
TAP	Total serum alkaline phosphatase
T-score	Bone mineral density comparison score
U	Urinary
WC	Waist circumference
WHO	World health organisation

Chapter 1 – Introduction

1.1. Introduction

The World Health Organization has described the prevalence of excess weight and obesity as ‘an epidemic’. It was reported in 2005 in Ireland that 39% of adults were classed as overweight and 18% as obese, with Irish girls aged 13-14 years as having levels of excess weight/obesity higher than the international average (Department of Health and Children, 2005). Recent research recorded 39% of 18-64 year olds as having normal weight, with 37% overweight and 24% obese; thus the number of individuals with normal weight is lower, and the occurrence of obesity is greater than ever before, with higher incidence of the latter reported in men aged 51-64 years. These results highlight obesity as a major health issue in Ireland (Walton, 2011).

Additionally, there is an overall increasing prevalence of osteoporosis worldwide (WHO, 2003) and despite the need to tackle incidences of excess weight and obesity, there is also concern about the effects of weight loss and aging on bone health. Extensive research has been carried out investigating Bone Mineral Density (BMD) with regard to advancing age, menopausal status and oestrogen levels, as well as weight loss. While a number of studies examining the influence of aging and menopause on women’s bone health concur on the progressive increase in bone loss with aging (Luisetto *et al.* 1993, Holm *et al.* 2002), it has been suggested that age related bone loss has only a minor influence on bone function compared to that of oestrogen withdrawal after menopause (Lukacs *et al.* 2003).

With regard to bone health and menopausal status, current research remains controversial; a number of studies agree that menopausal transitional women with irregular menstrual cycles experience more bone loss than both pre- and postmenopausal women (Mazzuoli *et al.* 2002; Holm *et al.* 2002; Ho *et al.* 2008). In

contrast, a longitudinal study, which measured BMD by DEXA scan on average every 25 months, reported BMD loss was fastest in women who became postmenopausal (Guthrie *et al.* 1998). However it is suggested that the techniques for detecting bone loss may have been inadequate (Finkelstein *et al.* 2008).

Bone loss has also been recorded during weight loss interventions; a study of overweight middle aged men observed 1.5% loss of BMD during a lipid reduction diet where weight loss was achieved (Prichard *et al.* 1996). A study of pre- and post-menopausal women also showed that BMD reduced by 1.2% during weight loss; this was a significant difference from the control group who maintained weight (Ricci *et al.* 2001). The measurement of bone density loss alongside weight reduction was similar between the pre-, peri-, and post-menopausal women who took part in the research of Jensen *et al.* (1994), and while it was not a significant loss there was a tendency for greater BMD loss among postmenopausal women without oestrogen replacement (Jensen *et al.* 1994). Interestingly, this study found BMD increased again when weight was regained. It is thought that increased body weight puts stress on the skeletal muscle which pulls against the bone thus increasing bone mass, this can be termed mechanical loading (Holm *et al.* 2002). It has also been suggested that there is a greater conversion of adrenal androgens to oestrogens in the subcutaneous tissue of heavier women (Wardlaw, 1996; Kaaks *et al.* 2002). Adipokins are reported as molecular pathways, independent of load-bearing, which also regulate bone mass and structure, leptin levels increase with increases in body fat and may stimulate bone formation (Hamrick & Ferrari 2008). A recent review (Biver *et al.* 2011) reported high levels of leptin as predictive of low risk fractures in postmenopausal women, and adiponectin was considered the most relevant adipokine negatively associated with BMD independent of gender and menopausal status (Biver *et al.* 2011). It is suggested

that inconsistent associations between adipokines and BMD may be mediated or confounded by body composition (Jurimae et al 2008)

Due to the augmented incidence of overweight and obesity dieting has become increasingly popular. Bish *et al.* (2007) found that among overweight American women who otherwise considered themselves healthy, approximately 60% were trying to lose weight. Meanwhile in Ireland approximately 53% of women aged 45-64 years were actively trying to manage their weight (SLAN, 2007), hence dieting practices are commonplace among Irish females. It has been suggested that many women lose weight for appearance rather than health reasons (Foster *et al.* 1997), with one of the most popular fad diets being the low-carbohydrate diet (Freeman *et al.* 2001).

Crowe and Cameron-Smith's (2005) study of 1200 subjects (which was representative of the Australian population), found 17% had tried, or intended to try, a low-carbohydrate diet. In addition the authors reported that 50% wrongly believed that a quarter of dietary intake should be carbohydrate and 70% believed that in order to lose weight, they should cut back on carbohydrate intake. This report may indicate a widespread misunderstanding of what constitutes a high carbohydrate food (Crowe & Cameron-Smith, 2005).

Low-carbohydrate diet books continue to be some of the biggest selling books worldwide. In America, the Atkins Centre claim to have sold more than 45 million copies over 40 years and the South Beach diet book was on the best sellers list for over 35 weeks in 2004 (Schnirring, 2004). In Australia, researchers involved in health and fitness gymnasiums suggest that more than 200 promote some variation of a low- or no-carbohydrate dietary regime (Bilsborough & Crowe, 2003). This pattern may be similar in the Republic of Ireland, as a recent study into the dieting practices of

adolescent females found a number of subjects were aware of the Atkins diet; one subject was able to describe halitosis and had also purchased the book (Mooney *et al.* 2009). In addition the low carbohydrate diet is very easily accessible on the Tesco website; specific menus are available each day in order for people who subscribe to the website to restrict their carbohydrate level to 20g/day. However it has been reported that there is a lack of scientific investigations into the effects and consequences of the low carbohydrate diet in those over 50 years of age and for diets of more than 90 days duration (Bravata *et al.* 2003).

The effect of weight loss and nutrient intake on bone health needs to be considered since it can influence bone health, due to the prolonged deficiency or excess of one nutrient, or the combination of several (Ilich & Kerstetter, 2000). Cashman (2007) suggests that many nutrients commonly consumed in the Western diet can potentially have either a positive or negative impact (or both) on bone health.

Beneficial factors	Potentially detrimental dietary factors
Calcium	Excess alcohol
Copper	Excess caffeine
Zinc	Excess sodium
Fluoride	Excess fluoride
Magnesium	Excess/insufficient protein
Phosphorus	Excess phosphorus
Potassium	Excess/insufficient vitamin A
Vitamin C	Excess <i>n</i> -6 PUFA
Vitamin D	
Vitamin K	
B vitamins	
<i>n</i> -3 Fatty acid	
Protein	
Novel bioactive food compounds	
Whey-derived peptides	
Phytoestrogens	
Non digestible oligosaccharides (especially inulin-type fructans)	

* Some nutrients could be categorized as being both beneficial and detrimental depending on dietary exposure level: insufficient or in excess.

(Cashman, 2007)

Some of the influences that nutrients may have on bone are altering the structure of bone, the rate of bone metabolism, pancreatic or endocrine systems and homeostasis of calcium (Cashman, 2007). There is controversy regarding the contribution of one or a group of nutrients on bone health, it is suggested that this is due to the complexity that many nutrients are co-dependant and simultaneously interact with genetic and environmental factors (Ilich & Kerstetter, 2000).

While many studies focus on the high-protein diet and biomarkers of bone health there is little research to date investigating the low-carbohydrate diet and biomarkers of bone health. Carter *et al.* (2006) conducted a 3 month dietary intervention, which excluded pre- and post- menopausal women. It consisted of 15 subjects who followed a low carbohydrate diet with 15 matched controls. Investigators measured two different biomarkers of bone health, Urinary N-Telopeptide (UNTx) for bone resorption and Bone Specific Alkaline Phosphatase (BSAP) for bone formation. Results showed no increase in bone turnover markers compared with controls at any time point, and no significant change in bone turnover ratio compared with controls (Carter *et al.* 2006). Coleman and Nickols-Richardson (2005) also conducted a 3 month dietary intervention involving pre-menopausal females who either followed a low-carbohydrate or high-carbohydrate diet. In contrast they found that the bone biomarker for formation (OC) and that for resorption (NTx) increased in both diet groups compared to baseline, with no differences recorded between groups, indicating that weigh loss may stimulate bone turnover (Coleman & Nickols-Richardson, 2005).

Conclusion

Individuals who undertake a low-carbohydrate diet may not be clinically monitored for any potential detrimental effects on their health, nor have their health status prior to taking up this diet clinically evaluated. In Ireland, there is a vital need for

investigation into the health effects of this dietary regimen in menopausal females. This is due to the increasing prevalence of osteoporosis in women and high incidence of Irish women managing their weight around menopausal age. These factors coupled with increased interest and popularity of low-carbohydrate diets warrants research into its effects on bone health.

1.2. Rationale for the study

It is unknown whether the nutrient intake of a low carbohydrate diet is supportive to bone health or not. Based on the results of the study by Carter *et al.* (2006) and Coleman and Nickols-Richardson (2005) it is clear that a low carbohydrate (LC) diet intervention trial of longer duration than 12 weeks, in addition to using a larger number of subjects, is necessary. Carter *et al.* (2006) did not include pre- or post-menopausal women as subjects in their intervention, it can be seen that these groups may be at risk of reduced bone health. Thus, we conclude that a low carbohydrate dietary intervention study that includes pre- and postmenopausal females and investigates bone health in parallel would be very valuable to the academic community.

1.3 Aims of the study

Primary aims:

- This research will examine the effect of a 6 month low carbohydrate diet on biomarkers of bone formation and resorption.

Secondary aims:

- This study will investigate the influence of a low-carbohydrate diet consumed over 6 months on weight loss and intake of macro- and micronutrients, which could affect bone health.
- This research aims to examine how a low carbohydrate diet could influence other aspects which could affect bone health such as urine pH and insulin like growth factors.

1.4 Hypothesis

When a low carbohydrate diet is followed for 24 weeks by menopausal woman then effects on bone formation and resorption may be observed when measuring biomarkers of bone health.

Chapter 2 – Literature Review

2.1. Bone

2.1.1. Bone Composition

Bone provides support, attachment of muscles, protection of vital organs and a store for minerals in the body. Bone is composed of an extra-cellular matrix, which consists of both an organic phase, mainly made up of a strong fibrous protein called collagen, and a mineral phase (Vasidaran, 2008). Approximately 65% of bone tissue is made up of a variety of minerals, including calcium, phosphorus, and fluoride; with stores being used when needed by the body. These minerals form hydroxyapatite crystals that gather around collagen fibres thus enabling the bones to bear body weight and act in response to movement (Thompson & Manore, 2010). Additionally there are two major bone cellular components;

- i) Osteoblasts, these are bone forming cells, and can differentiate either into osteocytes to become embedded in the bone matrix or into a lining cell on the bone surface (Canalis, 2000)
- ii) Osteoclasts, which are involved in the breakdown of existing bone (Vasidaran, 2008)

Two different types of bone make up the human skeleton; cortical and trabecular bone. Cortical bone is dense, comprises approximately 80% of the bone density, and is found on outer surfaces as well as on many small bones in the body. Trabecular bone is found in the ends of the long bones, inside the spinal cord, flat bones and pelvis, is porous and comprises approximately 20% of the skeleton (Thompson & Manore, 2010). Trabecular bone is also referred to as spongy bone as it has no apparent organisation; Thompson & Manore (2010) equate it to scaffolding, supporting the outer cortical bone.

2.1.2. Bone Formation

Osteoblasts produce bone matrix; they appear in clusters lining the bone surface. The osteoblasts produce and secrete the major protein type 1 collagen, in addition the major non-collagenous protein osteocalcin is produced, and alongside this an initial deposit of minerals occurs (Canalis, 2000). Osteoblasts also produce insulin-like growth factors (IGFs) and a range of other growth factors. As the osteoclast matures it begins to produce alkaline phosphatase followed by osteocalcin and osteopontin, which serve as markers of bone formation in serum and urine. During the next step in maturation, 15% of the cells differentiate into osteocytes and are embedded into the new bone matrix rather than remaining to line the bone surface (Canalis, 2000).

2.1.3. Bone Resorption

The breakdown or resorption of bone results in the release of its degraded collagen fragments into the bloodstream along with calcium and phosphorus, which are utilized by the body. Osteoclasts are the bone cells that perform this resorption, they secrete enzymes and acids that degrade the bone surface (Thompson & Manore, 2010). The plasma membrane of the osteoclast contains deep folds allowing the cell to attach to the bone, similarly, it also has a ring of contractile proteins for attachment to the bone to create a bone-resorbing compartment, which allows a high extra cellular concentration of resorption product within this resorption lacuna (Canalis, 2000).

Calcium is critical to many physiologic processes, one of which is bone resorption. The reserve of calcium stored in the bone acts to support these processes, as when it is required, calcium can be released into the bloodstream for bodily utilization by resorption. In addition, if a bone becomes fractured, the rough edges may be

smoothed by resorption, or alternatively the minerals needed to repair the fracture may be obtained by breaking bone down at another site (Thompson & Manore, 2010).

2.1.4. Bone Turnover

This describes the process of bone being broken down and replenished. In general, resorption and formation are coupled so there is no change in net bone mass. Trabecular bone is more sensitive to hormonal and nutritional factors and turns over more rapidly than cortical bone (Thompson & Manore, 2010).

2.1.4.1. Bone Modelling

Bone modelling is a process, which begins in early foetal life and lasts until early adulthood. This modelling determines the shape of the bone and even after a bone has reached its full length it can increase in thickness (Thompson & Manore, 2010). After puberty, bone growth and modelling cease to change significantly but bone density or the strength of the bone continues to develop until peak bone density is reached. 90% of females reach bone density by 17 years and the majority of males by early twenties; but for both genders, peak bone mass is reached before 30 years. Bone density remains relatively stable during the thirties however, by approximately 40 years it begins to decline (Thompson & Manore, 2010).

2.1.4.2. Bone Remodelling

Bone mass is regularly recycled by a process called bone remodelling which begins before birth and continues until death (Clarke, 2008). Bone formation and bone resorption are balanced as part of the turnover mechanism whereby older or damaged bone tissue is replaced by the formation of new tissue (Raisz, 1999). In a normal

young adult approximately 30% of the total skeletal mass is renewed every year (Canalis, 2000). Bone remodelling or bone turnover increases in peri- and early post-menopausal women, and while it is still at a faster rate than in pre-menopausal women, it slows with further aging and late menopause. Bone turnover is thought to increase mildly in aging men (Clarke, 2008). There are four phases to the remodelling cycle;

- i) Activation - increased demand for minerals such as calcium and phosphorus or bone damage can signal and activate a group of pre-osteoclasts that attach to the bone surface and fuse to form a multinucleated osteoclast which creates resorption cavities (Canalis, 2000; Vasidaran, 2008).
- ii) Resorption - collagen degradation products, such as N-terminal type 1 collagen telopeptide (NTx) and C-terminal type 1 collagen telopeptide (CTx) are released during this phase of the bone turnover and can be measured in serum and urine (Vasidaran, 2008).
- iii) Reversal – when resorption is complete, osteoclasts are replaced by reversal mononuclear cells which smooth the surface and cement the old and new bone (Canalis, 2000). Pre-osteoblasts are activated by an unknown signalling mechanism although bone matrix derived factors are suggested, among which are IGF-1 & IGF-2 (Clarke, 2008).
- iv) Formation – the former signalling proteins, promote the differentiation of pre-osteoblasts into osteoblasts which lay down collagen in the bone matrix, which then becomes mineralized (Canalis, 2000).

Critically, biomarkers that can be measured here for bone formation are bone specific alkaline phosphate (BSAP) and Osteocalcin (Vasidaran, 2008). This bone remodelling unit (BRU) normally balances bone resorption and bone formation,

however it is suggested that, bone resorption which is greater than bone formation is the main root of micro architectural deterioration (Canalis, 2000).

2.2. Osteoporosis

Osteoporosis may occur when a mismatch between the overall rate of breakdown versus formation of bone develops, it is characterised by low bone mass as well as deterioration of the micro and macro-architecture of the bone tissue, and has been referred to as a silent disease since there are no signs or symptoms until a fracture has occurred (Costa-Paiva *et al.* 2011). It has been predicted that by 2020 half of all Americans over 50 years of age will have weak bones (US Dept of Health & Humanities, 2004).

2.2.1. Type 1 Osteoporosis

There are two types of osteoporosis; Type 1 is related to the rate of bone loss due to decreased oestrogen levels before and after menopause in women; this may be due to the regulation of osteoblast function by oestrogen. In early post-menopause oestrogen administration is often the first line of therapy for osteoporosis, however further research is needed in this area (Canalis, 2000).

2.2.2. Type 2 Osteoporosis

Type 2 osteoporosis is age related and is due to an imbalance between bone resorption and formation (Vasikaran, 2008). As both men and women age they have a slow continuous indefinite phase of bone loss mainly mediated by the loss of oestrogen action on extra-skeletal calcium homeostasis, leading to net calcium wasting and secondary hyperparathyroidism. Five to ten years after menopause onset sees a phase

of rapid bone loss, due to oestrogen withdrawal, additionally in elderly men oestrogen deficiency may also be the principal cause of bone loss due to low serum bioavailable oestrogen and low testosterone levels (Riggs, 2002). In addition to oestrogen and testosterone deficiency, Riggs (2002) suggests that the production of growth hormone and IGF-1 decreases with age in both genders and contributes to reduced bone formation. Their research states that while other endocrine changes occur they are less important than IGF-1 in age-related osteoporosis (Riggs, 2002).

2.2.3. Other Risk Factors for Osteoporosis

Upon reviewing the literature, Brown and Josse (2002) identified four key factors that could predict osteoporosis related fracture, these are: low BMD, prior fragility fracture, age and family history of osteoporosis. Other factors such as a weight of 57kg or less, weight loss since age 25, high caffeine and low calcium intakes were not found to be consistent independent predictors of fracture risk, after taking age and BMD into account (Brown & Josse, 2002).

The prevalence of osteoporosis has increased sharply and is expected to increase further (WHO, 2003). Currently, the lifetime risk for fracture is 40% to 50% in women, and is 13% to 22% in men; these percentages may increase if a predicted worldwide increase in life expectancy occurs. It is estimated that the number of people aged 65 and older will increase from 323 million to 1555 million worldwide by the year 2050 (Dennisen *et al.* 2006).

2.3. Measurement of Bone Health

2.3.1. Dual Energy X-ray Absorptiometry (DEXA)

Dual Energy X-ray Absorptiometry (DEXA) measures the BMD of the whole body including specific joint sites. The method also provides an estimate of percentage body fat and lean muscle. DEXA is a simple, inexpensive and non-invasive procedure where bone density is measured using low level of x-rays. The technique is considered to be of minimal risk once the correct precautions are taken by both participants and operator.

Once bone density readings are collected, they are compared to the average peak bone density of a 30-year old healthy adult in order to establish the individuals risk for osteoporosis. This comparison is known as the T-score, which for a healthy adult is 0. A score of 50 represents the mean. A difference of 10 from the mean indicates a difference of one standard deviation. Thus, a score of 60 is one standard deviation above the mean, while a score of 30 is two standard deviations below the mean. If bone density is normal the T-score will range between +1 and -1; if the T-score is between -1 and -2.5 the person has low bone density and is at an increased risk for fractures, and is considered to have osteopenia; if the range is more negative than -2.5 the person is considered to have osteoporosis (WHO, 2003).

DEXA has been identified by clinicians and the academic community as the gold standard for Bone Mass Density (BMD) measurement, but it cannot distinguish between trabecular and cortical bone (Vasikaran, 2008). Research suggests that BMD measurement as an indicator of bone health may have limitations; for instance not all individuals with low BMD will sustain fracture, and large numbers of fractures occur in people with T-scores above -2.5 (Glendenning, 2011; Schuit *et al.* 2004). It is recommended that joint use of BMD measurement and biochemical markers may be

useful in risk assessment in those not identified as at risk by BMD alone (Garnero, 2008). Research confirmed this when it was seen that bone turnover markers (BTMs) may have the potential to assess fracture risk independent of BMD (Glendenning, 2011).

2.3.2. Bone Turnover Markers

While high levels of BTMs may be an independent risk factor for fracture in postmenopausal women (Vasikaran *et al.* 2011), it has been proposed that there is a need for international agreement on a standard BTM, in the same way that DEXA has been identified for bone density (Vasikaran, 2008; Vasikaran *et al.* 2011; Glendenning, 2011). Identifying a standard BTM is proving difficult, as there are a wide variety of modifiable and non-modifiable factors that effect BTMs (Meier *et al.* 2009). Some suggested sources of variability in BTMs, are summarized in Table 2.1.

Table 2.1: Variability in bone turnover markers

Technical sources:

Specimen and mode of sample collection
 Specimen handling and storage
 Thermo degradation
 Photolysis
 Timing of sample collection (see also diurnal variation)
 Between laboratory variation

Biological (subject related) sources:

Age
 Puberty, somatic growth, menopausal transition, menopause, ageing, frailty
 Gender
 Ethnicity
 Recent fractures (up to one year)
 Pregnancy/ Lactation

Drugs:

Anti-resorptive agent (e.g. HRT, bisphosphonates, strontium,)
 Anabolic agents (e.g. anabolic steroids, PTH, strontium)
 Glucocorticosteroids
 Anticonvulsants
 GnRH agonists
 Oral Contraception

Non-skeletal Disease:

Diabetes
 Thyroid disease
 Renal impairment (GFR < 20 ml/min/1.73 m²)
 Liver disease

Systemic inflammatory disease
Degenerative joint disease
Immobility / Loss of gravity (bed rest, space flight)
Diet
Exercise
Temporal variability:
Diurnal (circadian)
Menstrual
Seasonal

(Adapted from Seibel, 2005)

It is thought some of the most sensitive biochemical markers are serum Osteocalcin (OC); Bone Specific Alkaline Phosphatase (BSAP); N-terminal propeptide of type I collagen for bone formation; Crosslinked C (CTX) and N (NTX) teleopeptides of type I collagen for bone resorption (Garnero, 2008).

2.3.2.1. Alkaline Phosphatase – bone formation

Total Serum Alkaline Phosphatase (TAP) is made up of several enzyme isoforms derived from bone, liver, intestinal, placental and kidney tissues. Most of the alkaline phosphatase activity is from the liver and bone, and in a healthy individual it can be difficult to distinguish between the two isoforms. Thus, TAP measurements can lack sensitivity in conditions with a mild increase in bone turnover (Vasikaran, 2008).

Bone specific Alkaline Phosphatase (BSAP) is synthesised by osteoblasts and measurement of serum BSAP (bone formation) has been seen to be more sensitive in identifying a change in bone turnover due to remodelling compared to measurement of TAP (Bolarin, 2001). In an 8 week randomized crossover design study, healthy postmenopausal women were assigned controlled high and low meat diets. Data collected for bone-specific alkaline phosphatase (BSAP) using enzyme-linked immunoassays suggested that the diet did not affect BSAP (Roughead *et al.* 2003). However a low carbohydrate (LC) dietary intervention study in 10 men and women who consumed what is considered an average diet of 285g/day carbohydrates for 2

weeks (high carbohydrate diet (HC)), followed by what is termed the induction phase to the Atkins diet (19g/d carbohydrates (LC)) for 2 weeks was followed by 33g/d carbohydrates for 4 weeks (LC) found a significant decrease in TAP levels during both LC phases of the diet compared to that of the HC diet, but saw no change in the BSAP (Reddy *et al.* 2002). However, a study of parathyroid hormone (PTH) treatment in postmenopausal women saw increases in BSAP from baseline at 1, 3 and 12 months (Bauer *et al.* 2006). These studies confirm the utility of BSAP in determining changes in bone formation but also the lack of effect emphasizes the need for more than one measurement of bone formation to be used in bone health studies, due to the lack of gold standard available to measure bone turnover markers.

2.3.2.2. Osteocalcin – bone formation

Osteocalcin (OC) is thought to make up 20% of the non-collagenous protein in bone, and to be Vitamin D and Vitamin K dependant. While OC is synthesised by the osteoblasts and is thought to be a measure of bone formation, some OC may also be released into circulation due to bone resorption (Seibel, Robin & Belezikian, 2006). Serum OC reflects the 10-40% of OC produced that has not been incorporated into the bone matrix; intact molecules of OC can be detected in serum but these degrade rapidly, however, fragment molecules of OC can also accumulate in serum (Lee *et al.* 2000). One type of fragment which is seen to be more stable than others is the large N-terminal mid-fragment (Chen *et al.* 1996). Some researchers rightly indicate the importance of measuring intact OC as well as the N-terminal fragment as these assays are more sensitive and have more strength (Vasikaran, 2008). There seems to be a lack of consensus as to which is the most clinically informative fragment (Seibel, Robin & Belezikian, 2006). Measurements of OC are dependant on a number of

variables such as i) time of sampling e.g. increases can be seen in the mornings; ii) seasonality – OC measurements can be lower between January and July and increases to a peak during the winter; iii) increased measurements have been taken during the luteal phase of the menstrual cycle, iv) in children compared to adult, v) in males compared to females and vi) in a woman's 50th year and beyond (Neilson *et al.* 1990). The utility of OC as a measurement tool to indicate bone formation was observed in a study of 10 men and women recruited to investigate a low carbohydrate (LC) intervention of 6 weeks duration; OC levels were measured by immunoradiometric assay kit and found to be significantly lower during the LC diet, subject's total energy consumption in their usual diet was 2,314 kcal/d; in the induction diet 1,930 kcal/d and in the maintenance was 2,034 kcal/d, and it was not reported if the subjects were weight stable during this trial (Reddy *et al.* 2002). In contrast, a study of overweight menopausal women on a high protein-weight reducing diet saw no difference in OC markers from baseline at 6 and 12 months (Sukumar *et al.* 2011). It is possible some of the variables indicated above (Neilson *et al.* 1990) which may affect OC, could have influenced these results.

2.3.2.3. Pyridinoline Crosslinks – bone resorption

The work of Eyre (1992) on biomarkers of bone resorption as cited by Vasikaran (2011) and De la Piedra (1997) describes pyridinoline (PYD) and deoxypyridinoline (DPD) as cross links between collagen molecules, which are released during the degradation of bone and cartilage. DPD is more specific to bone as large amounts are found in bone alone whereas, PYD is present in other tissues also. The measurement of day to day variations in urine samples may be reduced by the pooling of two or three daily samples (Vasikaran, 2008; De la Piedra, 1997). Immunoassays can detect

free PYD (F-PYD) and free DPD (F-DPD) in urine (Seyedin *et al.* 1993; Robins, 1994) but due to the absence of an international standard, no cross-laboratory standardization of methods has been achieved (Vasikaran, 2008). In addition to these free forms there are also peptide bound crosslinks, which are sensitive markers; namely the crosslinked C- (CTX) and N- (NTX) telopeptides of type I collagen for bone resorption (Garnero, 2008). Results from a study that compared urinary calcium, F-PYR, F-DPD, CTX and NTX as markers of bone resorption suggest that F-DPD, CTX and NTX have the best sensitivity (De la Piedra, 1997).

2.3.2.4. C-terminal Cross Linking Telopeptide of Type 1 Collagen (CTX)

During bone collagen degradation by osteoclasts, fragments of CTX are released into circulation (Knott & Bailey, 1998). A study evaluating the effects of aging, menopause and osteoporosis on measurements of serum CTX and urine CTX, found that either immunoassay equally reflected the increase of bone resorption in pre- and post-menopausal women with vertebral and hip fractures (Kawana *et al.* 2002). Measurement of the urinary ratio of alpha to beta CTX, may reflect changes in the material property of bone and thought to be predictive of fracture risk independently of BMD and bone turnover (Garnero, 2008). Due to this specificity for type 1 collagen, CTX (and NTX) most often replaces the use of older resorption indices in the diagnostic assessment of bone disease (Terpos *et al.* 2010). A further study confirming its usage examined osteoporitic postmenopausal women after 1, 3 and 12 months of PTH treatment and observed levels of CTX increase by 5, 64 and 109% respectively (Bauer *et al.* 2006).

2.3.2.5. N-terminal Cross Linking Telopeptide of Type 1 Collagen (NTX)

Another type of small cross-linked peptide that collagen is degraded to is the N-terminal cross linking telopeptide of type 1 collagen. These NTX fragments are specific for bone tissue breakdown with an enzyme linked immunosorbent assay (ELISA) detecting urinary NTX (Hanson *et al.* 1992). Urinary NTX results are expressed relative to creatinine (Terpos *et al.* 2010). A recent study investigating bone health in women aged 50-70 years with BMI 25-40 measured NTX using ELISA and reported no significant changes between a normal protein diet (18% of total calories) and a high protein diet (30% total calories) at 6 and 12 months of a calorie restricted intervention. Although there was a slight increase in NTX levels in the normal protein group at 6 months, levels returned to baseline at 12 months (Sukmar *et al.* 2011). Although this study did not produce significant difference in NTX it does demonstrate its usage in recent studies as the bone resorption marker of choice.

2.4. Factors Affecting Bone Health

2.4.1. Hormonal Decline

Age related decreases in bone health are more significant in females than males (Vasikaran, 2008); this is thought to be due to the considerable reduction in oestrogen levels after menopause (Lukacs *et al.* 2003). Investigations, which found direct effects of oestrogen on osteoblastic cells *in vitro* suggest an important role for sex steroids in the development and function of the osteoblast lineage (Gray, 1989). Evidence that oestrogen deficiency is a key contributor to bone loss was supported by later research where, techniques used to isolate highly purified mammalian osteoclasts showed that oestradiol (E₂) was able to directly inhibit osteoclastic bone resorption (Kameda *et al.* 1997). Researchers suggest that these findings support the validity of using oestrogen

replacement therapy for treating postmenopausal osteoporosis (Kameda *et al.* 1997). One study examining the influence of aging and menopause in women's bone health reported the progressive increase in bone loss with aging at the spine as -0.38% per year at 45years; -0.81% per year at 50years; -1.3% per year at 55years, and -1.9% per year at 60years (Luisetto *et al.* 1993). A number of more recent studies conclude that

- i) Pre-menopausal women with regular cycles record a bone loss of approximately 0.6% per year
- ii) Menopausal transitional women with irregular cycles, a bone loss of approximately 2.5% per year and
- iii) Postmenopausal women with no menstrual cycle for 1 year, a bone loss of approximately 1.5% per year

(Mazzuoli *et al.* 2002; Holm *et al.* 2002; Ho *et al.* 2008)

In contrast, a longitudinal study, which measured BMD by DXA scan on average every 25 months reported BMD loss was fastest in women who became postmenopausal; next fastest was in women who became late peri-menopausal; and undetectable in pre- and early peri-menopausal women (Guthrie *et al.* 1998). It has been suggested that techniques being used were not adequate for detecting bone loss when losses were small in pre and early peri-menopausal years (Finkelstein *et al.* 2008). It has also been suggested that bone loss occurs cyclically – in seven-year cycles, and that this is only observed in menopause after oestrogen levels drop (Mazzuoli *et al.* 2002). This supported previous studies that indicated acute rates of change in bone mass over a year or two are not often persistent (He *et al.* 1993). While it is reported that osteoporotic fractures occur in men ten years later in age than

women, osteoporosis is now recognized as an important disorder in men and increased incidences of fracture are predicted in the future due to increasing life expectancy (Adler, 2011). In studies where men had low levels of oestrogen they were also had lower BMD and a faster rate of BMD loss. Additionally this rate of BMD loss increased significantly in men who had both low oestrogen and low testosterone levels, while low levels of testosterone alone saw little effect on bone loss (Gennari, 2003; Cauley *et al.* 2010). However a study investigating age-related bone loss in men concluded that age-related increases of PTH and decreases of Insulin-like growth factor-1 (IGF-1) are more likely to explain the effect of age on bone loss in men rather than hormone loss (Blain *et al.* 2004).

Oestrogen loss due to ageing may be compensated for in those with excess weight, research suggests there is oestrogen production by adipose tissue (Wardlaw, 1996). Postmenopausal women with excess weight are reported to have increased E₂ levels while both pre- and postmenopausal had increased E₁ (Kaaks *et al.* 2002).

2.4.2. Physical Activity

Regular weight bearing exercises such as walking, jogging, tennis and strength training makes muscle contract and pull on the bones and put stress on the bone tissue, stimulating an increase in bone density. If weights were carried during these activities it adds extra stress and results in higher bone mass in the bones of the legs, hips and lower back (Thompson & Manore, 2010).

2.4.2.1. Cell Activity During Exercise

The mechanism at work when weight bearing exercise shows increased bone density could be a combination of events in osteocytes (Bonewald, 2006). Osteocytes are old osteoblasts which occupy the lacunar space and are surrounded by the bone matrix.

There may be a communication between osteocytes and the bone surface (Rochefort *et al.* 2010). It is thought that osteocytes may provide feedback from strain in the matrix during exercise and influence adaptive modelling and remodelling (Lanyon, 1993). If osteocyte apoptosis occurs it may activate bone remodelling however it is thought that physical activity may have a positive impact on reduction of apoptosis and thus could reduce bone remodelling (Rochefort *et al.* 2010).

2.4.2.2. Type of Physical Activity

All exercise is not equally effective for bone health, static stresses and strains are not thought to initiate osteogenesis however dynamic loading may do so (Turner & Robling, 2005). Turner and Robling (2005) suggest that the latter form of exercise creates fluid movement in the bone's lacunar-canalicular network generating shear stresses which bone cells are highly sensitive to. Therefore it is high impact exercises that produce large rates of deformation of the bone matrix which best drive fluid through the lacunar-canalicular network system (Turner & Robling, 2005). To illustrate this, a year long study of 18 middle aged women tested an endurance dance program with weight training along side a control arm of endurance dance alone. This research found that the dance group accompanied with weight training had increased muscular strength compared to the control group, but did not have increased bone mass compared to them (Peterson *et al.* 1991). A larger study of 320 postmenopausal women who engaged in aerobic weight bearing and weight lifting exercise three times a week saw increases in BDM as measured by DEXA scan compared to the control group who did no exercise (Going *et al.* 2003). If no loading is applied to the skeleton, as when individual are bed ridden or astronauts are in space, this disuse results in osteocyte apoptosis and imbalanced bone resorption leading to rapid bone loss (Ksiezopolska-Orlowska, 2010).

2.4.2.3. Duration of Exercise

Bone cells may become desensitised to prolonged mechanical stimulation since bone will be strained less by the same or a similar load once mass and geometric adaptations to an exercise have taken place, thus once this ceiling is reached no further increase in bone strength is observed (Turner & Robling, 2005). A study of male premier league soccer players found that exercise duration was correlated with total levels and bone-specific levels of alkaline phosphatase and carboxyterminal cross-linked telopeptide of type 1 collagen in those who exercised 6 hours per week, whereas no correlation was seen in those exercising above this level. Researchers concluded that bone turnover adapts to the current activity needed to maintain bone strength but above that level, exercise has no additional benefits (Karlsson *et al.* 2003).

2.4.2.4. Osteogenic Index

Animal research by Turner & Robling (2003) has led to the development of the Osteogenic Index (OI). This is a method of predicting how effective an exercise regime may be to improve bone strength. It is based on the response of bone cells and tissue to certain types of loading (Adler, 2011).

The OI for a single session of exercise is defined as the intensity of skeletal exercise $\times \ln(N+)$, where N is the number of loading cycles (Turner & Robling, 2003).

Among the first studies to investigate the use of the OI in humans, included 69 young healthy females who partook in aerobic, resistance, or combined aerobic and resistance exercise programs conducted over eight weeks alongside a control group. One of the aims of the study was to determine whether an OI could be calculated for each of the exercise programs that would reflect biochemical changes. They measured

the biomarkers BSAP and CTx. Despite the exercise programs having different loading characteristics the weekly OIs were similar. They did not identify a clear comparative relationship between the calculated osteogenic potential and the observed changes in biomarkers of bone turnover (Lester *et al.* 2009).

2.4.3. Dieting - Effect on Bone Health

Jansen *et al.* (1994) reported 16.5g bone mineral loss per kg fat loss during dietary restriction with this decrease similar for pre- and post-menopausal women (Jensen *et al.* 1994). During a dietary intervention, overweight middle aged men who reduced their total dietary fat intake by 32% from baseline reportedly lost 1.5% of BMD with a 6.4kg reduction in body weight (Prichard *et al.* 1996). Results from a study of post menopausal women supported the previous findings; here a 10% loss in body weight on a weight reducing diet alongside a 1.2% reduction in BMD, was reported. This was a significant reduction compared to the control group who maintained weight (Ricci *et al.* 2001). Another study recorded bone loss during dieting with data showing no differences in bone loss in pre-, peri-, and post-menopausal women. There was a non-significant tendency for greater BMD loss among women without oestrogen replacement (Jensen *et al.* 1994). In addition this study found BMD increased when weight was regained, suggesting that as overweight individuals have a higher BMD than average, the reduction in BMD during weight loss “is likely to be a physiologic readjustment toward normal” (Jensen *et al.* 1994). It is believed increased weight puts stress on the skeleton due to mechanical loading and thus increases bone mass (Holm *et al.* 2002). However, it has also been suggested that at the biochemical level, there is a greater conversion of adrenal androgens to oestrogens in heavier women which could result in improved bone health (Wardlan, 1996).

When the independent negative effects of both aging and dieting on bone health are considered, the information presented thus far suggests that a menopausal female who is attempting to lose weight through dieting could run the risk of significantly reduced bone health reaching beyond the effects of aging and menopause.

2.4.4. Nutrients and Bone Health

Nutrition is a modifiable factor influencing bone mass and fragility fractures. Many osteology studies have focused on calcium and vitamin D however other nutrients are also important determinants of bone health, as well as factors influencing absorption and retention of these nutrients (US Dept Health & Humanities, 2004). A range of inorganic minerals such as; calcium, magnesium, phosphorus, sodium, potassium and vitamins such as; A, D, E, K, C, as well as protein and fatty acids, can influence the development of peak bone mass in addition to loss of bone in middle age, thus affecting fracture risk (Cashman, 2007). Researchers tested associations between BMD and a number of dietary factors which were not confounded by genetics, age or lifestyle; they investigated over 2000 postmenopausal matched twins and found that diet had “an independent but subtle effect on BMD” (Fairweather-Tait *et al.* 2011). The importance of considering cellular as well as systemic effects of nutrients on bone health has been identified for some time (Roughhead & Kunkel, 1991).

While a common method of covering nutritional information in research is to do so nutrient by nutrient there is a caution that this may characterise whole foods by the health effects of specific nutrients (Campbell & Campbell 2006 p. 271). The above researchers refer to this a “reductionism” and suggest that to study isolated chemical and food components may make it possible to take the information out of context and make sweeping assumptions about complex diet and disease relationships (p.286).

2.4.4.1. Macro-nutrients and Bone Health

2.4.4.1.1. Carbohydrate

The RDA for carbohydrate for adults over 19 years of age is 130g/day however, this is based on the quantity needed to supply adequate glucose to the brain rather than what is needed to support daily activities, the Institute of Medicine recommends that 45-65% of the total daily energy intake consists of carbohydrates (Thompson & Manore, 2010). In the Irish diet the greatest contributors to carbohydrate intakes in all adults were; bread 24%, potatoes 11% and breakfast cereals 9% (Walton, 2011).

Glucose is the preferred source of energy for the brain, but is an important source of energy for all cells (Thompson & Manore, 2010). When carbohydrates are in the form of monosaccharides they are absorbed from the small intestine into the blood stream, which is controlled by three hormones insulin, glucagon, and epinephrine. Holt *et al.* (1997) suggest that while the glycemic index (GI) ranks food according to the extent to which they increase blood glucose, it may not consider insulin responses. The hypothesis that equal quantities of carbohydrate in different types of carbohydrate foods do not necessarily stimulate insulin secretion (IS) to the same extent was investigated in a study of 41 healthy males and females. They had healthy eating patterns and an average BMI of 22, it was observed that in isoenergetic servings of pasta and potatoes, both containing the same percentage of carbohydrate (50g), the IS for potatoes was three times greater than pasta. The same study similarly compared porridge to yogurt and whole-grain bread to baked beans and found that each comparison produced dissimilar ISs. Researchers suggest that different foods containing equal quantities of carbohydrate will not have equal physiologic effects

and may not require equal amounts of exogenous insulin to be metabolised (Holt *et al.* 1997).

When blood sugar concentrations are low which occurs with low carbohydrate intake, stored glycogen is converted to glucose. This was reported in a 6 month high protein diet where subjects displayed increased stimulation of glucagon and insulin in the endocrine pancreas, accompanied by high glycogen turnover and gluconogenesis (Linn *et al.* 2000). Glucose can be synthesized from non-carbohydrates sources; lactic acid, some amino acids from protein (gluconogenesis) and glycerol from fat (ketosis).

In the case of restricted carbohydrate intake and exhausted glycogen stores, glucose may be obtained from metabolizing protein or lipid in the diet. These processes; gluconogenesis or ketosis generate blood products which have the potential to generate a sub-clinical chronic metabolic acidosis which can promote calcium mobilisation from the bone (Shils *et al.* 2005). In addition evidence suggests a possible functional role for insulin in osteoclast-mediated bone resorption however it was inconclusive if the decrease in osteoclast activity found was due to an effect on the osteoclasts or was mediated by osteoblasts, as both types of bone cells have insulin receptors (Thomas *et al.* 1998).

2.4.4.1.2. Protein

The updated Irish RDAs for protein are calculated depending on the body weight of the individual: 18-65+ years 0.75g/kg (FSAI, 1999). The national adult nutritional study (NANS) recently published in Ireland shows the main contributors to protein intakes in all Irish adults are; meat 41%, breads 12%, milk & yoghurt 1% and fish 6% (Walton, 2011).

Protein breaks down into amino acids which contain a form of nitrogen that the body can easily use. Dietary protein is essential for general cell growth, repair and maintenance and in particular is involved in the development of collagen in the bone (Thompson & Manore, 2010). Proteins from animal products contain sulphur amino acids e.g. cysteine and methionine which causes blood to become more acidic when these amino acids are being metabolised. The primary mechanisms with these responses could be the hepatic oxidation of sulphur containing amino acids and a reduction in blood pH (Remer, 2000). This may cause calcium to be taken from the bone as a defence mechanism to neutralise these acids and protect the kidneys, which can only excrete urine at pH 5 (Kerstetter *et al.* 2003). If more resorption than formation of bone occurs, the risk for osteoporosis increases (Vasikaran, 2008). It was suggested that the adverse effects of sulphur amino acids could be buffered by consumption of alkali rich foods or supplements (Barzel & Massey, 1998) however, while this theory may be considered feasible additional studies are required (Thorpe & Evans, 2011).

Results from many studies of increased protein intake have been inconsistent thus far. Reddy *et al.* (2002) investigated the hypothesis that a low-carbohydrate diet (high-protein) could provide an exaggerated acid load through incomplete oxidation of fat and resultant ketoanion production. Ten male and female subjects followed a 6 week Atkins'- type diet. Results from this study saw an increase of urinary calcium excretion, in addition urinary pH decreased significantly from baseline and net acid excretion increased significantly; however there was no increase in calcium oxalate, this may be due to the small number in the study or the high fluid intake during the diet (Reddy *et al.* 2002). Roughead *et al.* (2003) conducted a randomized crossover trial with 15 postmenopausal women each consuming a high meat diet for 8 weeks

followed by a low meat diet for 8 weeks. Results from this study indicate that calcium retention and absorption as similar in both diet periods, in addition there was no effect in bone formation (BASP and OC), nor bone resorption (NTx). Urinary Calcium loss between week 3 and 8 were unaffected by diet, this was despite a significant decrease in urinary pH in the high meat diet compared to the low meat diet. This difference continually decreased at 3, 5 and 8 weeks, the investigators suggest that these results indicate a need to allow time for adaptation when investigating changes in protein intake (Roughead *et al.* 2003). This group of researchers also found similar results in a later study which was a 2 x 2 crossover design with high and low calcium and high protein (20% total energy) and low protein (10%) diets, this included 27 postmenopausal women for a duration of 7 weeks (Hunt *et al.* 2009). Cao *et al.* (2009) had a similar subject profile, study design, duration and diet as described in Roughead *et al.* (2003), but while they also found no change in biomarkers of bone formation or resorption investigators saw increased fractional calcium absorption and urinary calcium excretion, however the net difference between the amount of calcium absorbed and excreted in urine did not differ between the two diet periods leading to the suggestion that high protein diet may have no adverse effects on bone health (Cao *et al.* 2009). Kersteter *et al.* (2005) conducted a ten day cross-over control with 13 female subjects (20–70 years). This isotopic intervention included diets with high protein (2.1g/kg) and moderate protein (1g/kg) intakes, they also controlled for calcium, sodium, and phosphorous. No significant difference in bone formation or bone resorption was observed between the diets, urinary calcium was increased in the high protein diet at day four and a 42% relative increase of calcium absorption was reported. Investigators suggested no detrimental damage to overall bone health, suggesting this could be due to increased absorption of calcium with high protein

diets (Kerstetter *et al.* 2005). Sukumar *et al.* (2011) undertook the longest dietary intervention investigating the effects of protein on bone health that to our knowledge has been published to date. The study of twelve months duration included 47 postmenopausal women 50 – 70 years. Subjects were randomly assigned to either high protein (30% of total calories) or low protein (18%) diets, with controlled and recommended intakes of calcium and vitamin D in both groups. Two methods of measuring bone were used - peripheral quantitative computed tomography (pQCT) and DEXA, thus measurements of both cortical and trabecular bone could be recorded. Bone formation was measured by OC and type 1 pro-collagen N-terminal pro-peptide (PINP) and resorption by DPD and PYD, in addition NTx was measured by ELIZA, IGF1 and IGFBP3 were measured by immunoradiometric assay. Both diet groups in this intervention reported weight loss over the year but there was no significant difference between the two groups. The high protein group increased their protein intake to 24% of their total calories which was less than the goal of the study design; however there was a 26g/day difference in protein intake between the groups. Investigators saw a greater decrease in trabecular vBMD and BMC in the normal protein group compared to the high protein group over time, additionally there was an increase in cortical vBMD in both groups with no difference between groups. Resorption markers (DPD & PYD) were both significantly increased in the normal protein group compared to the high protein group over time. Formation markers (OC & PINP) showed no significant difference between the two groups over time. There was a significant increase in serum IGF1 over time in the high protein group compared to the normal protein group. IGFBP3 was unchanged in the high protein diet but significantly decreased in the normal protein group (Sukumar *et al.* 2011).

Overall there has been a large body of research that set out to test the previous hypotheses that protein is harmful to the bone (Denke *et al.* 2001; Kerstetter *et al.* 2005; Layman *et al.* 2005; Heaney & Layman, 2008; Thorpe & Evans, 2011). In general these studies have seen either no effect or slight beneficial effects of high protein diets on biomarkers of bone health. As a result researchers are investigating the possibility of raising the RDAs/RDIs for protein intake especially among the elderly. Kerstetter (2005) suggests that the RDA for protein of 1g/d protein per 1kg body weight may be the minimum to maintain nutrient requirements but not the optimal level of protein for best possible health. While protein is a macronutrient and provides energy, it is a nitrogen source and is primarily utilized for the growth and repair of body cells. The primary source of energy for the body is carbohydrate, however if the supply is not adequate the body will make glucose from protein (gluconeogenesis), therefore it is necessary to consume adequate carbohydrate to spare protein for its true function and prevent gluconeogenesis (Thompson & Manore, 2010).

2.4.4.1.3. Fat

Neither the US nor the EU determines specific total fat or saturated fat intakes for the individual. General Irish guidelines for total fat intake gives an upper limit of 35% of total food energy with half of this as saturated fat, however NANS reported 63% of the population exceeding this. Results of the survey were; among 18-64 years fat provided 37% of food energy, contributors were meat 24%, spreads 11%, milk and yoghurt 8%; those aged 65+ years saw the same contributors except spreads which were higher 17% (Walton, 2011).

A study by Corwin *et al.* (2006) using a large cohort representative of Americans suggested that saturated fatty acids may have direct and indirect effects on reduction of calcium absorption from the intestine, the reduction of bone formation and that BMD is negatively associated with saturated fat intake particularly in younger men. The study controlled for dietary factors, among which were calcium, protein, PUFAs and vitamin C (Corwin *et al.* 2006).

In a study of postmenopausal women it was observed that those with a higher dietary fat intake also had a higher incidence of fracture over a 7 year period, however it was not recorded how these fractures occurred nor were BMD or bone turnover markers measured (Kato *et al.* 2000).

A dietary intervention investigated the effect of added dietary PUFAs on bone health. This 12 month intervention of healthy young women used two groups, one supplemented with only calcium, the other with calcium, primrose oil and marine oil. BMD measured by DEXA remained the same in both groups but changes were observed in bone turnover markers (BSAP & NTx). However researchers could not determine whether the changes were due to the calcium or the PUFAs supplement as the study design did not include a control group for 'no calcium supplement' (Bassey *et al.* 2000).

Orchard *et al.* (2010) suggest that fatty acids (FA) may be an important dietary component that modulates osteoporotic risk, and examined FA intake in relation to osteoporotic fractures. Subjects were post-menopausal women from the Women's Health Initiative studies 1993–1998, results saw that higher saturated FA consumption was associated with higher hip fracture risk and lower total fracture risk associated with high monounsaturated and polyunsaturated FA intakes. Investigators reported being surprised that high intake of n-3 was associated with increased total

fracture risk while high n-6 intake was associated with decreased total fracture risk (Orchard *et al.* 2010).

Other research compared bone health with varying ratios of omega 6 (*n*-6) and omega 3 (*n*-3) types of fatty acids. Americans general consumption ratio of n-6/n-3 is thought to be 9.8:1 (Kris-Etherton *et al.* 2000) whereas Bassey *et al.* (2000) used an n-6 to n-3 ratio of 10:1. Subjects in another study displayed ratios of 8.4:1 in men and 7.9:1 in women (Weiss *et al.* 2005). The results of this study suggests that an increased ratio of n-6 to n-3 fatty acids is significantly associated with lower BMD at the hip in men and women (Weiss *et al.* 2005). The researchers controlled for hormone therapy (HT) status, age, lifestyle and medication use; however it was recorded that men had a higher average body weight and n-6 PUFA intake, in addition no bone turnover markers were measured (Weiss *et al.* 2005). A dietary intervention which added walnuts and flaxseed as a means to decrease the n-6/n-3 ratio observed reduced serum NTx, with serum BSAP levels maintained, and suggests that adding sources of n-3 PUFA may provide health benefits to the skeletal and cardiovascular systems (Griel *et al.* 2007). Researchers have reviewed trials investigating factors such as osteoblast formation, lipid oxidation, bone IGF-1 as possible mechanisms at work with dietary fat in bone health, and suggest that the complexity of nutrients being co-dependent and simultaneously interacting with genetic and environmental factors as a reason why these findings are controversial or inconsistent. In addition various study designs and controversies over the human study outcomes make it difficult to draw any definite conclusion and to do so would need further investigation (Maggio *et al.* 2009; Salari *et al.* 2008; Das, 2000; Ilich *et al.* 2000).

2.4.4.2. Minerals and Bone Health

2.4.4.2.1. Calcium

Calcium is a major mineral, the dietary reference intake as indicated by the Institute of Medicine are; 9-18 years 1,300mg/day; 19-50 years 1,000mg/day and over 50years 1,200mg/day (US Dept Health & Humanities, 2004). In Ireland the RDAs are 1,200mg/d; 800mg/d and 800mg/d respectively for the groups above, this view was based on newer research findings and consideration of prevailing Irish conditions reported (FSAI, 1999). A group of researchers reviewed studies of varying dietary calcium requirements and concluded that calcium requirements cannot be exactly defined; they also suggested that the body works to keep serum calcium levels within a narrow range as low intakes may cause secondary hyperthyroidism and high intakes have shown side effects such as kidney stones (Lips *et al.* 2010). Calcium comprises 1200g or 1-2%, of the average adult body weight; it is also the primary component of bone structure as 99% of calcium in the body is found in the teeth and bones (Cashman, 2002). It functions in conjunction with other major minerals, for example, calcium and phosphorus crystallize to form hydroxyapatite, the crystals pack together and build up the collagen foundation of bone, giving the necessary characteristics of hardness and flexibility of bone (Thomson & Manore, 2010). The remaining 1% of calcium in our bodies is found in the blood and soft tissues; and since calcium is alkaline it has a critical role in the body maintaining acid-base balance (Centre for Nutrition & Food Safety, 2002). If insufficient calcium is consumed or absorbed; osteoclasts erode bone so that calcium can be released into the blood thus maintaining normal blood calcium levels (Thomson & Manore, 2010). In a number of Western countries significant proportions of some population groups fail to achieve the recommended calcium intake (Cashman, 2002; Lips *et al.* 2010). Food consumption

reported in NANS (2010) indicates that the calcium intake is inadequate in 13% Irish women over 65 years; while it is suggested that those with adequate intakes may consume 995 mg/day (Walton, 2011).

In addition to the skeleton other systems and hormones work together to regulate a narrow range of calcium levels;

- i) In incidences of high serum calcium levels, the thyroid gland is activated to increase secretion of calcitonin (Felsenfeld *et al.* 1993) which inhibits the action of vitamin D and reduces resorption of bone and absorption of calcium from the intestines leading to reduced blood calcium levels (Thomson & Manore, 2010; Cashman, 2002).
- ii) Low calcium levels stimulate the production of parathyroid hormone (Miki *et al.* 1998) and activation of vitamin D (Thomson & Manore, 2010). This increases the re-absorption of calcium by the kidneys, increases the resorption of bone and increases the absorption of calcium from the intestines thus increasing blood calcium levels (Cashman, 2002).

It is indicated that intestinal absorption of calcium will rise when calcium urine loss increases, and will subsequently fall when urinary loss is reduced; however, it is not known if the absorptive compensation is adequate to offset high loss and it may depend on a high dietary calcium intake (Heaney, 2006). Low calcium levels stimulate the production of parathyroid hormone (Miki *et al.* 1998) and activation of vitamin D (Thomson & Manore, 2010). This increases the re-absorption of calcium by the kidneys, increases the resorption of bone and increases the absorption of calcium from the intestines thus increasing blood calcium levels (Cashman, 2002).

2.4.4.2.2. Magnesium

The DRIs for magnesium are: 10-30 years 400mg/day, 31 + years 420mg/day (US Dept Health & Humanities, 2004). Half of total body magnesium is found in the bone, the other 50% being found in tissues and organs with only 1% normally present in the blood. In addition to being intrinsic to the structure of bone, magnesium regulates calcium balance. Magnesium deficiency results in low calcium, altering calcium metabolism as well as calcium regulatory hormones (Elisaf *et al.* 1997). Magnesium acts as a cofactor for over 300 biochemical reactions and supports vitamin D metabolism and function (Fontenot, 1989). Low magnesium levels may cause increases in its absorption from the small intestine and kidneys or resorption from the bone store of magnesium, this helps maintain the necessary narrow serum range by exchanging part of its content with extra cellular fluid (Laires, 2004). Food consumption reported in NANS (2010) indicates that magnesium intakes in Irish women over 65 years may be inadequate at 262 mg/day (Walton, 2011).

2.4.4.2.3. Phosphorus

The Food Safety Authority of Ireland's (1999) RDAs for phosphorus is 18-65+ years 550mg/day. Phosphorus has a critical role in bone formation. It acts as part of the mineral complex of bone and along with calcium it forms hydroxyapatite crystals. 85% of the body's phosphorus is stored in the bones and the rest is stored in soft tissues such as muscles and organs (Shapero & Heaney, 2003). Phosphorus makes up 1% of total body weight and is obtained in high amounts from protein based foods. It is also an additive in many processed foods including soft drinks, therefore the intake frequently exceeds the recommended (Heaney, 2004). Decreases in serum phosphate

may lead to resorption of phosphorus from the bone through the activity of PTH and vitamin D (Farrow & White, 2010). How critical the ratio is between calcium and phosphorus remains controversial, it was thought that with elevated serum calcium levels, highly abundant phosphorus could bind to it, suggesting calcium would not be available for absorption thus triggering resorption of calcium from the bone. However results from other studies do not see increased bone turnover (Heaney, 2004; Heaney & Rafferty, 2001; Shapero & Heaney, 2003). Food consumption reported in NANS (2010) indicates the phosphorus intake in Irish women over 65 years is more than adequate at 1173mg/day (Walton, 2011).

2.4.4.2.3. Potassium

The RDA for potassium is: 3.1g/day for those aged 11 and over (FSAI, 1999). Potassium is abundant in fresh foods, especially in fruit and vegetables. However, a large proportion of individuals do not intake adequate amounts, possibly due to a high consumption of processed foods which are low in potassium (Thompson & Manore, 2010) and a low intake of fruit and vegetables (New *et al.* 2000). Among its functions potassium regulates acid-base balance, potassium citrates from fruit and vegetable intake neutralize the endogenous acid production associated with excessive protein intake that can be associated with the Western diet (New *et al.* 2000). The work of Frassetto *et al.* (1998) found that the ratio of protein to potassium in the diet may be a reliable predictor of diet net endogenous acid production (NEAP). It is now established that potassium can act as an indicator of NEAP and fruit and vegetable intake (Mardon *et al.* 2008), and research suggests that potassium may exert a modest influence on markers of bone health and over a life time may contribute to a decrease in osteoporosis (Zhu *et al.* 2009).

2.4.4.2.5 Sodium

1g sodium is equivalent to 2.5g of salt (FSAI, 2005). The US daily recommended intake (DRI) for sodium is 1500mg (65mmol)/day (US Dept. Health & Humanities, 2004) whereas in Europe the RDA is 1600mg (70mmol)/d (4g salt) for adults (FSAI, 1999). The FSAI acknowledges the EU RDA as sufficient to meet the requirements of 97% of the population however data from Ireland at the time showed the average daily salt intake was approximately 8.3g/day in adults therefore the FSAI considered 2.4g (100mmol)/day sodium (6g salt) to be an achievable target by the Irish population (FSAI, 2005). Data from NANS indicates that present salt intakes are lower than previous surveys however; the new average intake of 7.4g/day salt still exceeds the target of 6g/day (Walton, 2011). Irish data estimates that 15-20% of total dietary sodium intake is from discretionary sources (salt added in cooking and at the table), 15% from naturally occurring sodium in unprocessed foods and approximately 65-70% from manufactured foods, mainly processed meats and breads (FSAI, 2005).

The average adult human body is thought to contain from 90-130g of sodium, half of which is in the bone and the remainder in extracellular fluid where it maintains body fluid homeostasis. Heaney (2006) also cites Walser (1961) who demonstrated that sodium and calcium compete for the same reabsorption mechanism in the proximal renal tubule (Heaney, 2006). However, other research suggests that the effect of sodium intake on urinary calcium excretion mainly reflects changes in the filtered calcium load rather than changes in renal sodium handling, thus suggesting that the proximal tubule has a capacity to dissociate calcium reabsorption from that of sodium (McCarron *et al.* 1981).

A dietary intervention in young healthy females tested the hypothesis that sodium chloride may increase urinary calcium (Ginty *et al.* 1998). Data suggested an increase

of sodium chloride from 80 to 180 mmol/d increased urinary calcium excretion in some individuals, however there was no corresponding change in bone turnover markers (Ginty *et al.* 1998). This lack of bone turnover it was suggested may have been due to an adequate adaptation process of increased calcium absorption even in the presence of restricted calcium intake (Evans *et al.* 1997; Ginty *et al.* 1998). Similarly, in research with postmenopausal women, increased sodium intakes from 60 to 170 mmol/d saw no change in turnover markers, however it was suggested that the urinary hydroxyproline biomarker used as the marker of bone resorption was not specific or sensitive enough (Lietz *et al.* 1997). In contrast, a study of postmenopausal women with higher increases of sodium intake (50-300 mmol/day) did see an increase in bone turnover markers; perhaps explained by mal-adaptation processes to high Na intake associated with post-menopause (Evans *et al.* 1997).

Considering intake trends of lower than recommended calcium intake and consistently high sodium intakes this data suggests that the interaction between calcium and sodium is important (Heaney, 2006), especially in terms of bone health. Harrington *et al.* (2004) investigated the effect of high sodium, high protein (calciuric) diets on urinary calcium and biomarkers of calcium and bone metabolism in 10 healthy post-menopausal women using HRT and 10 not using HRT. In this randomised cross-over trial the calciuric (180mmol/d Na, 90g/d protein) and the basal (70mmol/d Na, 70g/d protein) diets were consumed for 4 weeks, urinary NTx, calcium (Ca), sodium (Na), potassium (K), nitrogen (N), BSAP and OC were measured. Results found significant increases for Na, Ca and N during the calciuric diet for both groups of subjects. During the calciuric diet women without HRT had significantly higher levels of urinary NTx, however those on HRT did not. The calciuric diet had no effect on OC or BSAP in either group (Harrington *et al.* 2004).

The researchers in the above study suggest that a moderate protein and sodium diet is to be recommended for postmenopausal women, especially if they do not have HRT.

2.4.4.3. Vitamins and Bone Health

2.4.4.3.1. Vitamin D

Vitamin D is a fat-soluble vitamin and a hormone. Recommended levels of intake (with inadequate exposure to sun) are; 200 IU (5µg)/day for all ages under 50years; 400 IU (10µg)/day 50-70 years; 600 IU/day (15µg) 70+ years (FSAI, 1999; US Dept. Health & Humanities, 2004).

Two forms of vitamin D are active in the human body. The first, is mainly synthesised by the action of the sun on 7-dehydrocholesterol in the skin and leads to its transformation to pre-vitamin D₃, which is then converted to vitamin D₃ (cholecalciferol) (Hollick, 2004). Small amounts of D₃ are also found in animal foods products, the other form vitamin D₂ (ergocalciferol) is only found in plant foods; In general food sources supply very little vitamin D therefore many foods are fortified with it (Thompson & Manore, 2010).

Substantial proportions of the Irish population may have low vitamin D intakes, it is reported that in the age group 18 – 64 years 72% of men and 78% have intakes of less than 5ug/d, with an average of 90% having less than 10ug/d; among those over 65 years an average of 50% have intakes less than 5ug/d, additionally 87% men and 77% women reported intakes less than 10ug/d (Walton, 2011).

1,25 dihydroxy vitamin D then interacts with its vitamin D receptor (VDR) in osteoblast cells to maintain calcium homeostasis by increasing the efficiency of intestinal calcium absorption and mobilizing calcium stores from the skeleton (Hollick, 2004). Decreased levels of 1,25 dihydroxy vit D may increase PTH

secretion (Brown *et al.* 1989) causing excretion of phosphorus into urine thus depleting serum phosphorus levels. Therefore there may not be adequate calcium/phosphorus product needed for mineralization phase in the production of bone matrix (Hollick, 2007). *In utero* and childhood vitamin D deficiency can cause growth retardation, skeletal deformities, rickets and increased risk of fracture in later life. In adults it can precipitate or exacerbate osteopenia and osteoporosis, cause osteomalacia (Hollick, 2007). Vitamin D deficiency is an unrecognized epidemic among both children and adults in the US and Ireland (Hollick, 2004; FSAI, 2007).

2.4.4.3.2 Vitamin C

RDA for vitamin C is 60mg/day for those aged 11 and over (FSAI, 1999). Vitamin C may contribute to increasing bone density by affecting bone formation at a cellular level. It is thought that it could do this by stimulating procollagen and enhancing synthesis of collagen in the bone (Roughhead & Kunkel, 1991). A biochemical link has been established between increased oxidative stress and reduced bone density (Basu *et al.* 2001) with oxidative stress increasing bone resorption (Baek *et al.* 2010). Vitamin C could act as an antioxidant by donating electrons to free radicals thus preventing cell and tissue damage signifying biological plausibility for the positive effect of vitamin C on bone health (Thompson & Manore, 2010). Another hypothesis suggests that vitamin C can induce changes in calcium absorption (Freudenheim *et al.* 1986). This was tested in a large study of postmenopausal women where researchers took into account the dietary, supplement and total vitamin C intakes, controlled for a host of possible confounding factors and measured BMD at various sites. They did not measure vitamin C serum levels. The results showed no evidence of a relationship between vitamin C and BMD by itself but there was an increase in femoral neck and

whole BMD among a sub-group of women on hormone therapy (HT) who had high intakes of vitamin C (Wolf *et al.* 2005). Similar beneficial findings of increased vitamin C supplementation were observed in sub-groups of postmenopausal women who were on HT or calcium supplements (Morton *et al.* 2001). Similarly another large study only found associations in sub-groups of older men with low calcium or low vitamin E intakes whereas no possible protective role of vitamin C for bone health was found among women (Sahni *et al.* 2008). It is proposed that research findings could provide a rationale for further studies investigating the role of vitamin C in osteoporosis (Basu *et al.* 2001).

2.6. The Low Carbohydrate Diet (LC)

Scientific evidence with regard to the ideal method of weight loss remains controversial; researchers who investigated the 'efficacy and safety' of low carbohydrate diets suggest that results of 107 studies reviewed present insufficient evidence to make recommendations for or against the low carbohydrate diet (Bravata *et al.* 2003). Alternatively evidence from a large body of randomized control trials dating from the 60s to late 90s suggests that a moderate fat, low-calorie diet prevents weight gain and results in weight loss and weight maintenance (Freedland *et al.* 2004).

Significant improvements in obesity-related conditions have been seen with 5-10% weight loss even when an individual remains overweight (National Heart, Lung & Blood Institute, 1998). In spite of scientific evidence, overweight individuals may be susceptible to new diets that promise quick dramatic results, obese women have reported unrealistic expectations of a 32% weight loss and furthermore their goals for weight loss were based on appearance and physical comfort rather than change in

medical condition or weight suggested by a doctor or health care professional (Foster *et al.* 1997). Many obese individuals have been reported to having lost weight, but maintaining this weight loss is nearly impossible (Freedland *et al.* 2004).

Among the most popular fad diets are those which recommend low carbohydrate intakes; Dr Atkins' book in 1972 and revised in 1992 popularized a low-carbohydrate, high-protein, ketogenic diet which individuals could use on their own rather than in a medical setting (Freedman *et al.* 2001). Freedland *et al.* (2004) used the search words "weight loss" on Amazon.com and found 1214 matches, additionally many of the top 20 best sellers at Amazon.com promote some form of carbohydrate restriction. Some of these are the south beach diet, protein power, the carbohydrate addict's diet, Dr Bernstein's diabetes solution and life without bread to name but a few. While this popularity of books and websites attest to a high level of interest and demand it is difficult to estimate the number of people who have followed low carbohydrate diets (Bravata *et al.* 2003).

In general with weight loss studies, drop out rates (attrition) are high, however this has been seen to be the case regardless of the diet used in studies (Foster *et al.* 2003). Improved results for adherence and attrition may require more frequent follow up, patient and professional involvement (Shai *et al.* 2008; Yancy & Boen, 2006), however, there is a concern that this may not replicate the approach of most dieters in a free living situation without monitoring and motivation (Foster *et al.* 2003).

2.6.1. Nutritional Content of the LC Diet

The description of what a low carbohydrate diet is varies between the different diet researchers (Bilsborough & Crowe 2003; Bravata *et al.* 2003). Since many authors of these diets indicate a high percentage of calories from protein (25-30%) and fat (55-

60%) they have been referred to as high-protein or high-fat diets, however it is suggested that the absolute intake of protein or fat, while still higher than RDAs, is often not as high as the percentages imply (LC 105g/day protein v's RDA 82.5g/day protein; LC 94g/day fat v's RDA 85g/day fat) (Freedland *et al.* 2004). It is possible a higher than recommended intake of protein in a LC diet could increase both calcium absorption and excretion as shown in previous studies (Kersteter *et al.* 2005; Cao *et al.* 2009) The total effect of this change on bone health is controversial. The effect of a higher fat intake than recommended on bone health would depend on the type of fat consumed, as has been shown already in this review (Orchard *et al.* 2010; Greil *et al.* 2007).

It is thought that most LC diets are hypoenergetic, total energy intake is reduced as a result of carbohydrate restriction, and energy balance is not maintained by an increase of protein and fat (Bilsborough & Crowe, 2003). In a short randomised crossover trial which aimed to maintain a stable body weight, the carbohydrate:protein:fat ratio of the control diet was 55:15:30 whereas the test diet was 20:30:50. However data revealed body weight decreased on average by 4kg in both diets (Gannon & Nutall, 2004). An examination of a large number of adults found that those who consumed low carbohydrate diets had 'a low diet quality' as measured by the healthy eating index (HEI) plus they had lower than recommended energy intakes (Kennedy *et al.* 2001). Lower energy intake which has resulted in weight loss has already been presented and is associated with reduced bone health (Jensen *et al.* 1994; Prichard *et al.* 1996; Ricci *et al.* 2001). Low carbohydrate diets may be inadequate for vitamin E, vitamin A, thiamine, vitamin B6, folate, calcium, magnesium, iron, potassium and dietary fibre levels in addition to low total calorie intake (Freedman, 2001). It can be seen that many of the vitamins and minerals which may be lower in the low

carbohydrate diet have been presented as being associated with bone health. Many of the micronutrient deficiencies may be due to a lack of fruits, vegetables and grains. A number of authors of low carbohydrate diets suggest supplemental multivitamins, however this could lead to a deficiency in important biologically active phytochemicals present in the missing foods (Denke, 2001). It can also be suggested the bioavailability of nutrients from supplements may be reduced compared to consuming them through food in the diet (Clarke *et al.* 2011).

Table 2.2 shows the macronutrient and calorie intake of some popular lower carbohydrate diets which shows energy and carbohydrate intake is reduced and protein and fat intake is increased. Reduced energy intake may increase bone loss and resorption, Shapses & Riedt (2006) suggest that data supports the occurrence of bone loss during energy restriction in postmenopausal women and possibly older men, but indicate that mechanisms regulating bone due to weight reduction are not well understood and risk may depend on initial body weight, age, gender and physical activity (Shapses & Riedt, 2006).

Table 2.2: Macronutrient and Caloric Assessment of Weight Reducing Diets

<i>Diet</i>	Total calories/d	Carbohydrate g/d (% Kcal)	Protein g/d (% Kcal)	Fat g/d (% Kcal)
Average American diet	2,200	275 (50)	82.5 (15)	85 (35)
Low-carbohydrate diet				
Atkins Diet -Induction phase	1,152	13 (5)	102 (35)	75 (59)
Atkins Ongoing phase	1,627	35 (9)	134 (33)	105 (58)
Atkins Maintenance phase	1,990	95 (19)	125 (25)	114 (52)
Moderate-carbohydrate diet				
<i>Carbohydrate Addict's Diet</i>	1,476	87 (24)	84 (23)	89 (54)
Low-glycemic-index diet				
<i>Sugar Busters!</i>	1,521	176 (46)	89 (23)	44 (26)

Adapted from Freedman *et al.* (2001)

It can also be seen that low carbohydrate diets can vary between high fat ketogenic and low fat non-ketogenic high protein diets. In a 6 week trial, 20 obese adults were randomly assigned to a ketogenic LC diet (60% energy as fat; 5% as CHO) or a conventional LC diet (30% fat; 40% CHO) with protein content accounting for approximately 30% of total energy in both diets. Data from this research showed that serum ketones were directly related to LDL-cholesterol concentrations and elevated inflammatory risk; additionally both diets showed similar reduction in total body mass and insulin resistance hence it was concluded that severe restrictions in dietary carbohydrate are not warranted (Johnston *et al.* 2006).

2.6.2. Effectiveness of LC diet in Weight Loss

Several studies have demonstrated that low-carbohydrate diets are effective as a weight loss technique (Gardner *et al.* 2007; Foster *et al.* 2003) but there was a variation in the length of time and the amount of weight lost. For example, a number of 4 week trials reported total weight loss of 2.94 – 3.8kg over that period (Vander Wal *et al.* 2007; Rankin, 2007) and a 6 month study recorded a 10% weight loss from baseline (Westman *et al.* 2002). After 12 months the weight loss varied from 2 - 4.7kg (Dasinger *et al.* 2005; Gardner *et al.* 2007). Another long term 24 month study recorded a loss of 4.7 kg, with carbohydrate consumption at 20g/day for 2 months, gradually increasing to 160g/day (Vander Wal *et al.* 2007). These studies concur with the findings that significant weight loss at 3 and 6 months is not sustained at 12 months in a LC diet, this may be due to lost weight regained owing to difficulty adhering to the diet (Foster *et al.* 2003). While many studies have demonstrated that the LC diet helps weight loss, numerous others have shown no additional benefit on

weight loss when LC diets are compared to traditional low-fat diets (Swenson *et al.* 2007; Delbridge *et al.* 2009; Sacks *et al.* 2009).

2.6.3. Metabolic Effects of LC Diet

The metabolic changes involved with excess weight and obesity are so complex that to look at one in isolation would present a very unbalanced view (Bilsborough & Crowe, 2003). It is thought that a reduction in calorific intake by 500 kcal/day could result in a 0.45–0.9 kg weight loss each week but a low carbohydrate high protein diet may yield a 2–3 kg loss in the first week; however this is due to metabolic processes. Critically though, this is not maintained when normal food consumption patterns are resumed (Denke, 2001).

2.6.3.1 Glycogenolysis and Gluconeogenesis

When dietary carbohydrates are limited, glucose for energy is derived from glycogen stored in the muscles and liver (glycogenolysis). If dietary intake of carbohydrate is not increased, glycogen stores will be depleted within 24-48 hours (Bilsborough & Crowe, 2003). Each gram of glycogen is bound to 3g of water and this is released upon glycogen metabolism giving rise to a diuretic effect which is usually limited to the first week of a low carbohydrate diet (Denke, 2001). If the diet does not provide sufficient carbohydrate and glycogen stores are depleted, the body can make its own glucose from protein; a process is called gluconeogenesis (Thompson & Manore, 2010). A dietary trial that investigated three altered carbohydrate intakes on different occasions in the same subjects found that a low-carbohydrate diet mainly affected postabsorptive glucose production by modulation of glycogenolysis and only minimally by gluconeogenesis (Bisschop *et al.* 2000). In another randomized cross-over study of a low carbohydrate diet, an increase in energy expenditure was reported

suggesting 42% of the reported increase in energy expenditure was explained by an increase in gluconeogenesis (Velhorst *et al.* 2009).

2.6.3.2 Ketosis

With limited dietary carbohydrates and depleted glycogen stores, energy demands cannot be met by gluconeogenesis, the body increases fat oxidation and glycerol liberation from triglyceride breakdown to produce a form that can be utilized; this process is called ketosis (Thompson & Manore, 2010). Fatty acids are oxidized by the liver for energy production, or partially oxidized forming acetoacetate which is converted to beta hydroxybutric acid. Collectively termed ketone bodies, these molecules provide alternative energy to the brain during periods of fasting, low carbohydrate intake or vigorous exercise (Pan *et al.* 2000). Ketones can potentially be used by all tissues however, there is no consensus on a carbohydrate intake which induces ketosis, thus it may vary on an individual basis (Bilsborough & Crowe, 2003). One study recorded no ketosis in a diet with a ratio of 20% CHO: 30% Protein: 50% Fat (Gannon & Nutall, 2004). Ketone bodies are filtered by the kidneys and cause renal loss of sodium and thereby increase water loss (Denke, 2001). High levels of ketones may cause the blood to become acidic leading to a condition called ketoacidosis which can occur in individuals with untreated diabetes (Thompson & Manore, 2010). Adverse events to the LC diet have included dehydration, constipation, hypoglycaemia, vitamin deficiencies, hyperlipidemia, increased LDL cholesterol, bad breath, shakiness (Freeman *et al.* 1998; Foster *et al.* 2003). Ketones are thought to have an effect of satisfying hunger which may help compliance in low carbohydrate diets (Bilsborough & Crowe, 2003).

2.6.3.3. Insulin Response

Thompson & Manore (2010) describe insulin as a key that opens the cell membrane for glucose to enter, as without it, the glucose molecule is too large to cross the cell membrane of the tissues. The rate of glucose entering the blood circulation is balanced with the rate of glucose removal (circulating glucose concentrations) and is kept within a relatively narrow range. Insulin is a glucoregulatory hormone whereby in response to glucose entering the circulation it signals muscle to increase their uptake of glucose (Arnoff *et al.* 2004). Insulin can act on the liver to promote glycogenesis and it can inhibit secretion of the hormone glucagon which signals the liver to stop producing glucose through glycogenolysis and gluconeogenesis; insulin is only secreted when glucose concentrations increase beyond 3.3 mmol/L (Arnoff *et al.* 2004).

In a 5 week randomized crossover study of men with mild untreated type II diabetes, the following diets were compared; 20% CHO: 30% Protein: 50% fat to that of 55:15:30. Data showed a decrease to near normalized glucose concentrations in the low-carbohydrate diet; in addition glucagon response increased and insulin response decreased after the low carbohydrate meals (Gannon & Nuttal, 2004). Increased plasma concentrations of some amino acids, such as arginine, leucine and lysine can also stimulate insulin secretion (Aronoff *et al.* 2004). The role of diet composition on insulin concentrations is controversial. Some studies comparing low and high-carbohydrate diets found no difference in insulin concentrations (Foster *et al.* 2003; Veldohorst *et al.* 2009) whereas another one did find significantly different levels they found no difference in weight loss between the groups (Golay *et al.* 1996).

2.6.4. Low Carbohydrate Diet & Acid Load

The body does not have the ability to store excess protein. If calories from a low carbohydrate diet are replaced with calories from high protein foods, amine groups are removed from the amino acids (deamination), the nitrogen is then removed and excreted in the urine; allowing the remaining components to be used as energy (Thompson & Manore, 2010). There is a concern that when high quantities of amino acids are consumed it may result in the production of acid (Denke, 2001) altering the body's acid balance (Swenson *et al.* 2007; Delbridge *et al.* 2009; Sacks *et al.* 2009). Additional response to high dietary protein intake may be urinary calcium losses with corresponding increases in bone resorption (as described previously). The link between weight loss and osteoporosis could be exacerbated by the effect of extra protein consumed in the LC diet (Kreipe & Forbes, 1990). This could be due to the acidifying effects of sulphur containing amino acids in the protein foods, it is thought that for every gram of protein consumed 1mg of urinary calcium is excreted (Heaney & Layman, 2008). However, not all foods containing sulphur amino acids have the same quantity of sulphur; therefore it is possible to limit foods that have high sulphur content, for example soy has a low ratio of sulphur to protein at 39.8mEqSol/100g protein compared with pork which is 73mEqSol/100g protein (Thorpe *et al.* 2008). It is suggested that some factors related to increased protein in the diet may help to offset the negative effects of sulphur amino acids on bone, one of these may be the alkalizing effects of fruit and vegetables in the diet (Thorpe *et al.* 2008; Heaney & Layman, 2008). Other positive aspects of protein in relation to bone health are; that it is a substrate for collagen deposition, and also because there may be an increase in circulating insulin-like Growth Factor with increased protein which has a positive effect on BMD (Thorpe *et al.* 2008).

2.6.2. Low Carbohydrate Diet & Kidney Function

If over a long period of time energy consumed is too low for energy demands, the body begins to use protein for energy (Thompson & Manore, 2010). In this instance protein is utilized from the blood, liver and skeletal muscle, deamination occurs and the resulting nitrogen transported to the kidneys where it is excreted in the urine as urea. In this instance it is important for individuals who consume a high protein diet to drink more water as adequate fluid is needed to flush excess urea from the kidneys (Thompson & Manore, 2010). While it is considered important for athletes to counterbalance high sweat losses, an American trial used creatinine as a clinical measure of renal function after brief heavy exercise in trained athletes. The subjects consumed intakes of 2.8g protein/kg body weight with only slight changes in plasma levels of creatinine. Critically, results ‘remained in the upper limit of normal’ and did not indicate renal stress (Poortmans & Dellalieux, 2000). In a small metabolic study of a low-carbohydrate high-protein weight reducing diet where fluid intake was fixed, an enhanced acid load was detected. While the intervention was for six weeks only and a number of subjects had normal body weight, clinical implications of increased kidney stone risk was suggested by the researchers (Reddy *et al.* 2002). A large prospective study of women age 42-68 years found that a high protein intake was not associated with a decline in renal function in women with normal kidney function. However, in those with mild renal insufficiency, while small differences in protein intake may not have clinically meaningful implications, sustained high intakes particularly of non-dairy animal protein were found to have accelerated renal function decline (Knight *et al.* 2003). A study of obese women on an energy restricted high-protein, low-fat diet investigated the diets effect on renal function when compared to a conventional high-carbohydrate diet. Low-fat diet investigators found that serum

creatinine levels did not change from baseline to the end of the diet at 12 weeks (Noakes *et al.* 2005). It has been suggested that ‘adaptive alterations in renal size and function without indications of adverse effects’ may explain these lack of changes in renal function during weight loss induced by high versus low-protein low-fat diets in overweight subjects (Skov *et al.* 1999). Recent evidence has suggested that protein has beneficial effects such as increasing intestinal calcium absorption and circulating IGF-1, and may lower serum parathyroid hormone sufficiently thus offsetting the negative effects of the acid load on bone health (Cao, Nielsen & Forrest, 2010).

2.6.3. Low Carbohydrate Diet & Bone Health

2.6.3.1 Biomarkers of bone resorption and absorption

There are a limited number of research studies investigating the low-carbohydrate diet and biomarkers of bone health. Carter *et al.* (2006) conducted a 3 month dietary intervention, which excluded pre- and post- menopausal women. It consisted of 15 subjects who followed a low carbohydrate diet with 15 matched controls. Investigators measured two different biomarkers of bone health, Urinary N-Telopeptide (UNTx) was measured at baseline, 1 month and 3 months, and Bone Specific Alkaline Phosphatase (BSAP) was measured at baseline and 1 month. Therefore bone turnover ratio (BSAP/UNTx) could only be measured for the four weeks of the study. Results showed no increase in bone turnover markers compared with controls at any time point, and no significant change in bone turnover ratio compared with controls (Carter *et al.* 2006). Coleman & Nickols-Richardson (2005) also conducted a 3 month dietary intervention, here the subjects were pre-menopausal women, and subjects either followed a low-carbohydrate or high-carbohydrate diet. They found that bone biomarkers (serum OC and urinary NTx) increased in both diet

groups compared to baseline, with no differences recorded between groups, indicating that weight loss may stimulate bone turnover (Coleman & Nickols-Richardson, 2005). Research may now be shifting towards the effects of the high protein diet on bone health, with results suggesting limited negative effects and in some cases a slight benefit on the biomarkers of bone health (Kerstetter, 2009). Darling *et al.* (2009) conducted a systematic review and meta-analysis on literature from the previous three decades that evaluated dietary protein and bone health in healthy humans. Researchers concluded that while the weight of evidence shows the effect of dietary protein on the skeleton may be favourable or at least not harmful, a reduction in fracture risk was not seen. They suggest that until more research is undertaken current recommendations with regard to protein intake may be appropriate dietary advice (Darling *et al.* 2009). While the plethora of high protein diet trials focus on increasing the protein intake and reducing the carbohydrate intake compared to those recommended in healthy eating guidelines, the average carbohydrate intake recorded was not as low as the directions in the popular LC diets such as Atkin-like diets (Denke *et al.* 2001; Kerstetter *et al.* 2005; Layman *et al.* 2005; Heaney & Layman 2008; Thorpe & Evans, 2011).

2.6.3.2 IGF-1 & IGFBP-3

A number of investigators have demonstrated that increased protein may increase IGF-1 levels (Yakar *et al.* 2002; Cao, Nielsen & Forrest, 2010). Dawson-Hughes *et al.* (2004) recruited 33 men and women over 50 years to investigate a high protein low-carbohydrate diet for 63 days. OC and IGF-1 were measured by RIA, NTx by ELISA and serum and urine creatinine were measured colorimetrically. Results indicated a decrease in NTx in the high protein, low-carbohydrate group. In addition,

OC and calcium excretion had no significant difference between the two groups; however an increased meat protein intake from 0.78 – 1.6g/kg/d resulted in a 25% increase in IGF-1 levels. No differences in IGF-1 levels were found between men and women. It was concluded that a higher meat intake may potentially improve bone mass especially older men and women (Dawson-Hughes *et al.* 2001). In contrast to these results a study by Roughead *et al.* (2003) of 18 menopausal women 50-75 years for 8 weeks who also investigated an increased protein diet found no changes in BASP, OC, NTx nor IGF-1, however it has been suggested this could have been due to the small number of subjects or to different starting protein intake levels at baseline (Kerstetter, 2009). The twelve month study by Sukumar *et al.* (2011) with 47 women 50-70 years on a 30% protein diet reported there was a significant increase in serum IGF1 over time in the high protein group compared to the normal protein group. Correspondingly it is suggested that a decrease in protein and in calories may decrease IGF-1 levels (Sukumar *et al.* 2011). This concurs with a study on rats where a two week low protein diet saw decreased levels of IGF-1 (Dubois-Ferriere *et al.* 2011).

It has been suggested that trials of high protein diets investigating effects of amino acid metabolism to bone may have reported limited detrimental effects to bone due to increased IGF-1 (Heaney & Layman 2008; Kerstetter, 2009; Darling *et al.* 2009; Sukumar *et al.* 2011) as IGF-1 has been associated with increased bone health (Clarke, 2008). It is thought that IGF-1 regulates bone size, shape and composition; and regulates the individual's ability to adapt bone structure to mechanical loads during growth and development (Yakar *et al.* 2009a). A study in mice by Yakar *et al.* (2009a) saw that changes in IGF-1 levels had an effect on the bone microarchitecture as well as the cortical and trabecular bone. This study observed a decrease in bone

strength in mice with low levels of IGF-1 in the serum (Yakar *et al.* 2009a). The study in rats by Dubois-Ferriere *et al.* (2011) related the decrease in IGF-1 with a corresponding decrease in relative bone volume and trabecular thickness (Dubois-Ferriere *et al.* 2011).

Researchers suggest that in addition to circulating serum IGF-1 the distribution of IGF-1 in the tissues plays a role in the effects of IGF-1 on the skeleton (Yakar *et al.* 2009b). By binding to IGF-1 in serum, IGFBP-3 may regulate IGF-1 bioavailability, and thus either reduce or enhance the effectiveness of IGF-1 (Yakar *et al.* 2009b).

Higher levels IGF-1 levels are observed in individuals recording a BMI range of 25-27, and reduced levels in those with a lower or higher BMI, as most circulating IGF-1 is produced in the liver the former may be due a low supply of nutrients to the liver and the latter due to compromised liver function (The Endogenous Hormones and Breast Cancer Collaborative Group, 2010).

Changes IGFBP-3 gene expression can effect carbohydrate metabolism, it may act as a mediator of apoptosis in insulin secreting cells (Chen & Ferry, 2006). The previously mentioned yearlong study by Sukumar *et al.* (2011) found IGFBP-3 was unchanged in the high protein diet but significantly decreased in the normal protein group. While still controversial increases in IGF-1 levels have been associated with cancer risks (Kaaks *et al.* 2002; Schernhammer *et al.* 2005).

Concerns have been raised about commercially available assays used to measure IGF-1 levels, researchers found a wide variance among these, despite being calibrated against IGF-1 WHO international agent 87/518, in addition considerable variations between individuals were observed making it difficult to establish standard normal values for IGF-1 (Ranke *et al.* 2005; Frystyk *et al.* 2010).

Summary:

Optimum bone health aims for a balance in bone resorption and bone formation; however bone turnover is sensitive to hormonal and nutritional factors. When the rate of resorption exceeds that of formation the risk of developing osteoporosis or bone fracture is increased. The DEXA scan is considered the gold standard for measuring bone health, however due to its limitations detecting changes to bone in the short-term, other biomarkers of bone health are used in combination with it. While there is a myriad of these biomarkers a number are utilized more regularly by researchers based on specific merits.

A decline in oestrogen level is thought to contribute to bone loss and may explain the higher incidence of osteoporosis among females than males; however age related increases of parathyroid hormone and decreases in IGF-1 may also contribute to bone loss in either gender. In addition to this weight loss and weight bearing exercise also affect bone strength, the latter being a positive affect.

The most apparent nutritional factor influencing the strength of bone has been calcium and Vitamin D, as it aids the absorption of calcium. However researchers are investigating the importance of protein in bone health as it is involved in the development of bone. Controversially however the possible production of amino acids associated with the consumption of high protein diets may increase bone resorption. In addition, lipids may have an affect on bone health as an excess may lead to the absorption of calcium being reduced.

Data from studies of high protein and low carbohydrate diets are indicating low rates of bone loss, lending the question, whether increased levels of IGF-1 are increasing bone formation thus balancing bone resorption and protecting the bone.

Chapter 3 - Methodology

3.0. Introduction

This low carbohydrate diet study was a randomized control crossover trail which involved 24 overweight women. The low and normal carbohydrate phases of the diet lasted 6 months each. Dietary information as well as fasting urine and serum samples were collected. ELIZA assays were performed to measure biomarkers of bone resorption and absorption.

3.1. Ethical Approval

Ethical approval for this study was obtained from the ethics committee in Waterford Institute of Technology (WIT). Data collected from volunteers remained confidential and was stored securely. Each volunteer signed a written consent form (Appendix A) which outlined the procedures involved and any potential health risks.

3.2. Subjects

Subjects completed a medical history screening form (Appendix B) to ascertain eligibility for the study and whether there were any factors (diseases, injuries or medications) which may affect bone health.

3.2.1. Inclusion Criteria

Females in late reproductive and post-menopausal stages, age range 39 – 65 years and who were overweight with Body Mass Index (BMI) between 25 and 30.

3.2.2. Exclusion Criteria

Females were excluded if they were pregnant or lactating, taking hormone replacement therapy, engaging in intense physical activity, had a history of chronic

menstrual irregularities or had a hysterectomy. They were also excluded if their T score was less than -1 or if they had diabetes mellitus, kidney disease, chronic illness, inflammatory conditions, renal, gastrointestinal or hormonal disorders or were excessive smokers or alcohol drinkers. Additional conditions which excluded females from the study were taking drugs known to affect bone metabolism, other prescribed drugs such as diuretics, antibiotics, antacids and Cox-2 inhibitors. Those suffering from rheumatoid arthritis, osteo-arthritis or metabolic diseases of the bone e.g. osteoporosis or Paget's disease were also excluded. If the subject had engaged in dieting practices in the 6 months prior to the study; including a low carbohydrate diet, they were also excluded from participating.

3.2.3. Recruitment

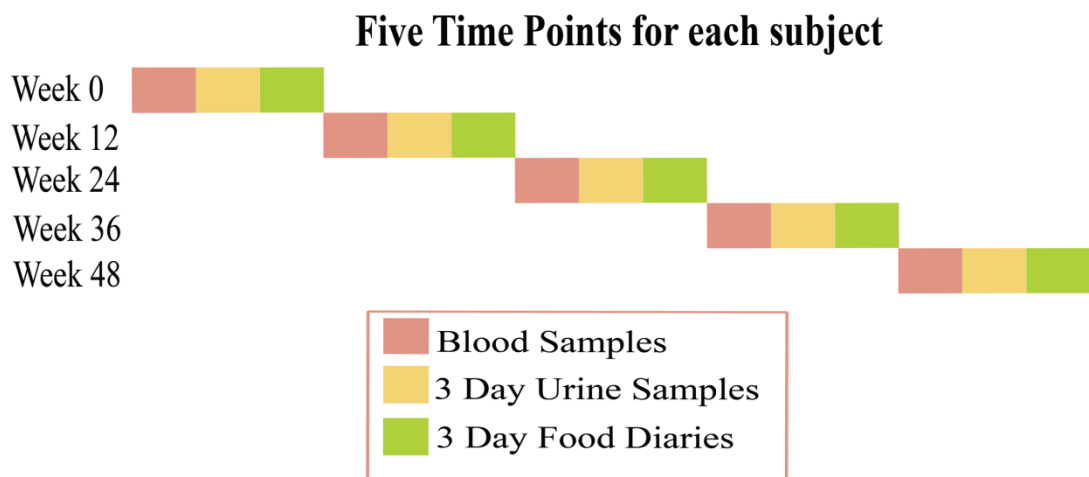
Subjects were recruited through a variety of methods; posters in WIT college campus which all staff and students had access to; posters in the local community centre where an annual health day had measured BMI levels; advertising in local newspaper; a generic email was sent to subjects whom had previously taken part in trials at WIT; word-of-mouth. It was necessary to recruit an approximately even number of pre- and postmenopausal women to compare the bone health of the two groups. A total of 98 women were screened from which 40 volunteers were recruited, of these 24 completed the full study protocol; 13 pre-menopausal women (age 44.2 ± 3.6 years, range 39 – 52 y) and 11 post-menopausal (age 53.3 ± 5.1 y, range 44 – 60 y).

3.3. Study Protocol

Once subjects were deemed eligible for the trial based on the health screening form, their height and weight were measured in order to calculate their BMI. They were

then given a DEXA scan to assess bone health. This form was obtained from Daly and Bass (2000). At the start of the study subjects also completed a 3 day food diary (Appendix C) to assess dietary intake and a record of supplement use was also taken. During the 3 days that subjects recorded their dietary intake they provided 3 first morning urine samples which were measured for urinary pH and ketone levels. Several aliquots of urine were acidified with 3% HCl and then stored at -20°C. Blood samples were taken by a qualified phlebotomist, processed to serum and then stored at -80°C until required for analysis. At the start of the study serum follicle stimulating hormone (FSH) and oestrogen levels were measured to confirm self-reported menopausal status.

Figure 1: Sampling for study participants



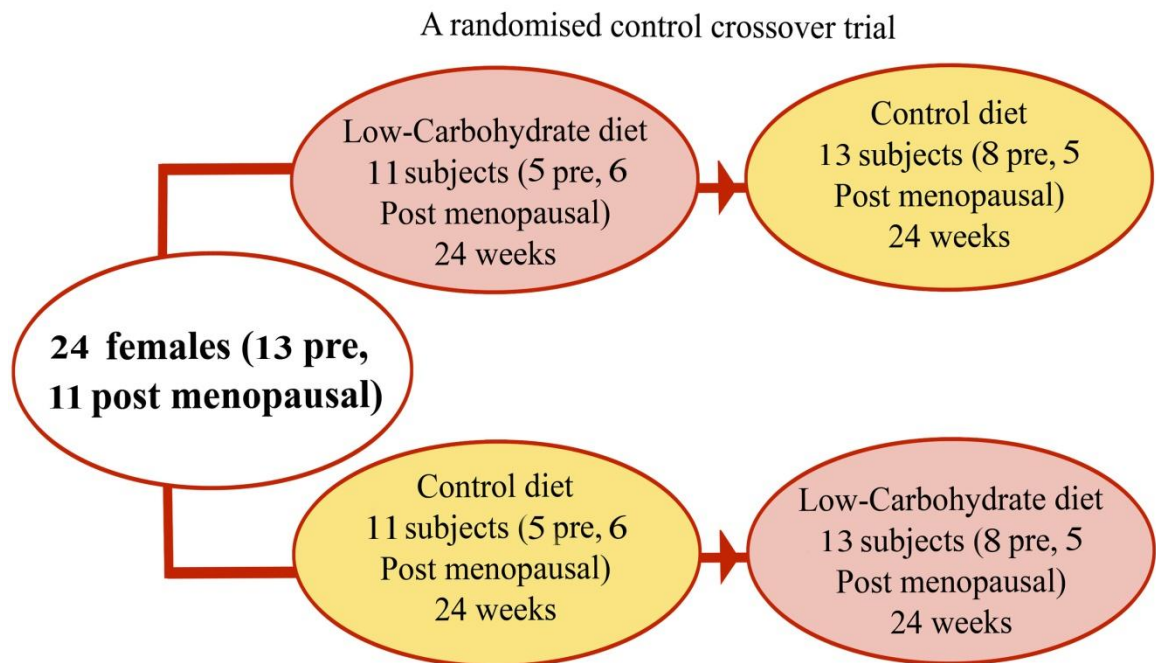
3 day food diaries, serum samples and 3 day urine samples were taken at weeks 0, 12, 24, 36 and 48 as shown in figure 1. Serum was analysed for creatinine, insulin, BSAP, osteocalcin, crosslaps, IGF-1 & IGFBP-3 levels. Calcium was measured on acidified

urine. Unacidified urine stored at -20°C was subsequently measured for urinary creatinine and NTx concentrations.

3.4. Study Design

The study was a randomized control crossover trial to involve 24 overweight women, 13 pre-menopausal and 11 post-menopausal; each group acting as its own control. It was intended to have equal numbers of pre- and post- menopausal women, when one pre- met the criteria for entry to the trial they were assigned to control first, the next to diet first; when one post- met the criteria that subject was assigned to control first and the next to diet first. However due to attrition rates the final numbers were uneven.

Figure 2: Study Design



As can be observed from Figure 2, 8 pre-menopausal and 5 post-menopausal women were advised to continue with their normal diet for 6 months, during which they filled

in a food frequency questionnaire (FFQ) (Appendix D). They returned every 12 weeks to repeat baseline procedures as outlined in the study profile.

The other 5 pre- and 6 post-menopausal women were given a detailed explanation on how to follow a low carbohydrate diet; the carbohydrate content of this was 40g/day, to prevent ketosis. The information was given in an individual session with the subject, it included a sheet explaining what constituted a carbohydrate food; as well as a list of the macronutrient content in the most commonly consumed foods. A booklet with low-carbohydrate recipes, meal plans and advice (Appendix E), adapted from Atkins and Holford diets (Atkins 2004; Holford 2006) was also explained and a copy given to each subject when they understood how to use it. In addition they attended cooking classes in the teaching kitchens at WIT, showing how to make low-carbohydrate foods that were not readily available on the market.

All subjects were asked to weight their food portions in order to record the food diaries accurately, this only had to be done for the first week or two, it was demonstrated or explained that once weighed the food could then be put into a common cup or bowl to see it's level and then this could later be used to calculate the exact measures effortlessly. A food atlas calculating portions size in relation to the hand was also given to each subject so they could record accurately when they ate out.

They also filled in a weekly FFQs, this was intended to aid compliance to diet if subjects inclined to lapse and as a reminder of the detail needed in the 3 day food diaries.

Subjects returned after 6 weeks on the diet and gave urine and blood samples to measure creatinine levels and ensure kidney function was not affected; all other of these measurements being taken at 12 week intervals. At the end of 6 months the

subjects then crossed over; those who had been following their normal diet then followed the low-carbohydrate diet, with the same support and reporting procedures in place.

It was not intended to include a washout period, as statistical analysis, checking for carryover would mean that interaction over time was examined and removed.

Physical activity was assessed in the screening questionnaire, subjects were requested not to alter their pattern of activity during the trial, and to record any activity that did alter, to minimise any effect this could have on the results.

3.5. Anthropometry

Height was measured to the nearest cm by a free-standing stadiometer and weight was measured to the nearest 0.1 kg using an electronic balance. BMI was calculated as:

$$\frac{\text{weight (kg)}}{[\text{height (m)}]^2}$$

3.6. Dietary Assessment

At baseline subjects completed a food diary for 3 days which was then analyzed using CompEat™. Subjects were instructed to maintain normal dietary habits and to estimate the food quantities as accurately as possible; they were provided with information on how this could be best achieved. During the control period subjects were instructed to keep their dietary habits as close to the baseline estimates as possible and to return every 12 weeks with 3 day food diaries completed. During the low-carbohydrate period subjects were instructed to reduce their carbohydrate intake to 40g/day, which was to include as many vegetables as possible, and to increase their

protein and fat content to meet recommended energy levels. They returned every 12 weeks with their completed 3 day food diaries. Average daily intake of selected macro-nutrients (energy, protein, fat, and carbohydrate), micro-nutrients (calcium, magnesium, potassium, phosphate, vitamin C, vitamin D and sodium) and essential and non-essential amino acids were measured in this way. Estimates from the weekly food frequency questionnaires were not analysed since these were collected as an aid for adherence to the diet only.

3.7. Net Endogenous Acid Production (NEAP)

NEAP was calculated by the following method described by Remer *et al.* (2003):

$$\text{Estimated NEAP (mEq/d)} = \text{PRAL (mEq/d)} + \text{OA}_{\text{est}} \text{ (mEq/d)}$$

whereby PRAL denotes potential renal acid load and OA_{est} denotes estimated urinary organic anions, with the 2 components calculated as follows:

$$\text{PRAL (mEq/d)} = 0.49 \times \text{protein (g/d)} + 0.037 \times \text{phosphorus (mg/d)} - 0.021 \times \text{potassium (mg/d)} - 0.026 \times \text{magnesium (mg/d)} - 0.013 \times \text{calcium (mg/d)};$$

$$\text{OA}_{\text{est}} \text{ (mEq/d)} = \text{individual body surface area}^1 \times 41/1.73.$$

¹Body surface area was calculated according to the formula of Du Bois and Du Bois (Wang *et al.* 1992) as follows:

$$\text{body surface area (m}^2\text{)} = [0.007184 - \text{height (cm)}^{0.725} - \text{weight (kg)}^{0.425}].$$

3.8. Sulphur Intake

The sulphur content of the diet was computed from the formula described by Sebastian *et al.* (1994) as follows:

$$\text{Sulphur (mEq/diet)} = 2 \times [(\text{mg methionine}/149.2) + (2 \times \text{mg cysteine}/240.3)].$$

3.9. Bone measurements

Whole body and hip Bone Mineral Density (BMD), Bone Mineral Content (BMC); and percent Lean Body Mass (%LBM) were determined by dual-energy X-ray Absorptiometry (DEXA) using an Excell™ DEXA scanner (*Norland Medical Systems, NY, USA*). Subjects T-score was also established. Scans were undertaken by the author on completion of DEXA training and were performed at Waterford Institute of Technology. These measurements were taken at the start of the study to ascertain study eligibility.

3.10. Blood Samples

Fasting blood samples were drawn from a vein between 08:00 and 10:00 h. Within 1 hour of collection samples were centrifuged (CR422 Jouan Inc. VA, USA) at 4°C for 10 minutes at 3,000 rpm and serum was separated and stored at -80°C until required for further analysis. Serum markers of bone turnover (bone-specific alkaline phosphatase (BSAP), osteocalcin (S-OC) and C-terminal peptide of collagen type-1 (S-CTx)), insulin, IGF-1 & IGFBP-3 were all measured by Enzyme-Linked Immuno Sorbent Assay (ELISA). Serum creatinine was measured spectrophotometrically.

3.10.1. Serum Bone-Specific Alkaline Phosphatase (BSAP)

Serum BSAP was measured using Ostase® BAP ELISA by Immuno Diagnostic Systems. The intra- and inter- assay reproducibility is shown in table 3.1.

Table 3.1: Precision of Ostase® BAP ELISA by Immuno Diagnostic Systems

Sample	Intra-Assay Precision			Inter-Assay Precision		
	Mean	SD (ng/ml)	CV %	Mean	SD (ng/ml)	CV %
1	8.4	0.47	5.8	7.4	0.48	6.5
2	29.2	1.88	6.4	27.3	0.78	2.9
3	55.6	2.03	3.7	52.7	1.35	2.6
4	81.1	4.92	6.1	79.5	3.54	4.5

3.10.2. Serum Osteocalcin (S-OC)

S-OC was measured using N-Mid® Osteocalcin ELISA by Immuno Diagnostic Systems. The intra- and inter- assay reproducibility is shown in Table 3.2.

Table 3.2: Precision of N-MID® Osteocalcin ELISA by Immuno Diagnostic Systems

Sample	Mean	Intra-Assay Precision		Inter-Assay Precision	
		SD (ng/ml)	CV %	SD (ng/ml)	CV %
1	6.7	0.1	1.3	0.2	5.1
2	26.2	0.4	1.8	0.7	2.7
3	53.9	1.2	2.2	2.3	4.2

3.10.3. Serum Crosslaps (CTx)

Serum CTx was measured using Serum Crosslaps® ELISA by Immuno Diagnostic Systems. The intra- and inter assay reproducibility is shown in Table 3.3.

Table 3.3: Precision of Serum Crosslaps® ELISA by Immuno Diagnostic Systems

Sample	Mean	Intra-Assay Precision		Inter-Assay Precision	
		SD (ng/ml)	CV %	SD (ng/ml)	CV %
1	0.121	0.004	3.0	0.013	10.9
2	0.444	0.007	1.7	0.043	9.7
3	1.967	0.035	1.8	0.050	2.5

3.10.4. Serum Insulin

Serum Insulin was measured using Serum Insulin ELISA by Diagnostic Automation Inc. The intra- and inter assay reproducibility is shown in Table 3.4.

Table 3.4: Precision of Serum Insulin ELISA by Diagnostic Automation Inc.

Sample	Mean	Intra-Assay Precision		Inter-Assay Precision	
		SD (ng/ml)	CV %	SD (ng/ml)	CV %
1	15.2	1.59	10.40	1.54	11.8
2	51.4	3.23	6.29	3.6	7.67
3	124	10.53	8.50	6.87	5.80

3.10.5. Serum Insulin-like Growth Factor-1 (IGF-1)

Serum IGF-1 was measured using Serum Human IGF-1 ELISA by Life Research. The average intra- and inter assay reproducibility obtained from 3 samples with low, middle and high level human IGF1 was <10% for intra-assay CV and <12% for inter assay CV.

3.10.6. Serum Insulin-like Growth Factor Binding Protein-3 (IGFBP-3)

Serum IGFBP-3 was measured using Serum Human IGFBP-3 ELISA by Life Research. The average intra- and inter assay reproducibility obtained from 3 samples with low, middle and high level human IGFBP-3 was <10% for intra-assay CV and <12% for inter assay CV.

3.10.7. Serum Creatinine

Serum Creatinine was measured with Quantichrom™ Creatinine Assay kit (DICT-500) by BioAssay Systems. The intra- and inter- assay CV is shown in Table 3.5.

Table 3.5: Precision of Quantichrom™ Serum Creatinine Assay kit (DICT-500)

Sample	Intra-Assay Precision		Inter-Assay Precision	
	Creatinine Conc. (mg/dL)	CV %	Creatinine Conc. (mg/dL)	CV %
1	8.92	2.8	9.04	2.3
2	4.08	1.3	4.18	2.7
3	1.94	2.5	2.03	3.9
4	1.11	3.0	1.18	3.7

3.11. Urine Samples

Each participant collected 3 first-morning urine samples on 3 separate days and was instructed to store samples immediately at -4°C. Freshly thawed samples were measured for pH and ketone presence using a digital urine analyser (Clinitex). Samples were then acidified (1:20 dilution) using 3% HCL and stored at -20°C until required for further analysis.

3.11.1. Urinary Calcium (U-Ca)

U-Ca was measured spectrophotometrically [QuantiChrom™ Calcium Assay Kit (DICA-500) by Bioassay Systems]. The intra- and inter- assay CV is shown in Table 3.6.

Table 3.6: Precision of Calcium Assay by Bioassay Systems.

Sample	Intra-Assay Precision		Inter-Assay Precision	
	Calcium Conc. (mg/dL)	CV %	Calcium Conc. (mg/dL)	CV %
1	4.01	2.4	4.78	3.15

3.11.2. Urinary creatinine (U-Cr)

U-Cr was measured spectrophotometrically with Quantichrom™ Creatinine Assay kit (DICT-500) by BioAssay Systems. The intra- and inter- assay CV is shown in Table 3.7.

Table 3.7: Precision of Quantichrom™ Creatinine Assay kit (DICT-500)

Sample	Intra-Assay Precision		Inter-Assay Precision	
	Creatinine Conc. (mg/dL)	CV %	Creatinine Conc. (mg/dL)	CV %
1	8.92	2.8	9.04	2.3
2	4.08	1.3	4.18	2.7
3	1.94	2.5	2.03	3.9
4	1.11	3.0	1.18	3.7

3.11.3. Urinary Cross-linked N-Telopeptides of Type 1 Collagen (NTx)

Urinary NTx was measured with Osteomark™ ELISA by Wampole Laboratories. The intra- and inter- assay CV is shown in Table 3.8.

Table 3.8: Precision of Osteomark™ ELISA

Sample	Intra-Assay Precision		Inter-Assay Precision	
	Mean (nM BCE)	CV %	Mean (nM BCE)	CV %
1	26	19	79	5
2	417	5	412	3
3	1113	5	1167	4

3.12. Statistical Analysis

Statistical analysis was carried out using SPSS (Statistical Package for Social Sciences) version 18.0. The mean (\pm SD) baseline level of each parameter measured in the study was initially calculated. In order to ascertain whether carryover effects were present a modified method suggested by Jones and Kenward (2003) was utilized. This was used due to the fact that a difference resulting from 3 months on the low-carbohydrate diet, could be carried into the control period, meaning the

difference during the control period for those who started the low-carbohydrate diet first, would be different to those who did the control period first. This analysis was completed for all parameter measured. Differences between week 0 and week 24 and week 24 and 48 were calculated for each parameter measured. For each parameter these differences were summated and an independent samples t-test tested whether the total difference for the subjects going from control to low-carbohydrate diet was different to the total difference for subjects going from the low-carbohydrate to control direction of the study. If there was no carryover the total change should be the same. Where a *P* value of < 0.05 is found the total change in the control to low-carbohydrate group over 48 weeks was not equivalent to the change in the low-carbohydrate to control group over the 48 week period, so carryover is present. Where carryover effects were evident the subjects going in the low-carbohydrate to control direction were removed and the analysis was completed on 12 subjects only (carryover effects were evident for NTx, Fibre, Carbohydrate and Waist Circumference so analysis was only completed on 12 subjects only starting the control period first and then undertaking the low-carbohydrate period).

In order to check for treatment effect data was first checked for normality. When data was normally distributed a paired samples t-test was used to check for differences between changes over the control period (24 weeks) for all 24 subjects versus changes over the low-carbohydrate period (24 weeks) for all 24 subjects. When data was not normally distributed Wilcoxon Signed ranks was utilized. Similar analysis was completed where carryover was evident except 12 subjects only were used.

It was also possible that changes occurred only in the initial 12 weeks of the trial (week 0-12, due perhaps to better adherence to the trial) rather than during week 12-24 of the trial. This could also be said for weeks 24-36 and week 36-48. In order to tests for possible differences over 12 weeks (rather than 24 weeks) between the control and low-carbohydrate groups an independent samples t-test was utilized, due to the fact that different subjects were being compared to each other.

According to calculations completed through the DSS research website this study had a statistical power of 98% to detect changes in weight as a result of consuming the low carbohydrate diet. Power to detect a change in crosslap's as a result of the low carbohydrate diet was 23.3%.

Chapter 4 – Results

4.1. Baseline Results

The baseline parameters for all subjects who completed the study are shown in the tables that follow.

Table 4.1: Physical Measurements and Blood Pressure of Study Participants (n24) at Baseline

Parameter	Mean	SD
Age (y)	48.0	6.0
Weight (kg)	76.5	9.1
Height (m)	1.6	0.1
BMI (kg/m ²)	28.0	3.2
Body fat (%)	43.0	5.0
Waist circumference (cm)	89.9	6.6
Systolic blood pressure (mmHg)	128.9	9.3
Diastolic blood pressure (mmHg)	81.9	9.6

Table 4.2: Urine (U) and Serum (S) Measurements of Study Participants (n24) at Baseline

Parameter	Mean	SD
U-pH	6.0	0.5
U-Ketones	neg	
S-FSH (mIU/mL)	6.5	3.4
S-E2 (pg/ml)	277.8	373.5
S-Insulin (μ IU/ml)	20.2	11.2
S-Creatinine (mg/dL)	0.8	0.2
U-Creatinine (mg/dL)	152.9	186.9
S-IGF-1 (ng/ml)	722.94	771.20
S-IGFBP-3 (ng/ml)	8254.43	1251.70

Table 4.3: Bone Biomarker Levels of Study Participants (n24) at Baseline

Parameter	Mean	SD
BMD (g/cm ²)	1.00	0.08
T-score	0.61	0.21
S-Osteocalcin (ng/ml)	14.58	6.59
S-Crosslaps (ng/ml)	0.29	0.18
S-BSAP (mg/ml)	14.71	6.19
U-NTx (nM BCE/mMol Creatinine)	42.38	20.05
U-Calcium (mMol Ca/mMol Cr)	0.16	0.09

Table 4.4: Dietary Intakes (3 day average) and PRAL of Study Participants (n24) at Baseline

Parameter	Mean	SD
Energy (Kcal)	1687.35	376.23
Fat (g)	70.54	23.39
Protein (g)	68.4	16.84
Carbohydrate (g)	194.99	46.38
Fibre (g)	12.70	4.37
Na (mg)	2256.89	774.35
K (mg)	2898.05	634.36
Ca (mg)	813.56	340.78
Mg (mg)	253.82	57.98
Vitamin C (mg)	143.17	322.50
Phosphorus (mg)	1171.12	305.37
Vitamin D (ug)	2.34	2.27
Sulphur (mg)	14.10	7.09
PRAL (mEq/d)	-1.18	16.73

Table 4.5: Non-Essential Amino Acid Intakes (3 day average) of Study Participants (n24) at Baseline

Parameter	Mean	SD
Total (g)	14.72	7.38
Cystine (g)	0.40	0.21
Tyrosine (g)	0.90	0.47
Arginine (g)	1.25	0.65
Alanine (g)	1.15	0.62
Aspartic acid (g)	2.05	1.08
Glutamic acid (g)	4.93	2.64
Glycine (g)	0.92	0.50
Serine (g)	1.30	0.68
Proline (g)	1.82	0.99

Table 4.6: Essential Amino Acid Intakes (3 day average) of Study Participants (n24) at Baseline

Parameter	RDA¹	Mean	SD
Total (g)		9.97	5.36
Isoleucine (g)	1.45	1.16*	0.13
Leucine (g)	3.21	1.98*	0.22
Valine (g)	1.84	1.40*	0.16
Phenylalanine (g)	2.52	1.18*	0.12
Tryptophan (g)	0.38	0.35	0.04
Histidine (g)	1.15	0.70*	0.07
Methionine (g)	0.46	0.60	0.07
Lysine (g)	2.91	1.59*	0.20
Threonine (g)	1.53	1.02*	0.10

¹ RDA for essential amino acids based on Institute of Medicine (IOM) 2005.

* Below RDA

4.2. Carryover Effects

Table 4.7: Analysis to Check for Presence of Carryover Effects in Physical Parameters Measured

Parameter	Control to Low Carbohydrate Group (<i>n</i> 12)		Low-Carbohydrate to Control Group (<i>n</i> 12)		<i>P</i> value
	Total Mean Change	SD	Total Mean Change	SD	
Systolic Blood pressure (mmHg)	-2.00	9.60	+4.73	16.80	0.247
Diastolic Blood Pressure (mmHg)	-1.91	12.29	+13.60	30.95	0.217
Waist Circumference (cm)	-4.53	2.53	+0.02	2.58	<0.001
Weight (Kg)	-6.34	6.56	-3.90	6.05	0.354

* $P < 0.05$

Table 4.7 shows carryover effects for physical parameters examined. Total change over the control and low-carbohydrate periods between those who started the control first versus those who started the low-carbohydrate first was analyzed using independent samples t-tests. There were no carryover effects evident in systolic blood pressure, diastolic blood pressure or weight measurements ($P > 0.05$). Carryover was evident in waist circumference measurements ($P < 0.001$) with a total change of -4.53 ± 2.53 cm when going from control to low-carbohydrate phase of the trial but a total change of $+0.02 \pm 2.58$ cm was evident when subjects went from the low carbohydrate to control trial phase. Only participants (n 12) from the control-low-carbohydrate phase of the trial were further analyzed for treatment effects for waist circumference results.

Table 4.8: Analysis to Check for Presence of Carryover Effects in Urine and Serum Parameters Measured

Parameter	Control to Low Carbohydrate Group (n12)		Low-Carbohydrate to Control Group (n12)		P value
	Total Mean Change	SD	Total Mean Change	SD	
U-pH	-0.17	0.80	-0.17	0.72	0.994
S-Insulin (μ U/ml)	-1.00	11.07	+0.60	7.29	0.717
S-Creatinine (mg/dL)	+0.14	0.11	+0.14	0.51	0.965
S-IGF-1 (ng/ml)	-124.33	436.19	-59.28	-216.88	0.725
S-IGFBP-3 (ng/ml)	-10.33	752.34	-219.43	630.67	0.596

Table 4.8 shows carryover effects for serum and urine parameters examined in the study. Independent samples t-test was performed as previously described. There were no carryover effects evident in urine pH, serum insulin, creatinine, IGF-1 and IGFBP-3 ($P > 0.05$). Hence all subjects could be analyzed for treatment effects for these parameters.

Table 4.9: Analysis to Check for Presence of Carryover Effects in Dietary Intakes and Potential Renal Acid Load

Parameter	Control to Low Carbohydrate Group (n12)		Low-Carbohydrate to Control Group (n12)		P value
	Total Mean Change	SD	Total Mean Change	SD	
Energy (Kcal)	-279.40	258.21	114.31	688.07	0.128
Fat (g)	+7.87	24.76	+0.68	43.58	0.649
Protein (g)	+12.60	19.85	+1.2	25.43	0.264
Essential AA (mg)	+165.00	643.00	+100.00	737.00	0.850
Non-Essential AA (mg)	+100.00	816.00	+198.00	939.00	0.806
Carbohydrate (g)	-107.84	56.30	-19.91	41.08	<0.001*
Fibre (g)	-3.80	4.42	+1.33	4.97	0.017*
Na (mg)	+3.35	872.40	+236.97	988.69	0.574
K (mg)	-270.80	476.11	-8.44	943.37	0.416
Ca (mg)	-101.07	255.66	-215.28	461.38	0.541
Mg (mg)	-21.75	42.25	+30.03	117.39	0.182
Vitamin C (mg)	-2.59	67.89	-22.50	41.97	0.440
P (mg)	+50.96	266.00	+93.81	610.36	0.833
Vitamin D (µg)	+0.44	1.08	-0.30	1.99	0.328
Sulphur (mg)	0.02	3.21	1.06	4.29	0.519
PRAL (mEq/d)	+15.21	16.66	+3.49	13.15	0.098

* $P < 0.05$

Table 4.9 shows carryover effects for dietary aspects examined in the study. Independent samples t-test was performed as previously described.

There were no carryover effects evident in energy, fat, protein, essential amino acid, non-essential amino acid, Na, K, Ca, Mg, Vitamin C, P,

Vitamin D, Sulphur or PRAL levels ($P > 0.05$). Hence all subjects were analyzed for treatment effects for these parameters. There were

carryover effects for carbohydrate ($P < 0.001$) where a total change of -107.84 ± 56.30 g was evident when subjects started the control-low

carbohydrate phase but a total change of $-19.91 \pm 41.08\text{g}$ was evident when subjects started the low-carbohydrate phase and then went to the control phase. Similarly carryover effects were also evident with fibre intake ($P < 0.017$)??? with a total change of $-3.80 \pm 4.42\text{g}$ when subjects started the control-low carbohydrate phase but a total change of $+1.33 \pm 4.97\text{g}$ when subjects started the low-carbohydrate phase and then went to the control phase. Hence only subjects from the control-low carbohydrate phase were analyzed for changes in carbohydrate and fibre intake.

Table 4.10: Analysis to Check for Presence of Carryover Effects in Bone Biomarkers

Parameter	Control to Low Carbohydrate Group (<i>n</i> 12)		Low-Carbohydrate to Control Group (<i>n</i> 12)		<i>P</i> value
	Total Mean Change	SD	Total Mean Change	SD	
S-Osteocalcin (ng/ml)	-1.08	5.17	-0.24	8.42	0.773
S- Ctx (ng/ml)	+0.07	0.16	+0.13	0.22	0.477
S-BSAP (mg/ml)	-0.55	1.88	-0.27	3.28	0.813
U-NTx (nM BCE/mMol Creatinine)	-0.54	13.78	17.74	12.94	0.008*
U-Ca (mMol Ca/mMol Cr)	+0.03	0.16	-0.07	0.15	0.181

* $P < 0.05$;

Table 4.10 shows carryover effects for bone biomarkers examined in the study. Independent samples t-test was performed as previously described. There were no carryover effects evident in S-Osteocalcin, S-Ctx, S-BSAP and U-Ca ($P > 0.05$). Hence all subjects were analyzed for treatment effects for these parameters. There were carryover effects for U-Ntx ($P = 0.008$) with a change of -0.54 ± 13.78 when subjects went from the control to low-carbohydrate phase but a change of $+17.74 \pm 12.94$ when subjects went from the low-carbohydrate to control phase. Hence only subjects from the control-low carbohydrate phase were analyzed for changes in urinary Ntx.

4.3. Treatment Effects

Table 4.11: Analysis to Check for Presence of Treatment Effects in Physical Parameters Measured (*n*24)

Parameter	Control Period		Low-Carbohydrate Period		<i>P</i> value
	Total Mean Change	SD	Total Mean Change	SD	
Systolic Blood pressure (mmHg)	+2.91	12.60	-0.58	4.61	0.389
Diastolic Blood Pressure (mmHg)	+3.33	7.61	-3.04	7.75	0.021*
Waist Circumference (cm) ^a	-1.47	2.11	-1.42	1.96	0.962
Weight (kg)	+0.52	4.09	-5.64	4.14	<0.001*

^a *n* 12; * *P* < 0.05

Table 4.11 shows treatment effects for physical parameters examined in the study. Comparison of change in the control period versus change in the low-carbohydrate period was analysed by paired t-test when data was normally distributed and Wilcoxon signed ranks for data not normally distributed. There was no significant change in systolic blood pressure or waist circumference during the control compared to the low carbohydrate period (*P* > 0.05). There was a significant difference in diastolic blood pressure (*P* = 0.021) with a reduction during the low-carbohydrate period compared to an increase during the control period. Weight was also significantly reduced (*P* < 0.001) during the low-carbohydrate period compared to the control period.

Table 4.12: Analysis to Check for Presence of Treatment Effects in Serum and Urinary Parameters Measured (n24)

Parameter	Control Period		Low-Carbohydrate Period		P value
	Total Mean Change	SD	Total Mean Change	SD	
U-pH	-0.05	0.57	-0.11	0.61	0.319
S-Insulin (μ U/ml)	-0.44	9.19	+0.33	9.02	0.838
S-Creatinine (mg/dL)	0.00	0.23	+0.14	0.33	0.176
S-IGF-1 (ng/ml)	-144.60	283.16	+48.75	205.05	0.043*
S-IGFBP-3 (ng/ml)	+156.77	798.89	-279.69	883.29	0.329

* $P < 0.05$

Table 4.12 shows treatment effects for serum and urinary parameters examined in the study. Statistical analysis was performed as described in previous table. There was no significant change over the control period compared to the low-carbohydrate period for U-pH, S-Insulin, S-creatinine and S-IGFBP-3 ($P > 0.05$). There was a significant change in S-IGF-1 ($P = 0.043$) with levels being reduced during the control period but increased during the low-carbohydrate period.

Table 4.13: Analysis to Check for Presence of Treatment Effects in Dietary Intake and Potential Renal Acid Load (PRAL) (n24)

Parameter	Control Period		Low-Carbohydrate Period		P value
	Total Mean Change	SD	Total Mean Change	SD	
PRAL (mEq/d)	-0.504	16.51	+14.38	14.38	0.004*
Energy (Kcal/d)	+303.18	508.38	-385.73	537.74	0.017*
Fat (g/d)	+9.16	32.47	-5.21	29.97	0.203
Protein (g)	+3.89	23.76	+5.07	24.27	0.780
Essential AA (g/d)	-0.26	6.74	+1.63	9.20	0.541
Non-Essential AA (g/d)	+0.71	10.21	+0.85	13.00	0.975
Carbohydrate (g/d) ^a	-11.15	64.22	-107.95	69.29	<0.001*
Fibre (g/d) ^a	-3.31	3.98	-0.49	2.83	0.109
Na (mg/d)	+283.07	1206.55	-157.36	917.29	0.279
K (mg)	+282.52	908.71	-416.45	854.62	0.047*
Ca (mg/d)	+3.12	264.79	-167.64	293.40	0.096
Mg (mg/d)	+41.02	95.81	-35.75	72.68	0.005*
Vitamin C (mg/d)	-19.42	40.03	+5.31	57.88	0.215
Vitamin D (µg/d)	-0.58	1.39	+0.60	1.91	0.073
Sulphur (mg/d)	-6.22	8.81	-4.01	9.67	0.385
P (mg/d)	+48.71	413.21	+23.67	355.70	0.851

^a n 12; * P < 0.05

Table 4.13 shows treatment effects for dietary intakes and PRAL measured. Statistical analysis was performed as previously described. There were no significant differences ($P > 0.05$) in dietary intake of fat essential amino acids, non-essential amino acids, fibre, Na, Ca, vitamin C and P. There were significant differences in energy, carbohydrate, potassium, Mg and PRAL ($P < 0.05$) whereas energy, K and Mg intake increased in the control period it decreased in the low carbohydrate period. Carbohydrate intake decreased in both periods but significantly decreased during the low carbohydrate period. PRAL significantly increased during the low-carbohydrate period.

Table 4.14: Analysis to Check for Presence of Treatment Effects in Bone Biomarkers (n24)

Parameter	Control Period		Low-Carbohydrate Period		P value
	Total Mean Change	SD	Total Mean Change	SD	
S-Osteocalcin (ng/ml)	-0.54	5.37	-0.27	5.36	0.848
S- Ctx (ng/ml)	+0.04	0.14	+0.05	0.15	0.542
S-BSAP (mg/ml)	-0.16	2.99	-0.42	3.14	0.946
U-NTx (nM BCE/mMol Creatinine) ^a	-7.06	10.22	+6.52	11.77	0.019*
U-Ca (mMol Ca/mMol Cr)	+0.01	0.08	+0.04	0.09	0.542

^a n 12; * P < 0.05

Table 4.14 shows treatment effects for bone biomarkers measured. Statistical analysis was performed as previously described. There was no significant change ($P > 0.05$) in S-Osteocalcin, S-Ctx, S- BSAP and U-Ca between control and low-carbohydrate dietary periods. There was a significant difference ($P = 0.019$) in U-Ntx between control and the low-carbohydrate period. U-Ntx decreased during the control period but increased during the low-carbohydrate period.

4.4. Effects Over 12 and 24 Weeks

Table 4.15: Changes in Physical Parameters Measured Over 12 and 24 Weeks (n24)

Parameter	Δ week 0-12		Δ week 12-24		Δ week 0-24		Δ week 24-36		Δ week 36-48		week 24-48	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Body weight (kg)												
Control first	-1.26 ^a	2.56	-0.24	2.12	-1.50 ^a	3.96	-3.79 ^a	2.63	-1.06	2.66	-4.85 ^a	4.73
Low - Carb first	-5.64 ^b	2.70	+0.52	1.75	-5.12 ^b	3.49	+0.49 ^b	1.90	+0.73	1.73	+1.22 ^b	3.2
Waist circumference (cm)												
Control first	-0.81 ^a	1.26	-0.65	1.39	-1.47 ^a	2.11	-1.06 ^a	1.38	-0.36	1.41	-1.42 ^a	1.96
Low - Carb first	-2.53 ^b	1.53	-0.53	1.13	-3.07 ^b	1.70	+0.97 ^b	1.09	+0.47	1.32	+1.44 ^b	1.95
Systolic blood pressure (mmHg)												
Control first	-2.36	7.80	-0.18	10.71	-3.25	8.97	+3.91	13.61	-2.67	13.01	+1.25	13.35
Low - Carb first	+1.67	19.24	-4.08	13.37	-2.42	19.43	+5.36	17.48	+4.27	11.01	+9.60	12.86
Diastolic blood pressure (mmHg)												
Control first	-3.00	6.08	+4.54 ^a	5.88	+2.33	7.79	-1.00	7.60	-0.83	9.22	-1.83 ^a	5.58
Low - Carb first	4.58	12.90	-8.83 ^b	11.50	-4.25	9.55	+1.42	9.80	+2.90	8.78	+4.66 ^b	7.60

^{a,b} different superscripts indicate the presence of a significant difference ($P < 0.05$) between the control and low-carbohydrate group.

Table 4.15 shows the changes in physical parameters and whether changes occurred in the first and/or second 12 weeks of the control and low-carbohydrate periods. Independent samples t-tests were performed to compare changes. Body weight and waist circumference significantly changed in the first 12 weeks of the low-carbohydrate phase regardless of whether they started the low-carbohydrate first or the control period first. No changes occurred over the 12-24 week period for body weight measurements. The change in control, relative to change in low-carbohydrate diet period, for measurements of systolic blood pressure were insignificant ($P > 0.05$). Diastolic blood pressure significantly

reduced in week 12-24 when subjects started the low-carbohydrate diet first but had significantly increased in that group when they reverted to their control type diet, compared to subjects on a low-carbohydrate diet.

Table 4.16: Changes in Serum and Urinary Parameters Measured Over 12 and 24 Weeks (n24)

Parameter	Δ week 0-12		Δ week 12-24		Δ week 0-24		Δ week 24-36		Δ week 36-48		Δ week 24-48	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
U- pH Level												
Control first	-0.17	0.85	-0.22	0.69	-0.39	0.56	+0.23	0.51	+0.03	0.58	+0.25	0.49
Low - Carb first	-0.33	0.56	-0.01	0.30	-0.34	0.50	+0.18	0.51	-0.36	0.41	-0.17	0.55
S-Insulin (uIU/ml)												
Control first	-3.00	7.00	+2.00	6.00	-1.00	8.00	+3.00	13.00	-1.00	7.00	+2.00	10.00
Low - Carb first	-5.00	5.00	+5.00	7.00	0.00	7.00	-2.00	10.00	+3.00	8.00	+1.00	9.00
S-Creatinine (mg/dL)												
Control first	-0.01	0.02	+0.02	0.19	+0.00	0.22	-0.03	0.09	+0.11	0.08	+0.07	0.11
Low - Carb first	+0.00	0.46	+0.19	0.26	+0.19	0.46	+0.02	0.15	-0.05	0.31	-0.03	0.27
S-IGF1 (ng/ml)												
Control first	-57.00	248.00	-160.00	342.00	-216.00	333.00	+93.00	118.00	+21.00	242.00	+119.00	203.00
Low - Carb first	-45.00	166.00	+4.00	85.00	-42.00	182.00	+14.00	92.00	-32.00	115.00	-17.00	106.00
S-IGFBP3 (ng/ml)												
Control first	-95.00	865.00	+132.00	790.00	+27.00	717.00	-656.00	790.00	+444.00	649.00	-212.00	1275.00
Low-Carb first	-168.00	570.00	-185.00	570.00	-465.00	529.00	+63.00	1110.00	+188.00	1571.00	+245.00	864.00

Table 4.16 shows the changes in serum and urinary parameters and whether changes occurred in the first and/or second 12 weeks of the control and low-carbohydrate periods. Independent samples t-tests were performed to compare changes. There were no changes in control period, relative to changes in low-carbohydrate diet period for U-pH, S-Insulin, S-Creatinine, S-IGF-1 and S-IGFBP-3 ($P > 0.05$).

Table 4.17: Changes in Dietary Intake and PRAL Measured Over 12 and 24 Weeks (n24)

Parameter	Δ week 0-12		Δ week 12-24		Δ week 0-24		Δ week 24-36		Δ week 36-48		Δ week 24-48	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Energy (Kcal/d)												
Control first	+50 ^a	336	-56	381	-6 ^a	381	-472 ^a	495	+168	211	-304 ^a	364
Low-Carb first	-425 ^b	591	-66	621	-490 ^b	621	+435 ^b	411	+158	337	594 ^b	447
Fat (g/d)												
Control first	+3	18	-2	25	+1	26	-8	30	+13	18	+5	27
Low - Carb first	-2	39	-11	18	-13	30	+5	34	+9	31	+14	37
Protein (g/d)												
Control first	+5	16	+1	20	+6	17	-5	15	+14	13	+9	20
Low - Carb first	-1	27	+1	13	0	31	0	28	+2	18	+2	25
Essential Amino acids (g/d)												
Control first	-2	4	+3	8	+1	7	-1	9	+2	7	0	8
Low - Carb first	+2	8	+1	5	+3	10	-1	9	-1	6	-2	6
Non-essential Amino acids (g/d)												
Control first	-3	6	+3	12	+2	11	0	12	+1	8	-2	10
Low - Carb first	+1	11	+2	7	+3	14	-0	12	-1	8	-1	9
Carbohydrate (g/d)												
Control first	+5 ^a	48	-16	60	-11 ^a	64	-94 ^a	78	-3	33	-97 ^a	69
Low-Carb first	-107 ^b	48	+7	33	-100 ^b	60	+62 ^b	60	+17	73	+79 ^b	68
Fibre (g/d)												
Control first	-2	3	-1	2	-3	4	-2 ^a	3	+1	2	-1 ^a	3
Low - Carb first	-2	4	-3	4	-5	5	+3 ^b	3	+3	6	+6 ^b	2
Sodium (mg/d)												
Control first	+131	457	+56	723	+187	584	-635 ^a	811	+407	848	-228	898
Low-Carb first	+19	1252	-255	967	-236	1346	+167 ^b	927	+163	1171	+330	1165

^{a,b} different superscripts indicate the presence of a significant difference ($P < 0.05$) between the control and low-carbohydrate group.

Table 4.17 continued: Changes in Dietary Intake and PRAL Measured Over 12 and 24 Weeks (n24)

Parameter	Δ week 0-12		Δ week 12-24		Δ week 0-24		Δ week 24-36		Δ week 36-48		Δ week 24-48	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Potassium (mg/d)												
Control first	+21	678	-161	867	-140	616	-418	667	+287	286	-131	775
Low-Carb first	-591	770	-87	594	-678	870	+307	1053	+363	810	+670	982
Calcium (mg/d)												
Control first	-44	241	+79	278	+35 ^a	272	-69	182	+12	217	-57	262
Low-Carb first	-218	371	0	124	-218 ^b	298	+88	195	-17	183	+71	303
Magnesium (mg/d)												
Control first	+1	47	-13	68	-12	29	-45	39	+32	46	-9	46
Low - Carb first	-45	106	-14	67	-59	86	+39	91	+50	111	89	110
Vitamin C (mg/d)												
Control first	-2	53	-23	41	-25	44	+5	34	+19 ^a	37	+24	62
Low-Carb first	-5	41	-4	33	-9	53	+6	48	-13 ^b	33	-7	40
Phosphorus (mg/d)												
Control first	0	238	-27	364	-27	264	-79	308	+163	170	+78	258
Low-Carb first	-33	515	-48	301	-81	452	-5	424	+130	372	+125	525
Vitamin D (μg/d)												
Control first	-0.7 ^a	2	+0.3	1	-0.4	1	+0.2	1	+0.6	1	+0.9 ^a	1
Low-Carb first	+1.2 ^b	2	-0.9	2	+0.3	2	-0.1	2	-0.6	2	-0.7 ^b	1
Sulphur (mg/d)												
Control first	-1.6	5	+0.6	7	+0.8	6	+0.2	11	-9.9	7	-9.8	7
Low-Carb first	+0.3	9	+3.3	7	+2.5	12	-2.1	12	-8.7	6	-11.2	9
PRAL (mEq/d)												
Control first	+2.2	15.3	+3.3	13.7	+5.5	17.7	+6.9 ^a	12.2	+6.24 ^a	7.9	+13.1 ^a	12.1
Low-Carb first	+11.2	19.5	+1.3	9.9	+13.1	18.1	-8.6 ^b	17.5	-3.8 ^b	13.6	-12.4 ^b	15.3

^{a,b} different superscripts indicate the presence of a significant difference ($P < 0.05$) between the control and low-carbohydrate group.

Changes in Dietary Intake and PRAL Measured Over 12 and 24 Weeks (n24)

Table 4.17 shows the changes in dietary intake and PRAL and whether changes occurred in the first and/or second 12 weeks of the control and low-carbohydrate periods. Independent samples t-tests were performed to compare changes. There were no significant changes ($P > 0.05$) in fat, protein, essential amino acids, non-essential amino acids, K, Mg, vitamin C, P and Sulphur over any time-points. Energy and carbohydrate intake were significantly reduced during the first 12 weeks all subjects started the low-carbohydrate diet, but there was no difference in energy intake between control and low-carbohydrate groups during week 12-24 of the study period. Fibre and sodium intakes decreased when subjects started the low-carbohydrate diet compared to the control group. Subjects who started the low-carbohydrate diet first compared to those in the control group had a significantly reduced calcium intake over 24 weeks. Subjects who had completed the control period and then started the low-carbohydrate period increased vitamin C intake in week 12-24. Consuming the low-carbohydrate diet was associated with a significant increase in Vitamin D intake during the first 12 weeks when subjects started the low-carbohydrate diet first and increased vitamin D intake over the 24 weeks when subjects took up the low-carbohydrate diet after being on the control period. There was a significant increase in PRAL over week 0-12 and week 12-24 when subjects took up the low-carbohydrate diet after being on the control diet.

Table 4.18: Changes in Bone Biomarkers Measured Over 12 and 24 Weeks (n24)

Parameter	Δ week 0-12		Δ week 12-24		Δ week 0-24		Δ week 24-36		Δ week 36-48		Δ week 24-48	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
S-Osteocalcin (ng/ml)												
Control first	-1.08	5.52	-0.17	1.27	-1.25	5.64	-0.90	2.02	+1.54	2.02	+0.63	2.66
Low - Carb first	-0.45	8.05	-1.63	3.47	-2.09	6.68	+1.75	3.67	+0.17	5.23	+1.92	4.10
S-Crosslaps (ng/ml)												
Control first	0.0	0.12	-0.01	0.1	-0.01	0.12	+0.04	0.09	+0.05	0.07	+0.08	0.10
Low - Carb first	+0.07	0.19	-0.04	0.17	+0.03	0.20	-0.02	0.07	+0.11	0.13	+0.09	0.14
S-BSAP (mg/ml)												
Control first	+0.46	3.38	-0.49	2.17	-0.77	2.47	-0.44 ^a	1.27	+0.60	1.96	+0.16	2.21
Low - Carb first	+0.02	3.33	-0.37	1.57	-0.72	3.86	+0.96 ^b	1.85	-0.67	2.69	+0.44	3.45
U-NTx (nMol Ca/mMol Cr)												
Control first	+0.25	16.12	-7.31	14.75	-7.06 ^a	10.22	+8.17 ^a	12.01	-2.11 ^a	13.39	+6.06	12.46
Low - Carb first	+7.25	20.54	-0.64	20.57	+5.82 ^b	17.15	-15.74 ^b	21.31	+19.38 ^b	19.07	+4.04	27.67
U-Calcium (mMol Ca/mMol Cr)												
Control first	+0.03	0.06	+0.02	0.08	+0.04	0.07	+0.08 ^a	0.12	-0.01	0.16	+0.01	0.10
Low - Carb first	+0.04	0.14	-0.00	0.16	+0.06	0.08	-0.06 ^b	0.09	+0.07	0.11	-0.01	0.08

^{a,b} different superscripts indicate the presence of a significant difference ($P < 0.05$) between the control and low-carbohydrate group.

Table 4.18 shows the changes in bone biomarkers and whether changes occurred in the first and/or second 12 weeks of the control and low-carbohydrate periods. Independent samples t-tests were performed to compare changes. There were no significant changes ($P > 0.05$) in S-Osteocalcin and S-Crosslaps. There was a significant difference ($P < 0.05$) in S-BSAP with a decrease occurring when subjects had been on the low-carbohydrate diet for 12 weeks having gone through the control period. Similarly after coming off the low carbohydrate diet S-BSAP increased for weeks 0-12. Starting the low-carbohydrate diet compared to subjects consuming their control diet was associated with a significant increase in U-NTx change over 0-24 weeks compared to a decrease when subjects consumed the control diet.

There was a significant increase in urinary Ca for week's 0-12 for subjects who started the low-carbohydrate diet having been on the control diet, but this difference was not maintained over the 24 weeks.

Chapter 5 – Discussion

5.0. Introduction

The aim of this study was to investigate whether a low carbohydrate diet consumed over a six month period could influence weight loss and the intake of macro- and micronutrients which could affect bone health, and in addition to examine the effects of the diet on biomarkers of bone formation and resorption. In summary this research identified a significant weight loss and reduction in waist circumference as a result of consuming the low-carbohydrate diet. Consuming the low-carbohydrate diet as associated with a significant increase in bone resorption, measured through serum NTx. The low-carbohydrate diet resulted in significant differences in intakes of several nutrients compared to the control diet. These aspects are discussed in more detail below.

5.1. Baseline Demography

This study recruited 24 healthy Caucasian women whose mean age was 48 years and ranged between 38 and 60 years. Of these 13 were pre- and 11 were post-menopausal. Serum FSH for pre-menopausal subjects was 5.41 ± 3.85 mIU/mL while post-menopausal FSH levels were 15.09 ± 24.27 mIU/mL. Serum E2 levels for pre-menopausal subjects was 539.04 ± 638.54 pg/ml and for post-menopausal subjects was 240.39 ± 421.17 pg/ml. The decrease in oestrogen with age and the increase in FSH with age in midlife females are in accordance with previous research (Randolph *et al.* 2004).

At entry, their average weight was 76 kg, BMI was 28 kg/m^2 (range, 25 to 38 kg/m^2). Their average body fat was 43% (range, 34 to 54%) and mean waist circumference was 90cm. Bone Mass Density was on average within the healthy range at 1 with a mean T-score of + 0.6. Subjects were all overweight, however three were at the lower end of the BMI range describing overweight (25 kg/m^2), and none were in the healthy range of body fat (33%).

5.2. Effects of Low Carbohydrate Diet on Physical Parameters

5.2.1. Weight

One of the primary measurements in this study was weight. A significant difference in weight was observed in the low carbohydrate diet group compared to the control group. Data recorded for all 24 subjects during the six months on the low carbohydrate diet observed weight decrease by average of 5.6kg (± 4.14) or 7% from baseline. This weight loss is similar to other studies of low carbohydrate diets. The study by Carter *et al.* (2006) also saw a 7% decrease in weight from baseline over 3 months; Coleman & Nickols-Richardson (2005) 8% decrease over 3 months; Reddy *et al.* (2002) 5% decrease over 8 weeks. A study by Can *et al.* (2020) observed a weight loss of 2.4kg over 4 weeks on a low-carbohydrate diet. Data for the first twelve weeks show weight in the diet group was significantly different compared with that in the control group; however the difference was not significant during the second 12 weeks of the diet. Greater weight loss during the first half of the diet may indicate that compliance to the diet was easier during the first 12 weeks of the diet. Otherwise it could be due to the metabolic effect of the diet; each gram of glycogen is bound to 3g of water and this is released upon glycogen metabolism giving rise to a diuretic effect, however this is usually limited to the first weeks of a low carbohydrate diet (Denke, 2001). Other researchers also found a large initial weight loss, followed by a slowdown (Carter *et al.* 2005; Coleman & Nickols-Richardson 2005; Reddy *et al.* 2002) However, weight loss may be attributed more to energy restriction rather than to the low carbohydrate content of the diet as studies comparing different macronutrient content found no significant difference in weight loss between high protein and normal protein diets (Colman & Nickols-Richardson 2005; Sukumar *et al.* 2011).

It appears that subjects who did the control first may have lost weight throughout the control. However, this tendency does not appear in the control stage with subjects who did the low carbohydrate diet first; these seem to have gained weight throughout. This may signify that subjects were eager to lose weight or that the information on low carbohydrate diets, which had to be given in order to gain consent from the subjects may have been affected their original eating patterns. In a similar study controls were also reported to have lost an average of 1kg in 3 months (Carter *et al.* 2006).

In the present research subjects who did the diet first gained weight during the control period however, they did not return to their original weight (76 kg) after the control period (72 kg), this could indicate that in a reluctance to gain weight, they did not completely return to their normal diet.

5.2.2. Blood Pressure

Initial systolic and diastolic blood pressure was 128.9 ± 9.3 mmHg and 81.9 ± 9.6 mmHg. There was no significant change in systolic blood pressure between control and low-carbohydrate periods. There was a significant change in diastolic blood pressure with an increase during the control period ($+3.33 \pm 7.61$ mmHg) and a decrease during the low carbohydrate period (-3.04 ± 7.75 mmHg). Subjects who started the low-carbohydrate diet first had a greater decrease in diastolic blood pressure than subjects starting the diet after being on the control period. During the low-carbohydrate diet period there was a greater decrease in diastolic blood pressure during week 12-24, compared to weeks 0-12.

In a review by Bravata *et al.* (2003), few studies of low carbohydrate diets reported on blood pressure, on average those that did suggest no change in systolic blood pressure, although 4

studies saw a mean decrease of 0.7mmHg. Research comparing low-fat, Mediterranean and low-carbohydrate diets, found blood pressure fell in all with no significant difference between the diet types, but the lowest decrease was in the low carbohydrate diet where systolic blood pressure fell by 3.9mmHg and diastolic by 0.8mmHg (Shai *et al.* 2008). Other researchers suggest decreases in both systolic and diastolic may not be discerning to the low carbohydrate diet, as all subjects in their studies who followed a low energy diet reported lower blood pressure (Foster *et al.* 2003; Delbridge *et al.* 2009), so weight loss itself may have caused the decrease in blood pressure. It has been reported that a reduction of 1kg in weight causes a decrease in systolic and diastolic blood pressure of 1.05 and 0.92mmHg (Neter *et al.* 2003).

5.2.3. Waist Circumference

There was no significant change in the waist circumference in this study between control and low-carbohydrate groups with both groups losing approx 1.4 cm off their waistline. Due to carryover effects, results from 12 subjects only were used, which therefore reduces the power of the result. Within the subjects on the low-carbohydrate diet first versus subjects on the control first a significant decrease in waistline was observed during the first 12 weeks but significance was not maintained into the second 12 weeks of the diet. Low-carbohydrate diet over 4 weeks in overweight/obese females (BMI 30.4 kg/m²) has been associated with a 3cm decrease in waist circumference (Can *et al.* 2010). The subjects in the present study had a BMI of 28 kg/m² so may not have had as much potential to decrease waist circumference compared to the study by Can *et al.* (2010). A significant difference in WC between those on a low-carbohydrate diet and control has been seen in other studies (Carter *et al.* 2006; Shai *et al.* 2008), with a loss of 4.3cm over 3 months & 3.8cm after 24 months on a low-carbohydrate diet.

5.3. Effects of Diet on Bone Biomarkers

5.3.1. Urinary Calcium

Baseline urinary Ca was 0.16mMolCa/mMol Cr. This is in line with the normal reference range of 0.16-0.50mMolCa/mMol Cr (Yamamoto *et al.* 2000). There was no significant difference in urinary calcium in the low-carbohydrate diet versus the control period. However when subjects took up the low-carbohydrate diet, after being in the control period, there was a significant increase in urinary Ca between weeks 0 and 12 but this difference then disappeared, possibly after normalization to the diet. The lack of increase in urinary Ca may be due to a lack of significant increase in protein and thus sulphur containing amino acids, which has been proven to cause an increase in urinary Ca (Reddy *et al.* 2002). Reddy *et al.* (2002) found a significant increase in urinary Ca with consumption of a low-carbohydrate diet, but protein intake was increased in that study, in comparison to the current study. Similar increases in urinary Ca in response to a low-carbohydrate diet, which has increased protein, have also been observed (Reddy *et al.* 2004; Coleman & Nickols-Richardson, 2005).

5.3.2. Serum CTx

Serum CTx levels at baseline were 0.29ng/ml. This is at the lower end of the range for serum crosslaps which is 0.112-0.738ng/ml for premenopausal females and 0.142-1.353ng/ml for postmenopausal females (serum crosslaps®, IDS). Therefore bone resorption according to crosslaps levels was quite low for this population. There were no significant differences in serum crosslaps between subjects in the control and low-carbohydrate diet periods. One would expect bone resorption to increase due to significant weight loss. Increased bone resorption as shown through increased serum CTx has been observed as a result of weight loss (Prouteau *et al.* 2006;

Hinton *et al.* 2009). Increased calcium and magnesium have been associated with decreased serum CTx (Zik *et al.* 2001; Hilary *et al.* 2002) with increased PRAL being associated with increased Ctx (Buclin *et al.* 2001). Furthermore vitamin D insufficiency has been associated with increased CTx Levels (Mezquita-Raya *et al.* 2001) in humans with reduced sodium intake also being associated with reduced CTx levels (Lin *et al.* 2003). In the present study there were significant decreases in Ca and Mg intake but increased PRAL. This occurred together with increased Vitamin D intake and decreased sodium intake. These dietary factors could have cancelled each other out in terms of affecting bone resorption causing the lack of significance in serum CTx observed. No study could be found which measured the effect of a low-carbohydrate diet on serum crosslaps in humans. In rats short-term exposure to a low-carbohydrate diet also caused no change in serum crosslaps (Bielohuby *et al.* 2010), similar to the current research albeit applied to female humans.

5.3.3. Urinary NTx

Baseline urinary NTx levels were 42.38nM BCE/mM creatinine. This level equates well with the reference range which is 5-65nM BCE/mM creatinine among premenopausal females. Values towards the higher end of this scale are expected since females in this study had an average age of 48y. Carryover effects were evident for urinary NTx therefore analysis was carried out on 12 subjects only. Urinary NTx as a marker of bone resorption was seen to significantly increase during the low-carbohydrate period of the study compared to the control period.

An increase in bone resorption during the low carbohydrate diet has been observed in other research (Coleman & Nickols-Richardson, 2005). Other studies have found no significant reduction in NTx (Reddy *et al.* 2002; Carter *et al.* 2006) with low-carbohydrate consumption.

Most studies which investigated the effect of a low-carbohydrate diet had higher protein intake. However protein intake in the present study was not changed due to the low-carbohydrate diet. Rather the ratio of carbohydrate:protein was changed. For this reason the results of several studies cannot be realistically applied to the current study, due to their significant increase in protein intake.

The differences in results observed in the two bone resorption markers (CTx and NTx) could be related to the different aspects of bone resorption that each marker reflects. It may also reflect a difference in tissue specification of the markers as described previously (Knott & Bailey 1998; Hanson *et al.* 1992). Shan *et al.* (1997) failed to show any difference in the variability between measurement of CTx and NTx using the same kits used in the present research. In contrast Rosen *et al.* (2000) found urinary NTx showed much greater variability compared to serum CTx. Since NTx was measured in urine and could be more variable in NTx (according to Rosen *et al.* 2000) this may account for the difference in results between the 2 bone resorption markers. This does not take away from the fact increased bone resorption as demonstrated through increased NTx excretion, could result in poor bone health of people adapting a low-carbohydrate diet.

5.3.4. Serum Osteocalcin

Baseline serum osteocalcin was 14.58ng/ml. This is similar to the level of osteocalcin in pre-menopausal females at 17.9ng/ml (N-mid osteocalcin®, Nordic Biosciences Diagnostics). It is suggested osteocalcin decreases between the age 20-29 and 30-49y after which there is a progressive increase (Nabipour *et al.* 2008). Since subjects in this study had an average age of 48y serum osteocalcin may not yet have started to increase. There were no significant differences in serum osteocalcin between the control and low-carbohydrate diet periods. This indicates that

the low carbohydrate diet had no effect on bone formation. Coleman & Nickols-Richardson (2005) also found no significant difference in OC between a low-carbohydrate high-protein diet and a high-carbohydrate low-fat diet. However Reddy *et al.* (2002) found a significant decrease in osteocalcin following a low-carbohydrate diet. In the present study increased bone resorption without an increase in bone formation indicates potential bone loss for females on a low-carbohydrate diet, since bone loss is not being replaced by new bone.

5.3.5. Serum BSAP

Baseline BSAP was 14.71ng/ml. This level is similar to the highest reference level for premenopausal females at 14.5ng/ml (Ostase®, Immuno Diagnostic Systems). Overall there was no significant difference between control and low-carbohydrate dietary periods in terms of effects on BSAP. However in subjects who started the diet after the control period an initial decrease was observed - this was not maintained in the second 12 weeks of the diet, a corresponding increase was recorded for those coming off the low carbohydrate diet which indicates a significant change in serum BSAP only for weeks 24 to 36 of the study. This indicated the low-carbohydrate could potentially be associated with a period of low bone formation after starting the diet initially, but this reverts to normal levels of bone formation after 12 weeks of being on the diet, so overall the effect is negligible. These results confirm the lack of effect the low-carbohydrate diet had on bone formation producing a similar result to the other bone formation marker – osteocalcin. Other studies have also found a low-carbohydrate diet had no effect on BSAP (Reddy *et al.* 2002; Carter *et al.* 2006).

5.4. Effects of Diet on Energy & Macronutrients

It has been suggested that the weight loss observed while following a low carbohydrate diet is largely associated with decreased total calorie intake and increased diet duration but not with the carbohydrate content (Bravata *et al.* 2003).

5.4.1. Energy

Energy intake at baseline was 1687 ± 376 kcal/d. This intake is less than the reported intake of 2108 kcal/d for Irish females aged 45-64 years in the SLAN 2007 survey. However the intake is slightly greater than reported intake of 1463kcal/d for females involved in the low-carbohydrate study by Can *et al.* (2010). The subjects involved in the study by Can *et al.* (2010) had a greater BMI than subjects in the present study, but less reported energy intake indicating the possibility of underreporting in the study by Can *et al.* (2010). Underreporting food consumption is more common among obese subjects (Poppitt *et al.* 1998; SLAN, 2007).

Data in the present research was assessed for validity through the application of the Goldberg equations to determine levels of mis-reporting. Ideal energy intake at the start of the study for the group was 2219kcal/d. Actual recorded energy intake was 1717kcal. This indicated under-reporting of food consumption may have been present ($P < 0.001$), however this was a weight loss study and subjects may have been trying to lose weight as a primary goal, therefore reducing energy consumption.

A significant difference in total energy consumed in the low-carbohydrate phase versus the control period was observed. Energy consumption increased in the control period by 303kcal and decreased in the low-carbohydrate period by 386kcal. The decrease in calorie intake was largely observed during the first 12 weeks of the diet, but this decrease was maintained for weeks 12-24

of the diet. Energy consumption was on a self-reported basis however, these measurements correspond with the pattern of weight loss and gain recorded during the diet and control periods, therefore indicating that changes over time may be reliable even if subjects under/over reported in food diaries.

Can *et al.* (2010) reported an energy deficit of ~220kcal in females consuming the low-carbohydrate diet. This study is consistent with others indicating that diets lower in carbohydrate content also tend to have a lower mean energy intake (<60g/d carbohydrate; mean energy intake 1446 Kcal/d) (Bravata *et al.* 2009). As energy balance was not maintained results may concur with the theory of Bilsborough & Crowe (2003) that low carbohydrate diets are hypo-energetic. In contrast to this view the energy content of low-carbohydrate diets (0-30% calories) from a USDA food survey resulted in an energy intake of 2031kcal/d, which was ~200kcal higher than the energy intake of a high carbohydrate diet (>55% calories) (Bowman & Spence, 2002). However the study by Bowman and Spence (2002) was a population study and not focussed on weight reduction, like the current study.

5.4.2. Carbohydrate & Fibre

Carbohydrate consumption reported at baseline was 195g/d. This is comparable to the carbohydrate consumption of females from the study by Can *et al.* (2010) which was 192g/d at baseline. It is substantially less than the reported intake of 258g/d amongst similar aged Irish females (SLAN, 2007). Carryover effects were evident for carbohydrate and fibre, perhaps due to subjects not wanting to increase their carbohydrate intake after being on the low-carbohydrate diet. As a result only 12 subjects were used in these results thus reducing their power.

Carbohydrate consumption decreased significantly between diet and control groups; this was observed regardless of whether subjects started the diet immediately or at week 24 of the study. The mean reported carbohydrate consumption was 27% of energy consumption which is greatly lower than the recommended 50%. Carbohydrate consumption was reduced by 108g during the low-carbohydrate phase of the trial. Therefore this diet is consistent with the definition of a low-carbohydrate diet (provided 50-150g carbohydrate/d) (Westman *et al.* 2007). This level of carbohydrate decrease is consistent with observations from other low carbohydrate studies (Reddy *et al.* 2002; Shai *et al.* 2008; Can *et al.* 2010) and while Carter *et al.* (2006) did not report carbohydrate measurements decrease in carbohydrate consumption is indicated by the presence of urinary ketones at 4 weeks into the diet.

Fibre intake was very low among study participants at 12.7 ± 4.4 g/d. This is much lower than the recommended 25-35g/d (SLAN, 2007). It is also significantly less than the 27g/d fibre intake which has been reported for similar aged Irish females (SLAN, 2007), however much less fibre was reported to be consumed among females (17.4g/d) in the North South Food Consumption Survey in 2001. The reported fibre intake in a low-carbohydrate study by Brehm *et al.* (2003) was very similar to the present study at 12.03g/d at baseline. There was no significant difference in fibre intake between control and low-carbohydrate diet periods when only 12 subjects were considered, due to the presence of carry-over. This perhaps indicates subjects replaced breads with vegetables and seeds/nuts resulting in a similar carbohydrate intake between dietary periods. However the replacement was not enough to raise fibre intake to recommended levels. Low-carbohydrate diets have been found to have reduced fibre intake with an intake of 9g/d reported when 0-30% energy derives from carbohydrate (Bowman & Spence, 2002). Brehm *et*

al. (2003) also found fibre intake significantly reduced by 12.03 to 5.27 after 3 months on a low-carbohydrate diet contrary to the present results.

5.4.3. Protein, Amino Acids, PRAL and Sulphur

Protein consumption at baseline was 68.4g/d. This is lower than the reported protein consumption of similar aged Irish females which was 93g/d (SLAN, 2007). However protein intake is similar to that reported in the North/South Food Consumption Survey (2001) at 69.8g/d in Irish females. There was no significant difference in protein intake (including essential and non-essential amino acid intake) between the control and low-carbohydrate periods of the study. Subjects were instructed to consume significantly more protein than their normal diet but as observed in the data presented this did not occur. While subjects were given information on the protein (as well as fat and carbohydrate) content of the most common foods, three one-to-one contact sessions and cooking classes during the diet period, it is possible they did not understand the instructions to increase protein in order to compensate for reduced calories from carbohydrates. Alternatively they may have been conditioned by other methods of dieting where high protein foods are often associated with fat content and weight gain; if their main aim was to lose weight they may have been reluctant to increase these.

Based on studies investigating of the effects of high protein diets, the quantity of protein consumed in the diet period of this study may point towards the term ‘moderate protein diet’ (Kerstetter *et al.* 2005; Layman *et al.* 2005; Sukumar *et al.* 2011). However, subjects observed in these studies did not have as low a total energy intake and none had as low a carbohydrate intake (carbohydrate intakes were approx 305g/d; 198g/d; 153g/d respectively). From a low-carbohydrate diet perspective the amount of protein consumed in this diet during the low-

carbohydrate period at ~73g/d is lower than the amount consumed during low-carbohydrate diets at 102g/d (Bowman & Spence, 2002). However other studies have reported similar (78.15g/d, Brehm *et al.* 2003) and lower protein intakes (53g/d, Can *et al.* (2010) to the present study, during low-carbohydrate diet periods.

Essential amino acid intakes reported for females in this research was very low. When calculated according to the recommendations of the IOM (2005), insufficient intakes of isoleucine, leucine, valine, phenylalanine, histidine, lysine and threonine were found at baseline. This is interesting since protein intake was viewed to be sufficient providing 0.89g protein/kg body weight. Therefore protein quality of subjects involved in this study was insufficient. Lack of essential amino acids could pose health risks to these subjects since they have very important functions. Phenylalanine is an important regulator of enzyme activity (Young *et al.* 2000), leucine is described as being a regulator of protein turnover with protein transcription and translation affected (Young *et al.* 2000) with methionine being involved in skeletal muscle energy generation (Reeds, 2000). It can therefore be seen that regulation of normal body functions is not possible without sufficient essential amino acid intake.

Diets which are higher in meat (and lower in carbohydrates) are found to have higher sulphur content (Marsh *et al.* 1988) and higher PRAL (Cao *et al.* 2011). There was no significant change in sulphur consumption between the low-carbohydrate diet and control periods. As described previously sulphur content was determined by calculating the amino acids consumed. If protein from meat had been increased during the diet a difference would have been expected, however as reported previously the protein consumption was not increased perhaps explaining the similarity between diet and control periods.

PRAL significantly increased during the low carbohydrate period but the difference was more significant in subjects who completed the control first rather than the low-carbohydrate diet first. This would indicate that protein sources were increasing and alkaline nutrients were decreasing in the diet. Increased PRAL could have potential effects on bone health since it has been associated with increased calcium excretion but also increased calcium absorption in the same study, hence bone biomarkers were unaffected (Cao *et al.* 2011).

5.4.4. Fat

Fat intake at 70g/d is slightly below intakes reported in SLAN (2007) of 82g for Irish females but close to that reported in the North/South Ireland Food Consumption survey at 73.1g/d. In this research fat consumption was 37% of total energy at baseline and 44% during the low carbohydrate diet, due to energy reduction, not increase in fat intake. Both these intakes are above the RDA for fat at 33% energy intake. There was no significant difference in fat consumption between the low-carbohydrate diet and control periods. This is surprising since subjects were instructed to increase consumption of fats. This was in contrast to previous studies of low carbohydrate diets where significant increases in fat consumption were reported (Bowman & Spence, 2002; Reddy *et al.* 2002; Layman *et al.* 2005; Shai *et al.* 2008; Sukumar *et al.* 2011). However Reddy *et al.* (2002) used a metabolic diet in their study and Shai *et al.* (2008) used the support of the workplace, for weigh-in's, counselling and colour-coded labelling in the work cafeteria, whereas our subjects were free living.

5.5. Effects of Diet on Minerals

5.5.1. Calcium

The average calcium consumption at baseline of 813mg/d was below the RDA for this age group and concurs with research observations of lower Ca intake in females (Cashman 2002; Lips *et al.* 2010). Levels of Ca intake in similar study participants were very similar to the present results in SLAN (2007) at 870mg/d. There was an insignificant decrease in calcium consumption observed (-167mg/d) during the low-carbohydrate diet period of the study. This decrease was significant between those who consumed the low-carbohydrate diet first (-218mg/d) compared to those who did the control first (*n*12). Decreased calcium intake is well known to be associated with reduced bone health (Cashman, 2002). Increased protein intake can improve calcium absorption (Roughead *et al.* 2003) but protein intake of subjects in the present study remained the same with decreasing Ca intake. This could increase the likelihood of increased bone resorption markers in this study. Many low-carbohydrate studies have not reported calcium intake of study participants (Carter *et al.* 2006; Coleman & Nickols-Richardson, 2005). One study reported calcium intake of a 0-30% energy intake as carbohydrate diet as 590mg/1000kcal (Brehm *et al.* 2003). When equated with the calorie content of the current study subjects consumed 481mg Ca/1000kcal which reduced with the low-carbohydrate diet. A study by Reddy *et al.* (2002) found no significant difference in calcium intake between a usual diet and a low-carbohydrate diet with calcium intake of 805-809mg/d reported. However the study included men in comparison to the current research. This level of Ca consumption amongst subjects in the current research could lead to reduced bone health in the future.

5.5.2. Sodium

Baseline sodium intake was 2257mg/d. This is a safe level of consumption since the FSAI (2005) set the advised target for sodium at 2.4g/d. Consuming a low-carbohydrate diet had no significant effect on sodium consumption, however in the subjects who were on the control and then started the low-carbohydrate diet, sodium intake decreased for the first 12 weeks while on the low-carbohydrate compared to the control period. It is possible that the decrease may be attributed to the subjects consuming less bread which has a high salt content, or due to a reduction in convenience foods, as low carbohydrate foods are not readily available and subjects often had to make their own. Since decreased sodium intake has been associated with reduced urinary calcium (Blackwood *et al.* 2001; Lin *et al.* 2003) and reduced bone resorption (Lin *et al.* 2003), bone health of subjects during that period of the diet could be enhanced. However over the full 24 weeks there was no significant difference in sodium intake between low-carbohydrate diet and control diet and this is similar to previous studies (Reddy *et al.* 2002; Sukumar *et al.* 2011).

5.5.3. Phosphorous

Subjects' phosphorous levels at 1171mg/d were high at baseline compared to RDA of 550mg from the FSAI (1999). Similar phosphorus intake of 1173mg/d has been reported among Irish females (Walton, 2011). Consuming a low-carbohydrate diet had no significant effect on phosphorus intake compared to the control diet. In the current study in the face of lower than adequate Ca intake and almost twice the RDA for phosphorus being consumed bone health could be hindered. Kemi *et al.* (2006) found when P is abundant but Ca intake is low PTH is increased, stimulating bone resorption. This could be a very real problem among Irish females.

5.5.4. Potassium

Baseline levels of potassium were below the RDA of 3.1g/d (FSAI, 1999) at 2898mg/d. Intake of potassium in the current study are below that reported in SLAN (2007) (4082mg/d) for similar aged females. Inadequate amounts of potassium could possibly be due to a high consumption of processed foods which are low in potassium (Thompson & Manore, 2010) and low intake of fruit and vegetables (New *et al.* 2000). Reduced potassium intake for subjects in the current study could lead to reduced bone health since potassium has been proven to have a protective effect on bone (Zhu *et al.* 2009). Potassium intake was significantly decreased (-416mg/d) during the low-carbohydrate diet compared to the control period. This could be expected as consumption of fruit and vegetables which had carbohydrate content may have decreased. This decrease in potassium during the low-carbohydrate diet could result in poor bone health with increased bone resorption. In addition low level of serum potassium can be common on a low-carbohydrate diet and result in serious effects on health (Advani & Taylor, 2005). The fact subjects in the current study had low potassium intakes before starting the low-carbohydrate diet is worrying in a real life context.

5.5.5. Magnesium

At baseline the level of magnesium were 254mg below DRI of 420mg/d. Mg intake in this research was very similar to that found by other research in Irish women (255mg/d) (Walton, 2011). Lack of magnesium has been associated with poor bone health (Tucker *et al.* 1999) so regardless of the low-carbohydrate diet, Irish females are not consuming a diet with enough magnesium to sustain good bone health. Consuming the low-carbohydrate diet caused a significant reduction in magnesium intake compared to the control period. This could be due to cereals and whole grains being a very rich source of magnesium. This reduction in magnesium

with consumption of a low-carbohydrate diet could further exacerbate bone health problems in Irish females.

5.6. Effect of Diet on Vitamins

5.6.1. Vitamin C

Reported baseline values at 143mg/d were well within the RDA of 65mg/d (FSAI, 1999). The level of vitamin C intake reported in this study is very similar to that found in the National Adult Nutrition Survey (NANS) at 141mg/d (Walton, 2011). Higher levels of vitamin C intake have been suggested to be associated with better bone health (Roughhead & Kunkel, 1991; Baek *et al.* 2001; Base *et al.* 2001; Morton *et al.* 2001). Hence vitamin C level in Irish females is supportive to bone health. Consuming a low-carbohydrate diet produced no significant effect on vitamin C intake. It may be expected that vitamin C could decrease during a low carbohydrate diet as many decrease consumption in vegetables; however quantities of low carbohydrate fruit and vegetables were recommended to subjects each day in the current study, which while reducing overall carbohydrates may have prevented a decrease in vitamin C. Reduced vitamin C intake as a result of consuming the low carbohydrate diet has been reported (Brehm *et al.* 2003).

5.6.2. Vitamin D

The baseline values for vitamin D provided by diet were very low at baseline with an intake of 2.34µg/d. This is less than half the 5µg/d recommendation (FSAI, 1999). This intake is even lower than reported for Irish women in the NANS study where an intake of 3.9µg/d was found (Walton, 2011). The dietary reference intake for vitamin D is under review with suggestions that the recommended intake should be increased (Cashman, 2012). This means the majority of

females are falling below the current daily recommendations and will be even more so if the recommended intake is raised. Lack of vitamin D is associated with poor bone health and can cause osteomalacia and exacerbate osteoporosis (Hollick, 2007). This is recognised as a significant health problem among Irish people. Consuming a low-carbohydrate had no significant effect on vitamin D consumption over the full study, but there were suggestions that it may slightly increase vitamin D intake. When examined in 12 week intervals consuming a low-carbohydrate diet initially significantly increased vitamin D intake for 12 weeks but this difference diminished for the last 12 weeks of the study. For subjects who started the control first and then changed to low-carbohydrate diet vitamin D intake did significantly increase over the entire 24 weeks of the research. This suggests the low-carbohydrate diet may slightly increase vitamin D intake which may benefit bone somewhat and enhance Ca absorption. Enhanced Ca absorption would be needed in the present subjects due to low dietary intake of Ca, especially during the low-carbohydrate period. However the increase in vitamin D intake with a low-carbohydrate diet is $1\mu\text{g}/\text{d}$ so is insufficient to bring vitamin D intake up to the recommended level.

5.7. Effects on other Serum and Urine Parameters Measured

5.7.1. Serum Creatinine

Serum creatinine at baseline was 0.8mg/dl. This level equates well with the reference for human serum of 0.79mg/dl (QuantichromTM DICT-500). There was no significant change in serum creatinine as a result of consuming the low-carbohydrate diet. A low-carbohydrate diet has been suggested to reduce serum creatinine in subjects with type-II diabetes (Nielsen *et al.* 2006).

5.7.2. Urinary pH

Urine pH was 6.0 at baseline. This level is very similar to urine pH reported by Welch *et al.* (2008) at pH 5.9. There were no significant differences in urine pH between the control and low-carbohydrate diet groups. Urine pH has been proven to be an indicator of dietary acid load (Welch *et al.* 2008). Therefore the lack of change in urine pH may be due to the lack of significant increase in protein and associated sulphur consumption. When a low carbohydrate diet also has increased protein intake it has been associated with decreased urinary pH (Reddy *et al.* 2002; Reddy *et al.* 2004).

5.7.3. Serum Insulin

Baseline serum insulin was 20.2 μ IU/ml. This level is more in line with someone who might run the risk of becoming a type II diabetic since a range of 0.7-25 μ IU/ml has been the suggested range for Type II diabetics (Diagnostic Automation Inc.). The average BMI of subjects in this study was 28kg/m² and even though no subjects were diagnosed type II diabetics, being overweight means they are at increased risk of developing diabetes (Hu *et al.* 2001). There were no significant differences between the control or low-carbohydrate group for serum insulin. These results are similar to those of other researchers (Foster *et al.* 2003; Veldohorst *et al.* 2009). Brahm *et al.* (2003) also found no change in serum insulin in subjects consuming a low carbohydrate diet. However in contrast to these results, Can *et al.* (2010) found reduced serum insulin upon consumption of a low carbohydrate diet. The reason for the difference in these studies is unclear although the subjects in the study by Can *et al.* (2010) had a higher BMI (30.4kg/m²) they lost less weight compared to subjects in the present research. The fact subjects

were obese in the study by Can *et al.* (2010) but overweight in the present research could help account for the difference in insulin results with a low-carbohydrate diet.

5.7.4. Serum IGF-1 & Serum IGFBP-3

Baseline serum IGF-1 and IGFBP-3 levels were 723ng/ml and 8254ng/ml. These appear to be higher than reported normal values (Ranke *et al.* 2005). While a uniform standard seems to be lacking for normal values of IGF-1, higher levels have been associated with the mean BMI of subjects in this study and in moderate alcohol drinkers (The Endogenous Hormones and Breast Cancer Collaborative Group 2010; Frystyk *et al.* 2010) as reported by many subjects in the screening form (Appendix C).

The large standard deviation in IGF-1 and IGFBP-3 values suggest considerable variations between individuals as found in other studies (Frystyk *et al.* 2010). This factor is more likely to explain our observations of high levels at baseline, a larger cohort would be necessary for accurate measurements. Alternatively the ELIZA kit used by the laboratory may not have had adequate instructions for use, it has been reported that not all kit inserts state that unknown samples are acidified and blocked with excess IGF-11 (Frystyk *et al.* 2010).

There was no significant difference in IGFBP-3, however, a trend of increase during the control and decrease in the diet period was observed.

A significant difference in IGF-1 was observed, this was due more to a decrease during the control than increases during the diet period. Significant increases were observed due to the low carbohydrate diet in other studies (Dawson-Hughes *et al.* 2004; Sukumar *et al.* 2011).

Limitations

There were several aspects which may have had the potential to affect study results. The number of subjects was low and compliance to a low carbohydrate diet for 6 months is difficult to achieve. In addition since subjects in the cohort were overweight and the low-carbohydrate diet is reported as a weight loss method they may have been more orientated towards weight loss than compliance to the macronutrient directions. It is possible that subjects may have been reluctant to regain weight lost during the low-carbohydrate period when they were directed to return to their normal dietary patterns for the control period of the intervention. Furthermore information given with regard to the low-carbohydrate diet in order to gain admission to the research may have had an influence on the subjects' normal dietary pattern when they were assigned to the control period of the intervention before partaking in the low-carbohydrate diet period. Finally dietary information for the subjects was on a self-reported basis and under/over reporting was not cross-checked using blood and urine samples. It was checked using the Goldberg equation but there was difficulty applying this due to this research resulting in significant weight loss.

Conclusion

Participants in this study managed to reduce their carbohydrate intake significantly. Consuming the low-carbohydrate diet caused a significant weight loss but this may have been due to decreased energy intake rather than increased protein intake, concurring with the theory that low carbohydrate diets are hypo-energetic. As a result of a reduced carbohydrate intake several other aspects of the diet changed. Fibre intake was reduced due to its being found substantially within carbohydrate rich foods. Some important minerals such as Ca, K and Mg were reduced, but PRAL was increased in the low-carbohydrate diet implying a risk of reduced bone health for people adopting this diet type. However other aspects which could potentially be beneficial for

bone health such as reduced Na and increased vitamin D which occurred in the low-carbohydrate diet situation. The diet therefore could have both negative and positive effects on bone health which could balance each other. There was also a significant decrease in diastolic blood pressure but this may have been due to significant weight loss rather than the low carbohydrate content.

The low-carbohydrate diet did not cause any changes in the markers of bone formation (osteocalcin and BSAP) but it did cause a significant increase in bone resorption when measured through urinary NTx. Increasing bone resorption but not formation means subjects consuming this diet are at risk of bone loss,. However weight loss could also be the reason for increased bone loss. Further work is needed to investigate if weight loss was the major contributing factor to bone loss, rather than the nutrient content of the diet.

The low-carbohydrate diet caused a significant difference in IGF-1, but no difference in IGFBP-3 compared to the control diet, implying a slightly positive effect on bone health, although this was not observed in this study through bone biomarkers.

Recommendations

The current study suggests adopting a low-carbohydrate diet may be hypo-energetic and cause changes in intake of several nutrients known to affect bone health. No other study has presented a comprehensive measurement of nutrient intake in a low-carbohydrate diet in comparison to usual dietary intake. The findings of reduced intakes of Ca, Mg, K and increased PRAL, as a result of consuming this diet needs to be further researched and high-lighted in the face of high-levels of osteopenia and osteoporosis in females. Further research to confirm low intakes of these nutrients is needed. Increases in bone resorption (Ntx) observed in this study makes it likely these changes, in addition to reduced calorie intake could cause reduced bone health over time.

Further research examining whether it was the low-carbohydrate diet or reduced energy intake which caused increased bone resorption needs to be undertaken.

Although protein intake for females in the current research was sufficient with recommendations, insufficient amounts of several essential amino acids were revealed at baseline. There is no data in Ireland on essential amino acid levels in the population. Research in the area of adequate consumption of essential amino acid intake could be explored further on a larger scale. This would be a worthwhile study since essential amino acid intake has been linked with improved immunity.

The low-carbohydrate diet caused a significant increase in serum IGF-1. Low-carbohydrate diets which adopt higher protein intake have also been associated with higher IGF-1. Although this diet did not adopt a higher protein intake the ratio of protein to carbohydrate was higher. This increase in IGF-1 could be beneficial to bone, however higher levels of IGF-1 have been associated with increased cancer risk. This is an area which warrants further research especially due to the high level of protein supplement consumption amongst athletes.

References

Adler R (2011) Osteoporosis in men: insights for the clinician. *Therapeutic Advances in Musculoskeletal Disease* **3**, 191-200.

Advani A & Taylor R (2005) Life-threatening hypokalaemia on a low-carbohydrate diet associated with previously undiagnosed hyperaldosteronism. *Diabetes and Medicine* **22**, 1605-1607.

Aronoff S, Berkowitz K, Shreiner B & Want L (2004) Glucose metabolism and regulation: Beyond insulin and glucagon. *Diabetes Spectrum* **17**, 183-190.

Atkins R (2006) *Atkins for life*. New York, USA: St Martin's Press.

Bae K, Oh K, Lee W, Lee S, Kim M, Kwon H, Rhee E, Han J, Song K, Cha B, Lee K & Kang M (2010) Association of oxidative stress with postmenopausal osteoporosis and the effects of hydrogen peroxide on osteoclast formation in human bone marrow cell cultures. *Calcified Tissue International* **87**, 226-235.

Barzel U & Massey LK (1998) Excess dietary protein can adversely affect bone. *The Journal of Nutrition* **128**, 1051-1053.

Bassey E, Littlewood J, Rothwell M & Pye D (2000) Lack of effect of supplementation with essential fatty acids on bone mineral density in healthy pre-and postmenopausal women: two randomized controlled trials of Efacalt v calcium alone. *British Journal of Nutrition* **83**, 629-635.

Bauer D, Garnero P, Bilezikian J, Greenspan S, Ensrud K, Rosen C, Palermo L & Black D (2006) Short-term changes in bone turnover markers and bone mineral density response to parathyroid hormone in postmenopausal women with osteoporosis. *Journal of Endocrinology & Metabolism* **91**, 1370-1375.

Basu S, Michaelsson K, Olofsson H, Johansson S & Melhus H (2001) Association between oxidative stress and bone mineral density. *Biochemical and Biophysiological Research Community* **228**, 275-279.

Bielohuby M, Matsuura M, Herbach N, Kienzle E, Slawik M, Hoeflich A & Bidlingmaier M (2010) Short-term exposure to low-carbohydrate, high-fat diets induces low bone mineral density and reduces bone formation in rats. *Journal of Bone and Mineral Research* **25**, 275-284.

Bilsborough S & Crowe T (2003) Low-carbohydrate diets: what are the potential short and long-term health implications? *Aisia Pacific Journal of Clinical Nutrition* **12**, 396-404.

Bish C, Michels Blanck H, Maynard M, Serdula M, Thompson N & Kettel Khan L (2007) Health-related quality of life and weight loss practices among overweight and obese US adults, 2003 behavioural risk factor surveillance system. *Medscape General Medicine* **9**, 35.

Bisschop P, Pereira Arias A, Ackermans M, Endert E, Pijl H, Kuipers F, Meijer A, Sauerwein H & Romijn J (2000) The effects of carbohydrate variation in isocaloric diets on glycogenolysis and gluconeogenesis in healthy men. *The Journal of Clinical Endocrinology & Metabolism* **85**, 1963-1967.

Blain H, Buillemin A, Blain A, Gullemin F, De Talance N, Doucet B & Jeandel C (2004) Age-related femoral bone loss in men: Evidence for hyperparathyroidism and Insulin-like growth factor-1 deficiency. *The Journals of Gerontology Series A Biological Sciences and Medical Sciences* **59**, 1285-1289.

Bonewald L (2006) Mechanosensation and Transduction in Osteocytes. *Bonekey Osteovision* **3**, Issue 10, 7-15.

Bowman SA & Spence JT (2002) A comparison of low-carbohydrate vs. High-carbohydrate diets: Energy restriction, nutrient quality and correlation to body mass index. *Journal of the American College of Nutrition* **21**, 268-274.

Bravata DM, Sanders L, Huang J, Krumholz HM, Olkin I, Gardner C & Bravata D (2003) Efficacy and safety of low carbohydrate diets: A systemic review. *Journal of the American Medical Association* **289**, 1837-1850.

Brehm BJ, Seeley RJ, Daniels SR & D'alessio DA (2003) A randomized trial comparing a very low carbohydrate diet and a calorie-restricted low fat diet on body weight and cardiovascular risk factors in healthy women. *Journal of Clinical Endocrinology and Metabolism* **88**, 1617-1623.

Brown A, Ritter C, Finch J, Morrissey J, Martin K, Murayama E, Nishii Y & Slatopolsky E (1989) The non-calcemic analogue of vitamin D, 22-Oxacalcitriol, suppresses parathyroid hormone synthesis and secretion. *The American Journal of Clinical Investigation* **84**, 728-732.

Brown JP & Josse RG (2002) 2002 clinical practice guidelines for the diagnosis and management of osteoporosis in Canada. *American Medical Association Journal* **167**, S1-S34.

Buclin T, Cosma M, Appenzeller M, Jacquet A, Decosterd L, Biollaz J & Burckhardt P (2001) Diets Acids and Alkalis influence calcium retention in bone. *Osteoporosis International* **12**, Issue 6, 493-499.

Campbell TC & Campbell TM (2006) *The China Study*. USA: Benbella books Inc.

Can AS, Uysal C & Palaoylu KE (2010) Short term effects of a low-carbohydrate diet in overweight and obese subjects with low HDL-C levels. *BMC Endocrine Disorders* **10**, 18.

Canalis E (2000) *Skeletal Growth Factors*. Philadelphia, USA: Lippincott Williams & Wilkins.

Cao J, Johnson L & Hunt J (2009) A diet high in meat protein and potential renal load increases absorption and urinary excretion of calcium, as well as serum IGF-I in postmenopausal women. *The FASEB Journal* **23**, 108.7.

Cao J, Neilsen & Forrest H (2010) Acid diet (high-meat protein) effects on calcium metabolism and bone health. *Current Opinion in Clinical Nutrition & Metabolic Care* **13**, 698-702.

Cao JJ, Johnson LK & Hunt JR (2011) A diet high in meat protein and potential renal acid load increases fractional calcium absorption and urinary calcium excretion without affecting markers of bone resorption and formation in postmenopausal women. *Journal of Nutrition* **141**, 391-397.

Carter J, Vasey F & Valeriano (2006) The effect of a low-carbohydrate diet on bone turnover. *Osteoporosis International* **17**, 1398-1403.

Cashman K (2012) Dietary reference intervals for vitamin D. *Scandinavian Journal of Clinical Laboratory Investigation Supplement* **243**, 136-43.

Cashman K (2002) Calcium intake; calcium bioavailability and bone health. *British Journal of Nutrition* **87**, S169-S177.

Cashman K (2007) Diet, nutrition and bone health. *The Journal of Nutrition* **137**, 2507S-2512S.

Cauley J, Ewing S, Taylor B, Fink H, Ensrud K, Bauer D, Barret-Conor E, Marshall L & Orwoll W (2010) Sex steroid hormones in older men: longitudinal associations with 4.5-year change in hip bone mineral density – The osteoporotic fractures in men study. *Journal of Clinical Endocrinology Metabolism* **95**, 4314-4323.

Centre for Nutrition and Food Safety (2002) Nutrition Society Medal lecture. The role of the skeleton in acid-base homeostasis. *Proceedings of the Nutritional Society* **61**, 151-164.

Chen J, Hosoda K, Hasumi K, Ogata E & Shiraki M (1996) Serum N-terminal osteocalcin is a good indicator for estimating responders to hormone replacement therapy in postmenopausal women. *Journal of Bone and Mineral Research* **11**, 1784-1792.

Chen X & Ferry R (2006) Novel actions of IGFBP-3 on intracellular signalling pathways of insulin secreting cells. *Growth Hormone IGF-1 Research* **16**, 41-48.

Clarke B (2008) Normal Bone Anatomy and Physiology. *Clinical Journal of the American Society of Nephrology* **3**, S131-S139.

Clarke D, Hsu A, Riedl K, Bella D, Schwartz S, Stevens J & Ho E (2011) Bioavailability and inter-conversion of sulforaphane and erucin in human subjects consuming broccoli sprouts or broccoli supplement in a cross-over study design. *Pharmacological Research* **64**, 456-463.

Coleman M & Nickols-Richardson S (2005) A low-carbohydrate, high protein diet induces bone mineral density and content losses at the hip in overweight pre-menopausal women. *Journal of the American Dietetic Association* **105**, 9.

Corwin R, Hartman T, Maczuga S & Graubard B (2006) Dietary saturated fat intake is inversely associated with bone density in humans: Analysis of NHANES III 1 2. *Journal of Nutrition* **136**, 159-165.

Crowe T & Cameron-Smith D (2005) Low Carbohydrate diets in Australia: prevalence and public perceptions. *Medical Journal of Australia* **182**, 594-595.

Darling A, Millward D, Torgerson D, Hewett C & Lanham-New S (2009) Dietary protein and bone health: a systematic review and meta-analysis. *American Journal of Clinical Nutrition* **90**, 1674-1692.

Das U (2000) Essential fatty acids and osteoporosis. *Nutrition* **16**, 386-390.

Dasinger M, Gleason J, Griffith J Selker H & Schaefer E (2005) Comparison of the Atkins, Ornish, Weight Watchers, and Zone diets for weight loss and heart disease risk reduction: a randomized trial. *Journal of the American Medical Association* **293**, 43-53.

Dawson-Hughes B, Harris S, Rasmussen H, Song L & Dallal G (2004) Effect of dietary protein supplements on calcium excretion in healthy older men and women. *Journal of Clinical Endocrinology and Metabolism* **89**, 1169-73.

De La Piedra C, Traba M, Cabrera C & Henriquez M (1997) New biochemical markers of bone resorption in the study of postmenopausal osteoporosis. *Clinica Chimica Acta* **265**, 225-234.

Delbridge E, Prendergast L, Pritchard J & Proietto J (2009) One-year weight maintenance after significant weight loss in healthy overweight and obese subjects: does diet composition matter? *American Journal of Clinical Nutrition* **90**, 1203-1214.

Denke M (2001) Metabolic Effects of High –Protein, Low-Carbohydrate Diets. *The American Journal of Cardiology* **88**, 59-61.

Department of Health & Children (2005) Report of the National Taskforce on Obesity: Obesity the policy challenge. *Government of Ireland: Department of Health & Children*.

Dubois-Ferriere V, Brennan T, Dayer R, Rizzoli R & Ammann P (2011) Calcitropic hormones and IGF-1 are influenced by dietary protein. *Endocrinology* **152**, 1839-1847.

Elisaf M, Milionis H & Siamopoulos K (1997) Hypomagnesemic hypokalemia and hypocalcemia: clinical and laboratory characteristics. *Mineral and Electrolyte Metabolism Journal* **23**, 105-112.

Evans C, Chughati A, Blumsohn A, Giles M & Eastell R (1997) The effect of dietary sodium metabolism in premenopausal and postmenopausal women. *European Journal of Clinical Nutrition* **51**, 394-399.

Farrow E & White K (2010) Recent advances in renal phosphate handling. *Nature Reviews Nephrology*, **6**, 207-217.

Fairweather-Tait S, Skinner J, Guile G, Spector T & MacGregor A (2011) Diet and bone mineral density study in postmenopausal women from the TwinsUK registry shows a negative association with a traditional English dietary pattern and a positive association with wine. *American Journal of Clinical Nutrition* **94**, 1371-1375.

Felsenfeld A, Machado L & Rodriguez M (1993) The relationship between serum calcitonin and calcium in the haemodialysis patient. *The Official Journal of the National Kidney Foundation* **21**, 292-299.

Kemi V, Karkkainen M & Lamberg-Allardt C (2006) High phosphorus intakes acutely and negatively affect Ca and bone metabolism in a dose-dependent manner in healthy young females. *The British Journal of Nutrition* **96**, Issue 3, 545-52.

Finkelstein J, Brockwell S, Mehta V, Lo J, Johnston J, Cauley J *et al.* (2008) Bone Mineral Density Changes during the Menopause Transition in a Multiethnic Cohort of Women. *Journal of Clinical Endocrinology & Metabolism* **93**, 861-868.

Food Safety Authority of Ireland (FSAI) (1999) *Recommended Dietary Allowances for Ireland*. Dublin: FSAI.

Food Safety Authority of Ireland (FSAI) (2005) *Salt and Health: Review of the scientific evidence and recommendations for public policy in Ireland*. Dublin: FSAI.

Food Safety Authority of Ireland (FSAI) (2007) *Recommendations for a national policy on vitamin D supplementation for infants in Ireland*. Dublin: FSAI.

Fontenot J, Allen V & Bunce G (1989) Factors influencing magnesium absorption and metabolism in Ruminants. *Journal of Animal Science* **67**, 3445-3455.

Foster G, Wadden T, Vogt R & Brewer G (1997) What is reasonable weight loss? Patients' expectations and evaluations of obesity treatment outcomes. *Journal of Consultants and Clinical Psychology* **65**, 79-85.

Foster G, Wyatt H, Hill J, McGuckin B, Brill C & Mohammed S (2003) A Randomized Trial of a Low-Carbohydrate Diet for Obesity. *The New England Journal of Medicine* **348**, 2082-2090.

Frassetto L, Todd K, Morris R & Sebastian A (1998) Estimation of net endogenous noncarbonic acid production in humans from diet potassium and protein contents. *American Journal of Clinical Nutrition* **68**, 576-83.

Freedland E (2004) Role of a critical visceral adipose tissue threshold (CVATT) in metabolic syndrome: implications for controlling dietary carbohydrates: a review. *Journal of Nutrition and Metabolism* **1**, 1-12.

Freedman M, King J & Kennedy E (2001) Popular diets: a scientific review. *Obesity Research* **9**, S1-S40.

Freudenheim J, Johnson N & Smith E (1986) Relationships between usual nutrient intake and bone-mineral content of women 35-36 years of age: longitudinal and cross-sectional analysis. *American Journal of Clinical Nutrition* **44**, 863-876.

Gannon M & Nuttall (2004) Effect of a high-protein, low-carbohydrate diet on blood glucose control in people with type 2 diabetes. *Diabetes* **53**, 2375-2382.

Gardner C, Kiazand A, Alhassan S, Kim S, Stafford R, Balise R *et al.* (2007) Comparison of the Atkins, Zone, Ornish and LEARN diets for change in weight and related risk factors among overweight premenopausal women: the A TO Z Weight Loss Study: a randomized trial. *Journal of the American Medical Association* **298**, 178.

Garnero P (2008) Biomarkers for osteoporosis management: Utility in diagnosis, fracture risk prediction and therapy monitoring. *Molecular Diagnosis & Therapy* **12**, 157-170.

Gennari L, Merlotti D, Martini G, Gonnelli S, Franci B, Campagne S, Lucani B, Dal Canto N, Valenti R, Gennari C & Nuti R (2003) Longitudinal association between sex hormone levels, bone loss, and bone turnover in elderly men. *The Journal of Clinical Endocrinology & Metabolism* **88**, 5327-5333.

Ginty F, Flynn A & Cashman K (1998) The effect of dietary sodium intake on biochemical markers of bone metabolism in young women. *The British Journal of Nutrition* **79**, 343-350.

Glendenning P (2011) Markers of bone turnover for the prediction of fracture risk and monitoring of osteoporosis treatment: a need for international reference standard. *Osteoporosis International* **22**, 391-420.

Going S, Lohman T, Houtkooper L, Metcalfe L, Flint-Wagner H, Blew R, Stanford V, Cussler E, Martin J, Teixeira P, Harris M, Milliken L, Figueroa-Galvez & Weber J (2003) Effects of exercise on bone mineral density in calcium replete postmenopausal women with and without hormone replacement therapy. *Osteoporosis International* **14**, 637-643.

Golay A, Allaz A, Morel Y, DeTonnac N, Tankova S & Reaven G (1996) Similar weight loss with low- or high- carbohydrates. *American Journal of Clinical Nutrition* **63**, 174-178.

Gray T (1989) Estrogens and the skeleton: cellular and molecular mechanisms. *Journal of Steroid Biochemistry* **34**, 285-287.

Green J, Booth C & Bunning R (2002) Impact of supplementary high calcium milk with additional magnesium on parathyroid hormone and biochemical markers of bone turnover in postmenopausal women. *Aisa Pacific Journal of Clinical Nutrition* **11**, Issue 4, 268-273.

Griel A, Kris-Etherton P, Hilpert K, Zhol G, West S & Corwin R (2007) An increase in dietary n-3 fatty acids decreases a marker of bone resorption in humans. *The Nutrition Journal*, **6**, Issue 2 [Internet] published on line 2007 January Available from < <http://www.nutritionj.com/content/6/1/2> [Accessed 11 December 2011].

Guthrie R, Ebeling P, Hopper J, Barrett-Connor E, Dennerstein L, Dudley E *et al.* (1998) A prospective study of bone loss in menopausal Australian-born women. *Osteoporosis International* **8**, 282-290.

Harrington M, Bennett T, Jakobsen J, Ovesen L, Brot C, Flynn A & Cashman KD (2004) The effect of a high-protein, high-salt diet on calcium and bone metabolism in postmenopausal and its interaction with vitamin D receptor genotype. *British Journal of Nutrition* **91**, 41-51.

Harrington J, Perry I, Lutomski J, Morgan K, McGee H, Shelley E, Watson D & Barry M (2008) SLÁN 2007: Survey of Lifestyle, Attitudes and Nutrition in Ireland. Dietary Habits of the Irish Population, Department of Health and Children. Dublin: The Stationery Office.

He Y, Davis J, Ross P & Wasnich R (1993) Declining bone loss rate variability with increasing follow-up time. *Bone and Mineral* **21**, 119-128.

Heaney R (2004) Phosphorus nutrition and the treatment of osteoporosis. *Mayo Clinic Proceedings* **79**, 91-97.

Heaney R & Creighton J (2006) Role of dietary sodium in osteoporosis. *Journal of the American College of Nutrition* **25**, S271-S276.

Heaney R & Layman D (2008) Amount and type of protein influences bone health. *American Journal of Clinical Nutrition* **87**, 1567S-1570S.

Heaney R & Rafferty K (2001) Carbonated beverages and urinary calcium excretion. *American Journal of Clinical Nutrition* **74**, 343-347.

Hinton P, Le Cheminant J, Smith B, Rector R, Donnelly J (2009) Weight loss-induced alterations in serum markers of bone turnover persist during weight maintenance in obese men and women, *Journal of the American College of Nutrition* **28**, Issue 5, 565-73

Ho S, Chan S, Yip Y, Chan C, Woo J & Sham A (2008) Change in bone mineral density and its determinants in pre- and peri-menopausal Chinese women: the Hong Kong perimenopausal women osteoporosis study, *Osteoporosis International* **19**, 1785-1796.

Holford P (2006) *The Holford Low-GL diet made easy*. London, UK: Piatkus Books Limited.

Holm K, Dan A, Wilbur J, Li S & Walker J (2002) A Longitudinal study of bone density in midlife women, *Health Care for Women International* **23**, 678-691.

Hanson D, Weis M, Bollen AM, Maslan S, Singer F & Eyre D (1992) A specific immunoassay for monitoring human bone resorption: Quantitation of type 1 collagen cross-linked N-telopeptides in urine. *Journal of Bone and Mineral Research* **7**, 1251-1258.

Hollick M (2004) Sunlight and vitamin D for bone health and prevention of autoimmune diseases, cancers and cardiovascular disease. *American Journal of Clinical Nutrition* **80**, S1678-S1688.

Hollick M (2007) Vitamin D deficiency. *New England Journal of Medicine* **357**, 266-281.

Holt S, Brand Miller J & Ptoch P (1997) An insulin index of foods: the insulin demand generated by 1000-kJ portions of common foods. *American Journal of Clinical Nutrition* **66**, 1264-1276.

Hunt J, Johnson L & Roughead F (2009) Dietary protein and calcium interact to influence calcium retention: a controlled feeding study. *American Journal of Clinical Nutrition* **89**, 1357.

Ilich J & Kerstetter J (2000) Nutrition and bone health revisited: A story beyond calcium. *Journal of the American College of Nutrition* **19**, 715-737.

Institute of Medicine (2005) Dietary reference intakes for Energy, Carbohydrate, Fiber, Fat, Fatty Acids, Cholesterol, Protein, and Amino Acids. Washington: National Academies Press.

Jensen L, Quaade F & Sorensen O (1994) Bone Loss Accompanying Voluntary Weight Loss in Obese Humans. *Journal of Bone and Mineral Research* **9**, 459-463.

Johnston S, Tjonn L, Swan P, White A, Hutchins H & Sears B (2006) Ketogenic low-carbohydrate diets have no metabolic advantage over non-ketogenic low-carbohydrate diets. *American Journal of Clinical Nutrition* **83**, 1055-1061.

Ju HS, Leung S, Brown B, Stringer M, Leigh S, Scherrer C, Shepard K, Jenkins D, Knudsen J & Cannon R (1997) Comparison of analytical performance and biological variability of three bone resorption assays. *Clinical Chemistry* **43**, Issue 9, 1570-1576.

Kaaks R, Lukanova A & Kurzers (2002) Obesity, Endogenous Hormones & Endometrial Cancer. *Cancer, Epidemiology, Biomarkers & Prevention* **11**, 1531.

Kameda T, Mano H, Yuasa T, Mori Y, Miyazawa K, Shiokawa M, Nakamaru Y, Hiroi E, Hiura K, Kameda A, Yang N, Hakeda Y & Kumegawa M (1997) Estrogen inhibits bone resorption by directly inducing apoptosis of the bone-resorbing osteoclasts. *The Journal of Experimental Medicine* **186**, 489-495.

Karlsson K, Karlsson C, Ahlborg H, Valdimarsson O & Ljunghall S (2003) The duration of exercise as regulator of bone turnover. *Calcified Tissue International* **73**, 350-355.

Kato I, Toniolo P, Zeleniuch-Jacquotte, Shore R, Koenig K, Akhmedkhanova A & Ribolic E (2000) Diet, Smoking and anthropometric indices and postmenopausal bone fractures: a prospective study. *International Journal of Epidemiology* **29**, 85-92.

Kawana K, Takahashi M, Hoshino H & Kushida K (2002) Comparison of serum and urinary C-terminal telopeptide of type 1 collagen in aging, menopause and osteoporosis. *International Journal of Clinical Chemistry* **316**, 109-115.

Kennedy E, Bowman S, Spence J, Freedman M & King J (2001) Popular diets: correlation to health, nutrition and obesity. *Journal of the American Dietetic Association* **101**, 411-420.

Kerstetter J, O'Brien K, Caseria D, Wall D & Insogna K (2005) The impact of dietary protein on calcium absorption and kinetic measures of bone turnover in women. *The Journal of Endocrinology & Metabolism* **90**, 26-31.

Kerstetter J, O'Brien K & Insogna K (2003) Low protein intake: The impact on calcium and bone homeostasis in humans. *The Journal of Nutrition* **133**, 855S-861S.

Kerstetter J (2009) Dietary protein and bone: a new approach to an old question. *American Journal of Clinical Nutrition* **90**, 1451-1452.

Knight E, Stampfer M, Hankenson S, Spirigelman D & Curhan G (2003) The impact of protein intake on renal function decline in women with normal renal function or mild renal insufficiency. *Annals of Internal Medicine* **138**, 460-467.

Kreipe R & Forbes G, (1990) Osteoporosis: a new morbidity, for dieting female adolescents. *Pediatrics* **86**, 478-80.

Kris-Etherton P, Taylor D, Yu-Poth S, Huth P, Moriarty K, Fishell V, Hargrove R, Zaho G & Etherton T (2000) Polyunsaturated fatty acids in the food chain in the United States. *American Journal of Clinical Nutrition* **71**, 179S-188S.

Ksiexopolska-Orlowska K (2010) Changes in bone mechanical strength in response to physical therapy. *Polish Archives of Internal Medicine* **120**, 368-373.

Laires M, Monteiro C, Bicho M (2004) Role of cellular magnesium in health and human disease. *Frontiers in Biosciences: A Journal and Virtual Library* **1**, 262-276.

Layman D, Evans E, Baum J, Seyler J, Erickson D & Boileau R (2005) Dietary Protein and Exercise Have Additive Effects on Body Composition during Weight Loss in Adult Women. *Journal of Nutrition* **135**, 1903-1910.

Lee A, Hodges S & Eastell R (2000) Measurement of Osteocalcin. *Annals of Clinical Biochemistry* **37**, 432-446.

Leitz G, Avenell A & Robins S (1997) Short-term effects of dietary sodium intake on bone metabolism in postmenopausal women measured using urinary deoxypyridinoline excretion. *British Journal of Nutrition* **78**, 73-82.

Lester M, Urso M, Evans R, Pierce J, Spiering B, Maresh C, Hatfield D, Kraemer W & Nindl B (2009) Influence of exercise mode and osteogenic index on bone biomarker responses during short-term physical training. *Bone* **45**, 768-776.

Lips P, Bouillon R, Van Schoor N, Van der Schueren D, Ver Schueren S, Kuchuk N, Milisen K & Boonen S (2010) Review article: Reducing fracture risk with calcium and vitamin D. *Clinical Endocrinology* **73**, 277-285.

Lin PH, Ginty F, Appel LJ, Aickin M, Bohannon A, Garner P, Barclay D & Svetkey LP (2003) The DASH diet and sodium reduction improve markers of bone turnover and calcium metabolism in adults. *Journal of Nutrition* **133**, 3130-3136.

Linn T, Santosa B, Gronemeyer D, Aygen S, Scholz N, Busch M & Bretzel R (2000) Effect of long-term dietary protein intake on glucose metabolism in humans. *Diabetologia* **43**, 1257-1265.

Lukacs J, Reame N, Kleerekoper M, Ansbacher R & Rolfes-Curl A (2003) Differential Influence of Aging and Menopause on Hip Bone Loss in Healthy Middle-aged Women. *Endocrine*

Society's annual meeting in Philadelphia. Clinical poster: Osteoporosis. Presentation Date: Friday, June 20, 2003.

Luisetto G, Zangari M, Tizian L, Nardi A, Ramazzina E, Adami S & Galuppo P (1993) Influence of aging and menopause in determining vertebral and distal forearm bone loss in adult healthy women. *Bone and Mineral* **22**, 9-25.

Malik V & Hu F (2007) Popular weight-loss diets: from evidence to practice. *National Clinical Practice of Cardiovascular Medicine* **4**, 34-41.

Maggio M, Artoni A, Lauretani F, Borghi L, Nouvenne A, Valenti G & Ceda G (2009) The impact of omega-3 fatty acids on osteoporosis. *Current Pharmaceutical Design* **15**, 4157-4164.

Mardon J, Habauzit V, Trzeciakiewicz A, Davicco M, Lebecque P, Mercier S, Tressol J, Horcajada, M, Demigne C & Coxam V (2008) Long-term intake of a high-protein diet with or without potassium citrate modulates acid-base metabolism, but not bone status in male rats. *The Journal of Nutrition* **138**, 718-724.

Marsh AG, Sanchez TV, Michelsen O, Chaffe FL & Fogal SM (1988) Vegetarian lifestyle and bone mineral density. *American Journal of Clinical Nutrition* **48**, 837-841.

Mazzuoli G, Marinucci D, D'Erasmus E, Acca M, Pisani D, Rinaldi M *et al.* (2002) Cyclical Behavior of Bone Remodeling and Bone Loss in Healthy Women after Menopause: Results of a Prospective Study. *Bone* **31**, 718-724.

McCarthy S, Robson P, Livingstone M, Kiely M, Flynn A, Cran G & Gibney M (2006) Associations between daily food intake and excess adiposity in Irish adults: towards the development of food-based dietary guidelines for reducing the prevalence of overweight and obesity. *International Journal of Obesity* **30**, 993-1002.

Metcalf MG (1988) The approach of menopause: as New Zealand study. *New Zealand Medical Journal* **101**, 103-106.

Maxwell M & Kleeman C (1962) *Clinical disorders of fluid and electrolyte metabolism*. New York: McGraw-Hill.

Mezquita-Raya P, Oz-Torres M, De Dios Luna J, Luna V, Lopez-Rodriguez F, Torres-Vela E & Escobar-Jime F (2001) Relation between Vitamin D insufficiency, Bone Density and Bone Metabolism in Healthy Postmenopausal Women. *Journal of Bone and Mineral research* **16**, Issue 8, 1408-15.

Miki H, Van Heerden J & Fitzpatrick L (1998) Regulation of production of parathyroid hormone. *Principles of Medical Biology* **10**, 191-205.

Mooney E, Farley H & Strugnell C (2009) A qualitative investigation into the opinions of adolescent females regarding their body image concerns and dieting practices in the Republic of Ireland (ROI). *Appetite* **52**, 485-491.

Morton D, Barrett-Connor E & Schneider D (2001) Vitamin C supplement use and bone mineral density in postmenopausal women. *Journal of Bone and Mineral Research* **16**, 135-140.

Nabipour I, Larijani B, Jafairi SM, Amiri M & Amiri Z (2008) Reference database of crosslaps and osetocalcin for a healthy Iranian population. *Archives of Iranian Medicine* **11**, 203-206.

National institutes of health, national heart, lung and blood institute. Obesity Education Initiative (1998). Clinical guidelines on the identification, evaluation and treatment of overweight and obesity in adults. *Obesity Research* **6**, 51S- 210S.

Neter JE, Stam BE, Kok FJ, Grobbee DE & Geleijnse JM (2003) *Hypertension* **42**, 878-884.

Nevitt M, Simon J, Stone K & Wactawski-Wende J (2005) Lack of a relation between vitamin and mineral antioxidants and bone mineral density: results from the women's health initiative. *American Journal of Clinical Nutrition* **82**, 581-588.

New S, Robins S, Campbell M, Martin J, Garton M, Bolton-Smith C, Grubb D, Lee S & Reid D (2000) Dietary influences on bone mass and bone metabolism: further evidence of a positive link between fruit and vegetable consumption and bone health? *American Journal of Clinical Nutrition* **71**, 142-51.

Nielsen H, Brixen K & Mosekilde L (1990) Diurnal rhythm and 24-hour integrated concentrations of serum osteocalcin in normals: influence of age, sex, season, and smoking habits. *Calcified Tissue International* **47**, 284-290.

Neilsen JV, Westerlund P & Bygren P (2006) A low-carbohydrate diet may prevent end-stage renal failure in type 2 diabetes. A case report. *Nutrition and Metabolism* **3**, 23.

Noakes M, Keogh J, Foster P & Clifton P (2005) Effect of an energy-restricted, high-protein, low-fat diet relative to a conventional high-carbohydrate, low-fat diet on weight loss, body composition, nutritional status, and markers of cardiovascular health in obese women. *American Journal of Clinical Nutrition* **81**, 1298-1306.

Nordmann AJ, Nordmann A, Briel M, Keller U, Yancy W, Brehm B *et al.* (2006) Effects of Low-Carbohydrate vs Low-Fat Diets on Weight Loss and Cardiovascular Risk Factors. *Archives of Internal Medicine* **166**, 285-293.

North/South Ireland Food Consumption Survey; Irish Universities Nutrition Alliance (2001) Food and nutrient intakes, antropometry, attitudinal data and physical activity patterns: Summary Report.

Nutrition Research Newsletter (May 2005) Urinary ketones and weight following a low-carbohydrate/high-protein diet. *Nutrition Research Newsletter* **24**, 11-12.

O'Dea J, Wieland R, Hallberg M, Llerena L, Zorn E & Genuth S (1979) Effects of dietary weight loss on sex steroid, binding sex steroids and gonadotropins in obese postmenopausal women. *Journal of Laboratory and Clinical Medicine* **93**, 1004-1008.

Orchard T, Caulley J, Frank G, Neuhouser M, Robinson J, Snetselaar L & Tylaysky F (2010) Fatty acid consumption and risk of fracture in the women's health initiative. *American Journal of Clinical Nutrition* **92**, 1452-1460.

Pan J, Rothman D, Behar K, Stein D & Hetherington H (2000) Human brain, hydroxybutyrate and lactate increase in fasting-induced ketosis. *Journal of Cerebral Blood Flow and Metabolism* **20**, 1502-1507.

Peterson S, Peterson M, Raymond G, Gilligan C, Checovich M & Smith E (1991) Muscular strength and bone density with weight training in middle-aged women. *Medicine and Science in Sports and Exercise* **23**, 499-504.

Poortmans J & Dellalieux O (2000) Do high protein diets have potential health risks on kidney function in Athletes? *International Journal of Sport Nutrition and Exercise Metabolism* **10**, 28-38.

Poppitt SD, Swann D, Black AE & Prentice AM (1998) Assessment of selective under-reporting of food intake by both obese and non-obese women in a metabolic facility. *Journal of the International Association for the Study of Obesity* **22**, 303-311.

Pritchard J, Nowson C & Wark J (1996) Bone loss accompanying diet-induced or exercise-induced weight loss: a randomised controlled study. *International Journal of Obesity* **20**, 513-520.

Proteau S, Benhamou L & Courteix D (2006) Relationships between serum leptin and bone markers during stable weight, weight reduction and weight regain in male and female jodoists. *European Journal of Endocrinology* **154**, 389-395.

Raisz LG (1999) Physiology and pathophysiology of bone remodelling. *Clinical Chemistry* **45**, 1353-1358.

Randolph JF, Sowers MF, Bondarenko IV, Harlow SD, Luborsky JL & Little RJ (2004) Change in estradiol and follicle-stimulating hormone across the early menopausal transition: Effects of ethnicity and age. *The Journal of Clinical Endocrinology and Metabolism* **89**, 1555-1561.

Rankin J & Turpyn A (2007) Low carbohydrate, high fat diet increases C-reactive protein during weight loss. *Journal of the American College of Nutrition* **26**, 163-169.

Reddy S, Wanh C, Sakhaee K, Brinkley L & Pak C (2002) Effect of Low-Carbohydrate High-Protein Diets on acid-base balance, stone forming propensity and calcium metabolism. *American Journal of Kidney Diseases* **40**, 265-274.

Reeds PJ (2000) Dispensable and indispensable amino acids for humans. *Journal of Nutrition* **130**, 1835S–1840S.

Remer T (2000) Influence of diet on acid–base balance. *Seminars in Dialysis* **13**, 221–226.

Ricci T, Heymsfield S, Pierson R, Stahl T, Chowdhury H & Shapses S (2001) Moderate energy restriction increases bone resorption in obese postmenopausal women. *American Journal of Clinical Nutrition* **73**, 347-352.

Riggs B (2002) Endocrine causes of age-related bone loss and osteoporosis. *Novartis Foundation Symposium* **242**, 247-259.

Rocheffort G, Pallu S & Benhamou C (2010) Osteocyte: the unrecognized side of bone tissue. *Osteoporosis International* **21**, 1457-1469.

Roduit R, Nolan C, Alarcon C, Moore P, Barbeau A, Delghingaro-Augusto V, Przybykowski E, Morin J, Masse F, Massie B, Ruderman N, Rhodes C, Poitout V & Prentkil M (2004) A role for the malonyl-CoA/Long-chain Acyl-CoA pathway of lipid signalling in the regulation of insulin secretion in response to both fuel and non-fuel stimuli. *Diabetes* **53**, 1007- 1019.

Rosen H, Moses A, Garber J, Iloputaife I, Ross D, Lee S & Greenspan (2000) Serum CTX: A new marker of bone resorption that shows treatment effect more often than other markers because of low coefficient of variability and large changes with bisphosphonate therapy. *International Osteoporosis Foundation* **66**, Issue 2, 100-103.

Roughead Z, Johnson L, Lykken G & Hunt J (2003) Controlled high meat diets do not affect calcium retention or indices of bone status in healthy postmenopausal women. *The American Society for Nutritional Sciences Journal for Nutrition* **133**, 1020-1026.

Roughead Z & Kunkel M (1991) Effect of diet on bone matrix constituents. *Journal of the American College of Nutrition* **10**, 242-246.

Sacks F, Bray G, Carey V, Ryan D, Anton S, McManus *et al.* (2009) Comparison of weight-loss diets with different compositions of fat, protein, and carbohydrates. *New England Journal of Medicine* **360**, 859-873.

Sahni S, Hannan M, Gagnon D, Blumberg J, Cupples A, Kiel D & Tucker K (2008) High vitamin C intake is associated with lower 4-year bone loss in elderly men. *The Journal of Nutrition* **138**, 1931-1938.

Salari P, Rezaie A, Larijani B & Abdollahi M (2008) A systematic review of the impact of n-3 fatty acids in bone health and osteoporosis. *Medical Science Monitor: International Journal of Experimental and Clinical Research* **14**, 37-44.

Schnirring L (2004) 'The south beach diet' *Physician and Sports Medicine* **32**, 9-11.

Schuit S, Van der Klift M, Weel A, De Laet C, Burger H, Seeman E, Hofman A, Uitterlinden A, Van Leeuwen J & Pols H (2004) Fracture incidence and association with bone mineral density in elderly men and women: the Rotterdam study. *Bone* **34**, 195-202.

Shai I, Schwarzfuchs D, Henkin Y, Shahar D, Witkow S, Greenberg I *et al.* (2008) Weight Loss with a Low-Carbohydrate, Mediterranean, or Low-Fat diet. *The New England Journal of Medicine* **359**, 229-241.

Shapiro R & Heaney R (2003) Co-dependence of calcium and phosphorus for growth and bone development under conditions of varying deficiency. *Bone* **32**, 532-540.

Shapses S & Riedt C (2006) Bone, Body Weight, and Weight Reduction: What Are the Concerns? *Journal of Nutrition* **136**, 1453-1456.

Schernhammer E, Holly J, Pollak M & Hankinson S (2005) Circulating levels of insulin-like growth factors, their binding proteins, and breast cancer risk. *Cancer Epidemiology, Biomarkers & Prevention* **14**, 699-704.

Shils M, Shike M, Ross C, Caballero B & Cousins R (2005) *Modern Nutrition in Health and Disease (10th ed)*. US: Lippincott, Williams & Wilkins.

Skov A, Toubro S, Bulow J, Krabbe K, Parving H & Astrup A (1999) Changes in renal function during weight loss induced by high vs low-protein low-fat diets in overweight subjects. *International Journal of Obesity* **23**, 1170-1177.

Smith S (2009) A look at the Low-Carbohydrate Diet. *New England Journal of Medicine* **361**, 2286-2288.

Stumvoll M, Meyer C, Kreider M, Perriello G & Gerich J (1998) Effects of glucagons on renal and hepatic glutamine gluconeogenesis in normal post-absorptive humans. *Metabolism* **147**, 1227-1232.

Sukumar D, Amboa-Sobhan H, Zurfluh R, Schlüssel Y, Stahl T, Gordon C & Shapses S (2011) Areal and volumetric bone mineral density and geometry at two levels of protein intake during caloric restriction: A randomized, controlled trial. *Journal of Bone and Mineral Research* **26**, 1339-1348.

Swenson B, Saalwachter Schulman A, Edwards M, Gross M, Hedrick T, Weltman A *et al.* (2007) The effect of a Low-Carbohydrate, High-Protein Diet on Post Laparoscopic Gastric Bypass Weight Loss: A Prospective Randomized Trial. *Journal of Surgical Research* **142**, 308-313.

Taffe JR & Dennerstein L (2002) Menstrual patterns leading to the final menstrual period. *Menopause* **9**, 32-40.

Terpos E, Dimopoulos M, Sezer O, Roodman D, Abildgaard N, Vescio R, Tosi P, Garcia-Sanz R & Davies F (2010) The use of biochemical markers of bone remodelling in multiple myeloma: a report of the international myeloma working group. *Leukemia* **24**, 1700-1712.

The Endogenous Hormones and Breast Cancer Collaborative Group. (2010) Insulin-like growth factor 1 (IGF1), IGF binding proein 3 (IGFBP£), and breast cancer risk: pooled individual data analysis of 17 prospective studies. *The Lancet Oncology* **11**, 530-543.

Thomas D, Udagawa N, Hards D, Quinn J, Moseley J, Findlay & Best J (1998) Insulin receptor expression in primary and cultured osteoclast-like cells. *Bone* **23**, 181-186.

Thompson J & Manore M (2010) *Nutrition an Applied Approach*. San Francisco: Pearson Benjamin Cummings.

Thorpe M, Mojtahedi C, Chapman-Novakofski K, McAuley E & Evans E (2008) A Positive Association of Lumbar Spine Bone Mineral Density with Dietary Protein Is Suppressed by a Negative Association with Protein Sulfur. *Journal of Nutrition* **138**, 80-85.

Thorpe & Evans (2011) Dietary protein and bone health: harmonizing conflicting theories. *Nutrition Reviews* **69**, 215-230.

Thorpe M, Mojtahedi M, Chapman-Novakofski K, McAuley E & Evans (2007) Associations of dietary protein, calcium, potential renal acid load, and bone mineral density in elderly women. *The FASEB Journal* **21**, 548.16.

Thorpe M, Mojtahedi M, Chapman-Novakofski K, McAuley E & Evans (2008) Dietary protein and sulfur from protein hold opposing effects on the intercept, but do not affect the rate of change of lumbar spine bone mineral density in postmenopausal women over 2 years. *The FASEB Journal* **22**, 883.4.

Turner C & Robling A (2003) Designing exercise regimens to increase bone strength. *Exercise Sport and Science Review* **31**, 45-50.

Turner C & Robling A (2005) Exercises for improving bone strength. *British Journal of Sports Medicine* **39**, 188-189.

Vander Wal J, McBurney M, Moellering N, Marth J & Dhurandhar N (2007) Moderate-carbohydrate low-fat versus low-carbohydrate high-fat meal replacements for weight loss. *International Journal of Food Sciences and Nutrition* **58**, 321-329.

Vasikaran S (2008) Utility of biochemical markers of bone turnover and bone mineral density in management of osteoporosis. *Critical Reviews in Clinical Laboratory Sciences* **45**, 221-258.

Walton J (Ed) (2011) National Adult Nutrition Survey, Summary Report on Food and Nutrient Intakes, physical measurements, physical activity patterns and food choice motives. *Irish Universities Nutritional Alliance (IUNA)*, March 2011.

Wardlaw B (1996) Putting body weight and osteoporosis into perspective, *American Journal of Clinical Nutrition* **63**, 43335-43365.

Welch AA, Mulligan A, Bingham SA & Khaw K (2008) Urine pH is an indicator of dietary acid-base load, fruit and vegetables and meat intakes; results from the European Prospective Investigation into Cancer and Nutrition (EPIC) – Norfolk population study. *British Journal of Nutrition* **99**, 1335-1343.

Westman E, Yancy W, Edman J, Tomlin K & Perkins C (2002) Effect of 6 month adherence to a very low carbohydrate diet program. *American Journal of Medicine* **113**, 30-36.

Westman EC, Feinman RD, Mavropoulos JC, Vernon MC, Volek JS, Wortman JA, Yancy WS & Phinney SD (2007) Low-carbohydrate nutrition and metabolism. *American Journal of Clinical Nutrition* **86**, 276-284.

WHO Scientific group (2003) Prevention and management of osteoporosis: report of a WHO scientific group, *WHO technical report series*, **921**.

Yakar S, Rosen C, Beamer W, Ackert-Bicknell C, Wu Y, Liu J, Ooi G, Setser J, Frystyk J, Boisclair Y & LeRoith D (2002) Circulating levels of IGF-1 directly regulate bone growth and density. *The Journal of Clinical Investigation* **110**, 771-781.

Yakar S, Canalis E, Sun H, Mejia W, Kawashima Y, Nassar P, Courtland H, Williams V, Bouxsein M, Rosen C & Jepsen K (2009a) Serum IGF-1 determines skeletal strength by regulating subperiosteal expansion and trait interactions. *Journal of Bone and Bone and Mineral Research* **24**, 1481-1492.

Yakar S, Rosen C, Bouxsein M, Sun H, Mejia W, Kawashima Y, Wu Y, Emerton K, Williams V, Jepsen K, Schaffler M *et al.* (2009b) Serum complexes of insulin-like growth factor-1 modulate skeletal integrity and carbohydrate metabolism. *The FASEB Journal* **23**, 709-719.

Young VR, Yu YM & Borgonha S (2000) Proteins, peptides and amino acids in enteral nutrition: overview and some research challenges. In: *Proteins, Peptides and Amino Acids in Enteral Nutrition*. Nestle' Nutrition Workshop Series Clinical & Performance Program **3**, 1-23.

Zhu K, Devine A & Prince R (2009) The effects of high potassium consumption on bone mineral density in a prospective cohort study of elderly postmenopausal women. *Osteoporosis International* **20**, Issue 2, 335-40.

Zik V, Haas T & Stepan J (2001) Acute effects in healthy women of oral calcium on the calcium - parathyroid axis and bone resorption as assessed by serum b – crosslaps. *International Osteoporosis Foundation* **68**, Issue 6, 352-357.

Appendix A: Consent form



Waterford Institute *of* Technology

INSTITIÚID TEICNEOLAÍOCHTA PHORT LÁIRGE

Consent by Subject for Participation in Research Protocol

Subject Number: _____ **Name of Volunteer:** _____

Title of Protocol:

The effect of a low-carbohydrate diet on the biomarkers of bone health in pre and postmenopausal women.

Researcher: Doreen Fitzmaurice
Phone: 086 3028743
Email: doreenfitz@hotmail.com

Supervisor: Dr. Lorna Doyle
Email: lmdoyle@wit.ie

You are being asked to participate in a research study. The researchers at Waterford Institute of Technology study the impact of dietary practices on possible disease development in an attempt to reduce further disease incidence. In order to decide whether or not you want to be part of this research study, you should understand enough about its risks and benefits to make an informed judgement. This process is known as informed consent. This consent form gives detailed information about the research study which will be discussed with you. Once you understand the study, you will be asked to sign this form if you wish to participate.

Osteoporosis and bone fragility affects one in three women and one in five men in Ireland. The incidence of osteoporosis is increasing among females. Low-carbohydrate diets continue to be a popular choice in weight loss; these diets involve increasing protein and fat intakes to maintain adequate energy levels. Increased protein intake increases urine acidity, calcium excretion, bone resorption, and ultimately may reduce bone health. Studies have demonstrated the effectiveness of low-carbohydrate diets in losing weight, but few have examined low-carbohydrate diets and

their potential effect on bone health. Due to increasing osteoporosis incidence in women, this study investigates the influence of low-carbohydrate consumption, on urine acidity, calcium excretion and bone health.

What does it involve?

Each subject will be screened to ensure they have no factors which could affect bone health and are well enough to take part in the study. Each subject will have a DEXA scan to ensure normal bone health and have FSH and oestrogen levels measured to establish hormonal status. If any risk factors are identified during the screening process a follow up letter will be sent by the researcher to the subject's G.P. informing them of the findings.

After screening each subject will complete a physical activity questionnaire (osteogenic index measurement) to assess their physical activity level, since this could have a beneficial effect on bone health. Each subject will then complete a food diary for 3 days (2 weekdays and 1 weekend day) and a Food Frequency Questionnaire (FFQ) which will be analyzed on the dietary analysis programme CompEat. While the 3 day diary is being completed each subject will collect 3 first morning urine samples for measurement of urine pH. A blood sample of 10ml (one tube) will be taken from each subject by a trained phlebotomist (doctor or nurse). This sample will be used to analyze serum creatinine (indicator of kidney function) bone formation and resorption indicators.

Subjects will be advised to continue with their normal diet for 6 months, and return each month to complete a FFQ and monitor bone health and kidney function at 8 week intervals.

or

Subjects will be advised on the low-carbohydrate diet to follow. Support for adherence to the diet will include quantities of carbohydrates in various foods, recipes, meal plans, and cooking classes to produce low carbohydrate foods that are not readily available. They will also return each month to complete a FFQ and monitor bone health and kidney function at 8 week intervals.

At the end of 6 months the subjects will give a blood sample for measurement of biomarkers of bone turnover and kidney function, complete a 3 day food diary, physical activity questionnaire, and 3 day urine sample for measurement of urinary pH. They will then be crossed over.

Subjects who have been following their normal diet will then follow the low-carbohydrate diet, and subjects who have been on the low-carbohydrate diet will revert to their normal diet for 6 months with the same reporting procedures as in the previous 6 months, in place.

At the end of 12 months the subjects will give a blood sample for measurement of biomarkers of bone turnover, complete a 3 day food diary, physical activity questionnaire, and 3 day urine sample for measurement of urinary pH.

How inconvenient will this study be to you?

Taking blood sometimes may cause bruising. Very rarely it may cause inflammation of the vein and possible infection. The doctor makes every effort to avoid these situations. You will be asked to fast overnight on occasions that you give blood samples, this entails not eating from approximately 9.00 pm the night before and delaying breakfast until after the blood sample (between ~8.00 - 9.00 a.m.) which will be taken here in Waterford Institute of Technology.

We will be glad to provide you with the results of this study including your dietary intakes. The information that we collect is only for our research and will be confidential. This information will be stored in a secure place and in any publications that arise from this research; volunteers will be identified by number codes only.

The DEXA scanner used to measure bone density emits a very small dose of radiation, about 0.01 mSv, which is about the same as the average person receives from background radiation in one day, so the potential carcinogenic effect of exposure to radiation is minimal.

A low-carbohydrate diet will initially induce rapid weight loss, this is mainly due to water loss. If carbohydrate levels go very low the body will use protein and fat for energy and a condition known as ketosis may occur. The diet in this study is designed to prevent this. The symptoms of ketosis may include tiredness or fatigue, headache, bad breath, metallic taste in the mouth, weakness, dizziness, nausea or stomach ache, sleep problems. Drinking plenty of water can prevent or ease ketosis. If any symptom occurs eat an appropriate mod/high carbohydrate food, such as a carrot or some red pepper to alleviate the symptoms.

We consider this study to involve only "minimal risk", that is we think the worst thing to happen would be minor bruising after the taking of blood.

Benefits to the volunteer

As a result of taking part in this research volunteers can get some benefits in terms of weight loss. Feedback will also be available on:

Dietary intake and advice

Bone health status

Body fat %

Hormonal status

Kidney function

Blood pressure

Your decision to take part in this study is entirely voluntary. You may leave the study at any time. If you have any questions concerning the study, you may contact Ms. Doreen Fitzmaurice at 086 3028743 who will deal with any queries you have.

Agreement to Consent

The research project and the treatment procedures associated with it have been fully explained to me. All experimental procedures have been identified and no guarantee has been given about the possible results. I have had the opportunity to ask questions concerning any and

all aspects of the project and any procedures involved. I am aware that participation is voluntary and I may withdraw my consent at any time. Agreement to consent to take part in this study adheres to the regulations of the Data Protection Act. Confidentiality of records concerning my involvement in this project will be maintained in an appropriate manner. No subject in this research will be referred to and will be assigned a code (subject number) when dealing with result presentation, in order to ensure confidentiality. When required by law, the records of this research may be reviewed by government agencies and sponsors of the research.

I, the undersigned, hereby consent to participate as a subject in the above-described project conducted at the Department of Sport and Exercise Science, Waterford Institute of Technology. I have received a copy of this consent form for my records. I understand that if I have any questions concerning this research, I can contact the researchers listed above.

After reading the entire consent form, if you have no further questions about given consent, please sign where indicated.

Researcher: _____

Signature of Subject: _____

Witness: _____

Date: _____

Time: _____

am/pm (circle)

Appendix B:

Screening Questionnaire



Waterford Institute *of* Technology
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Bone Health Screening Questionnaire

All information provided will remain confidential

Personal details

Name: _____

Address: _____

Phone: _____ Email: _____

Height: _____ Weight: _____

Age: _____

GP's name: _____

GP's address: _____

GP's number: _____

Medical History

1. Have you ever broken any bones or experienced stress fractures?

Yes No

If yes please give details.....

.....

.....

2. Have you ever been immobilized for more than two weeks?

Yes No

If yes please give details.....

Oral contraceptives **Yes No**.....

General Nutrient supplements **Yes No**.....

Soya products **Yes No**.....

Calcium supplements **Yes No**.....

8. Are you currently taking any other medication?

Yes No

If yes please give details

.....
.....
.....

9. Do you smoke?

Yes No

If YES;

How old were you when you started?

How many do you smoke per day on average over the last year?.....

If NO;

Have you ever smoked? **Yes No**

How old were you when you started?

How old were you when you stopped?.....

How many did you smoke/day on average?.....

10. Do you drink? Coffee **Yes No**

If YES: How many per day?

Tea **Yes No**

If YES: How many per day?

Coke/cola **Yes** **No**

If YES: How many per day?

11. Do you drink alcohol?

Yes **No**

If YES; How many days per week?.....

What do you drink and how much on an average day/night?

During week:

.....

At weekend:

.....

Menstruation history

12. Is there a possibility that you may be pregnant?

Yes **No**

If no: please give details.....

13. Approx what was the length of your menstrual cycle between the ages of 25 and 35 years?

.....

14. Did you have chronic irregularities at any stage during menstruation between the ages of 25 and 35 years? **Yes** **No**

If yes please give details.....

.....

.....

15. Has your menstrual cycle ever become 7 days longer or shorter than normal? **Yes No**

If yes: please state how long ago this started to occur

: If it still occurs

16. Has the length of time between your menstrual cycles ever changed by seven days?

Yes No

If yes: please state how long ago this started to occur

: If it still occurs

17. Have you ever had an interval of 60 days or more without having a period (not including pregnancy)? **Yes No**

If yes: please state how long ago this started to occur.....

: If it is still occurring.....

18. Have you been 12 months or more without having a period? **Yes No**

(Not including pregnancy)

If yes how long is it since your final menstrual period?.....

Activity and Dieting history

19. Do you or have you ever engaged in intense physical training? **Yes No**

If yes please give details

.....

20. How would you describe your level of activity between the age of 25 and 35 years?

Leisure: **very active moderate non active**

Household: **very active moderate non active**

Occupational: **very active** **moderate** **non active**

21.How would you describe your level of activity now?

Leisure: **very active** **moderate** **non active**

Household: **very active** **moderate** **non active**

Occupational: **very active** **moderate** **non active**

22.Do you use self-administered and self-monitored diets?

Yes No

If YES;

How old were you when you started?

How many times were you on a diet on average?.....

How many times have you been on such a diet over the last year?.....

If NO;

Have you ever? **Yes No**

How old were you when you started?

How old were you when you stopped?.....

How many times were you on a diet on average?.....

23.Have you been on a diet that was monitored by a health professional? **Yes No**

If yes please give details.....

.....

.....

24.Have you ever been on a Low-Carbohydrate diet? **Yes No**

If yes : approx how many times?.....

: How long ago?.....

: For how long?.....

: What were its effects?.....

.....

In accordance with the data protection act the subjects' name will appear on the screening form and on the consent form. In all other incidences the subject is allocated a code. The screening and consent forms will exit only as a single hard copy which will be confidential. The data will be destroyed seven years after the publication date of the study.

Appendix C:

Food Diary

Dietary Analysis

Record all foods eaten yesterday, beverages and the portion size of each.

	Food (Include all foods, drinks and supplements)	Amount/Portion Size (for example ½ cup, 3oz)	Symptoms (for example gas, bloating, diarrhoea, none)	Mood (for example anxious, calm, angry, sad, happy, excited, worried, bored)
Breakfast or 1 st meal Time:				
Snack Time:				
Lunch or 2 nd meal Time:				
Snack Time:				
Dinner or 3 rd meal Time:				
Snack Time:				

Appendix D:

Food Frequency Questionnaire

Question 1

Did you eat any breakfast cereals last week	Yes	NO
	Tick or highlight selection	

Question 2 (ignore if you answered NO to question 1)

Indicate which cereals most closely represent the type you ate last week and how often.

All-bran		Bran flakes		cornflakes	
Coco pops / honey smacks / crunchy nut c' flakes		Fruit'n fibre		Muesli no added sugar	
porridge		Puffed wheat / shredded wheat		Rice krispies	
Special K		Sugar puffs / frosties		weetabix	

Question 3

If you eat bread, indicate the type of bread, rolls, pitta etc. you ate last week and how much.

Slices – brown / wholemeal		brown / wholemeal large roll		brown / wholemeal small roll	
brown / wholemeal pitta		croissant		Slices white	
White large roll		White small roll		White pitta	
crumpet		Plain muffin / scone		Chapattis – without fat	
naan		Fried bread		Crisp bread / rice cakes	
oatcakes					

Question 4

How many times last week did you use butter, margarine or a low fat spread? Count each slice of bread, roll, biscuit and what you put on	0	1	2	3	4	5
	6	7	8	9	10	11
	12	13	14	15	16	17
	18	19	20	25	30	35
	40	45	50	55	60	65+

Tick or highlight selection

Question 5

Which of the following spreads do you usually eat?

Butter	Hard Margarine
Soft Margarine	Monounsaturated Marg.
Low Fat Spread	Very Low Fat Spread
	Polyunsaturated Marg.
Soya Margarine	

Tick or highlight selection

Question 6

Do you spread:

Thickly	
A Thin Scrape	

Tick or highlight selection

How many teaspoonfuls of marmalade, jam or honey did you eat last week?

0	1	2	3	4	5
6	7	8	9	10	11
12	13	14	15	16	17
18	19	20	25	30	35
40	45	50	55	60	65+

Tick or highlight selection

Question 8

If you eat pasta (noodles / spaghetti) or rice,

White rice		Brown rice		White / orange / green pasta	
Wholewheat pasta					

Question 9

If you eat potatoes / cassava / yams / plantain (include potatoes in soups.

Boiled / mashed		Jacket / boiled in skins		Mashed with spread	
roast		Croquettes / waffles			

Question 10

How often last week did you eat any of the following?

Oven chips		Retail / home-made chips		Shallow fried potatoes	
Fried plantain					

Question 11

Indicate the vegetables (fresh, frozen, tinned) which you ate last week and the number of servings. Count a portion of salad or home-made vegetable soup as a serving. Vegetables in cooked dishes e.g. stews and curries should be included as a serving of vegetable mixture.

Vegetable mixture		A portion of salad		Vegetable stir-fry mix	
Aubergine / artichoke		Beans: green, broad, runner		Broccoli / peppers	
Cabbage / cauliflower		carrots		Courgette / leeks	
Peas, fresh / frozen		mushrooms		onions	
Sprouts / parsnips / okra		Spring greens / spinach / kale		Swede / turnip / pumpkin	
Sweetcorn / sweet potato		Tomato, other than salad			

Question 12

If you ate any vegetables last week, were they fried?

Yes

NO

Tick or highlight selection

Question 13

How often do you eat Quorn, Tofu or TVP?

Daily	3 – 5 Times a week
1 – 3 Times a week	1 – 3 Times a fortnight
Fortnightly	

Tick or highlight selection

Question 14

How often did you eat beans (including baked beans), split peas, dahl or lentils last week?

Canned in water only		Canned with added salt only		Canned with added salt and added	
Canned in sauce e.g. tomato		Canned in sauce – reduced sugar		Canned in sauce – reduced sugar / reduced salt	
dried					

Question 15

If you eat any of the following vegetarian dishes, how often did you eat them last week?

beanburgers		falafel		Vegetable pie – pastry top sugar	
Lentil rissoles		Nut cutlets / roast		Vegebanger / vegeburger	
Vegetable samosa					

Question 16

Indicate the fruits (fresh, frozen, tinned) and dried fruit, e.g. raisins, you ate last week and how many portions.

Fruit salad, a bowl		Dried fruit, a small handful		Apples / Apricots	
avocado		Bananas		Small bunch grapes / plums	
Kiwi / nectarines		Melons, Mangoes		Oranges / Grapefruit	
Peaches / Pears		Pineapple / Rhubarb		Satsumas / Tangerines	
Soft fruits e.g. strawberries					

Question 17

How much milk, including soya milk and made-up powdered milk, do you have in a day? Include what is used in tea, coffee and sauces. You will be asked about milk drinks and milk puddings in another question.

	¾ Pint
½ Pint	¼ Pint
I seldom use milk	

Tick or highlight selection

Question 18

How much milk, including soya milk and made-up powdered milk, do you have in a day? Include what is used in tea, coffee and sauces. You will be asked about milk drinks and milk puddings in another question.

	Whole Milk (silver; red top)
Semi-Skimmed (red and silver striped)	Skimmed (blue and silver checked)
	Goats Milk
Soya Milk	Don't Know

Tick or highlight selection

Question 19

If you use cream, canned milks or coffee whitener, what kind did you use and how often last week? (Include what is used in cooking.)

Double cream (1 tbsp)		Single cream (1 tbsp)		Whipping cream (1 tbsp)	
Sour cream (1 tbsp)		Imitation creams (1 tbsp)		Condensed Milk, whole (1 tbsp)	
Condensed Milk, skim (1 tbsp)		Evaporated Milk (1 small tin)		Coffee whitener (per tea / coffee)	

Question 20

If you eat cheese, what kind did you eat last week and how often? A portion of hard cheese is equivalent to the size of a small match box. Include the cheese in sauces, etc.

Blue cheese (stilton)		Hard cheese (cheddar type)		Hard cheese, reduced fat	
Vegetarian cheddar		Cheshire / Caerphilly		Gouda / emmental / edam	
Brie / camembert		Fetta / mozzarella / ricotta		cream cheese (1 tbsp)	
Half fat cream cheese (1 tbsp)		cottage cheese (3 tbsp)		Plain fromage frais / quark (3 tbsp)	
Processed cheese		Soya cheese		Don't know the type	

If you eat eggs, how are they cooked and how many did you eat last week?

Boiled / Poached		Fried		scrambled	
2 egg omelette / soufflé		Egg mayonnaise filling		Scotch eggs	
Cheese and egg quiche					

Question 22

If you eat meat, indicate the types which you ate last week and the number of times you ate them. Remember to include what you ate in sandwiches.

2 bacon rashers or 1 sausage		Low fat sausage		Sausage rolls	
Beef / lamb / pork – lean + fat		Beef / lamb / pork – no fat		Chicken / turkey – with skin	
Chicken / turkey – no skin		Breaded veal / chicken		ham	
Liver / kidney etc		Luncheon meat		Meat pies / pastie	
Mince / stews		Beefburgers / corned beef		Low fat beefburgers	
pâté / liver sausage		Blackpudding			

Question 23

If you ate any meat last week, was any of it fried?	Yes	NO

Tick or highlight selection

Question 24

If you eat fish (fresh, frozen or tinned), indicate what you ate last week and how often.

Fried fish	<input type="text"/>	Fish steamed / grilled	<input type="text"/>	Fish fingers / coated fish	<input type="text"/>
Kipper/herring/mackerel/salmon	<input type="text"/>	Pilchards / sardines	<input type="text"/>	Shellfish (prawns / crab)	<input type="text"/>
trout	<input type="text"/>	Tuna in water	<input type="text"/>	Tuna in oil	<input type="text"/>
Fish pâté / fish paste	<input type="text"/>				

Question 25

Did you eat any of the following RESTAURANT or TAKE-AWAY meals last week and if so how often?

Chinese meal / prawn meal	<input type="text"/>	Chinese vegetable meal	<input type="text"/>	Meat / prawn curry meal	<input type="text"/>
Tandoori chicken meal	<input type="text"/>	Chicken tikka meal	<input type="text"/>	Vegetable curry meal	<input type="text"/>
Kebab, shish	<input type="text"/>	McDonalds / Burger King	<input type="text"/>	Home made / shop pizza	<input type="text"/>

Question 26

If you eat nuts and seeds, how many times last week did you eat the equivalent of one handful?

plain	<input type="text"/>	salted	<input type="text"/>	Dry roasted	<input type="text"/>
A mixture	<input type="text"/>				

How many times last week did you eat a level tablespoonful of nut or seed butter (e.g. peanut)?

0	1	2	3	4	5
6	7	8	9	10	11
12	13	14	15	16	17
18	19	20	25	30	35
40	45	50	55	60	65+

Tick or highlight selection

Question 28

How many times last week did you eat a packet of potato crisps or other savoury nibbles (e.g. Asian snacks)?

0	1	2	3	4	5
6	7	8	9	10	11
12	13	14	15	16	17
18	19	20	25	30	35
40	45	50	55	60	65+

Tick or highlight selection

How many times last week did you eat tinned or packet soups and sauces?

0	1	2	3	4	5
6	7	8	9	10	11
12	13	14	15	16	17
18	19	20	25	30	35
40	45	50	55	60	65+

Tick or highlight selection

If you eat any of the following, how many times last week did you eat the equivalent to 1 tablespoonful? Don't forget what you added to sandwiches.

Hummus / taramasalata

Low calorie dressing

Chocolate spread

Pickle / brown sauce / ketchup

Salad cream

Scrape of marmite

French dressing / mayonnaise

coleslaw

Question 31

If you eat biscuits and crackers, how many did you eat last week?

Cereal bars

Digestive biscuit, chocolate

Custard creams / bourbons

Wholemeal crackers

flapjack

Chocolate coated biscuits

Semi-sweet / rich tea

Jaffa cakes

Cream crackers

Digestive biscuit, plain

shortbread

Garibaldi / fig rolls

Bread sticks / water biscuits

Question 32

If you eat cakes and puddings, how many did you eat last week?

Doughnut / Danish pastry

gateau

Fruit cake / mince pie

Fruit pie or crumble	
Steamed / baked puddings	
Cream cakes	

Cheese cake	
Sponge cake	
Fancy iced cakes	

trifle	
Asian pastries	
Currant buns / teacakes	

Question 33

If you eat milk-based desserts, how many servings did you eat last week?

Custard on pudding	
Milk puddings	
Plain whole milk yoghurt	
Soya yoghurt	

Ice cream	
Plain low fat / diet yoghurt	
Fruit whole milk yoghurt	
Fromage frais, fruit	

mousse	
Fruit low fat yoghurt	
Greek yoghurt	

Question 34

Indicate which, if any, of the following confectionery you ate last week, and how many times.

Milky way / fudge / kitkat (2 bar)	
Twirl / spira	
Bounty / drifter	
Smarties / m & m's / minstrels	

Flake / maltesers / crème egg	
Double decker / kitkat (4 bar)	
Mars / snickers / twix	
Plain or milk chocolate (50g bar)	

Aero / wisper / crunchie	
Caramel / toffee crisp / lion bar	
yorkie	
toffees	

Question 35

How often did you eat a packet of sweets last week? Include mints.

Two packets a week	One packet a week

Tick or highlight selection

How many teaspoonfuls of sugar do you eat a day on cereals and in hot drinks? Do not include artificial sweeteners.

0	1	2	3	4	5
6	7	8	9	10	11
12	13	14	15	16	17
18	19	20	25	30	35
40	45	50	55	60	65+

Tick or highlight selection

Question 37

How many drinks of the following did you have last week?

Milky drinks e.g. cocoa	<input type="text"/>
Glass of milk	<input type="text"/>
Water / low calorie drinks	<input type="text"/>

Fizzy drinks (not low cal.)	<input type="text"/>
Squash / cordial	<input type="text"/>
Tea / coffee	<input type="text"/>

Fruit juice, unsweetened	<input type="text"/>
Fruit drinks	<input type="text"/>
Low cal. Hot instant drinks	<input type="text"/>

Question 38

If you drink alcoholic drinks, how many of the following did you drink last week?

Half pint of beer / larger / cider	<input type="text"/>
sherry	<input type="text"/>
Low alcohol beer	<input type="text"/>

Glass of wine	<input type="text"/>
Martini / port	<input type="text"/>
Low alcohol wine	<input type="text"/>

Single measure of spirits	<input type="text"/>
liqueurs	<input type="text"/>

Personal Details

Full Name	<input type="text"/>
Height	<input type="text"/>
Weight	<input type="text"/>
Age	<input type="text"/>
Sex	<input type="text"/>
Occupation	<input type="text"/>
Lifestyle	<input type="text"/>
country	<input type="text"/>
Nearest city	<input type="text"/>

Appendix E:

Diet Catalogue

Low-carbohydrate Diet Plan

14 days at approx 40g carbohydrate/day
(*Carbohydrate value is in brackets*)

Day 1

- Breakfast: 30g/1/3 cup uncooked porridge & 200mls/
2 cups milk (13g)
Egg in 60g/2 thick slices tomato & 20g cheese
(5g) Tea/Coffee with cream
- Lunch: Chicken & Bacon Caesar salad (3g)
& low-carb Croutons (3g)
- Dinner: Sirloin steak in garlic butter & 75g/1 cup
Broccoli (1g) 60g /1/3 cup Sweet corn (16g)
& celeriac chips (8g)
50g Mocha cream cheese (1g) & 200mls water

Day 2

- Breakfast: 30g All Bran & milk (14g)
Scrambled eggs & bacon (0g)
Tea/Coffee
- Lunch: 200mls Tomato & basil soup (7g) &
1 slice low carbohydrate bread (3g)
1 small apple (8g)
200mls water
- Dinner: 250g Moussaka & 50g/1 handful lettuce,
1 spring onion, 15g olives, 15mls dressing(10g)
140g Rhubarb & cream (2g)
200mls water

Tip : Drink a minimum of 8 x 200ml glasses of water per day—it is possible to mistake thirst for hunger

Day 3

Breakfast: 30g/1/3 cup uncooked porridge & 200mls/
2 cups milk (13g)
Baked egg, ham & cheese (1g)
Tea/Coffee

Lunch: 92g/1/2 tin tuna, 23g/4 slices cucumber,
34g/2 slices tomato, 50g/7 slices red pepper,
20g/ 5 lge slices lettuce, mayonnaise &
paprika salad (5g)
200mls water

Dinner: Baked chicken breast & Asian veg stir-fry (5g)
& 15g/3 teaspoons uncooked BrownRice (12g)
100g/4 tablespoons Mixed berry & cream (6g)
200mls water

Day 4

Breakfast: 18g cornflakes, 50mls milk (20g)
130g mushrooms, 46g ham, 25g cheese (trace)
Tea/Coffee

Lunch: 3 x chicken legs, lemon, garlic (3g),
& sml salad (3g)
200mls water

Dinner: 82g Baked salmon & red pepper mayonnaise(2g)
50g/1/3 cup Broccoli & 60g/1 cup Cauliflower
(2g) & 35g/1/4 cup canned chickpeas (6g)
50g/1 cup cantelope melon (2g)
200mls water

Tip : Put all the things you don't want to eat into a press that you are not going to use— Out of sight out of mind

Day 5

Breakfast: 44g/1/2cup Mushrooms & 50g Bacon (1g)

2 slices low carbohydrate bread (6g)

Tea/coffee

Lunch: 200mls Tomato & red pepper soup (10g)

20g herb cheese dip & 100g/3 sticks celery(3g)

200mls water

Dinner: 2x Lamb chops, 80g/1/2 cup roasted carrot&pesto

& 60g/1 small boiled potato (17g)

200mls water

Day 6

Breakfast: 30g/1/3 cup uncooked porridge & 200mls/

2 cups milk (13g)

1 fried egg & 100g Bacon

Tea/Coffee

Lunch: 200mls Cauliflower & cheese soup

1 slice low carbohydrate bread (8g)

200mls water

Dinner: 200g meatballs in spicy tomato sauce

1 x boiled potato (10g)

200mls water

Tip : Too much food in one meal? Split it and have the 2nd part as a snack later e.g. breakfast—have porridge & milk and keep the egg & bacon for mid-morning break

Day 7

Breakfast: Egg in tomato, cheese & 1 slice low carb bread (8g)

Tea/Coffee

Lunch: 46g turkey, 40g cheese, 170g tomato & mayo

Rolled in lettuce leaves (2g)

30g cream cheese dip & 80g/1 carrot sticks (6g)

200mls water

Dinner: 82g salmon in pesto, 40g spinach (4g),

90g/1 cup Roasted parsnip chips (11g)

100g/1cup Mixed berry crumble & cream (11g)

200mls water

Day 8

Breakfast: 20g cornflakes & 50mls milk (21g)

44g mushroom & 100g bacon (1g) Tea/Coffee

Lunch: 200mls chicken & sweetcorn chowder &

1 slice low carbohydrate bread (8g)

200mls water

Dinner: 170g Pork chop, 100g/1 cup sautéed leek &

60g/1/2cup green beans (5g) 1 boiled potato(10g)

200mls water

Day 9

Breakfast: 100g Bacon, 30g cream cheese,

1 slice low carbohydrate bread (3g)

Tea/coffee

Lunch: 1 small apple(10g)

92g/1/2 tin tuna & egg mayonnaise

& 1 slice low carbohydrate bread(3g)

200mls water

Dinner: 100g/1 cup Coconut chicken

& 15g/3 teaspoons uncooked Brown Rice (17g)

200mls water

Tip : Ramekin dishes are great for making single portions of food

Day 10

Breakfast: 30g All bran & 100mls milk (14g)

100g Bacon & 30g cream cheese

Tea/Coffee

Lunch: 50g/1/2 cup chicken tikka rolled in lettuce rolls(3g)

yogurt (10g)

200mls water

Dinner: Lamb chops in white wine, tomato & olive (4g)

60g/1/2cup green beans (6g) 1 potato (10g)

200mls water

Day 11

Breakfast: 30g/1/3 cup uncooked porridge & 200mls/

2 cups milk (13g)

Baked egg, 23g ham, 20g cheese (1g)

Tea/Coffee

Lunch: 200mls Tomato & basil soup & 1 slice low

Carbohydrate bread (10g) & 200mls water

Dinner: 120g Baked fish & rocket pesto (1g)

60g carrots (6g) chickpeas (16g)

200mls water

Day 12

Breakfast: 20g cornflakes & 50mls milk (21g)

Scrambled egg & 1 slice bread (3g) Tea/Coffee

Lunch: Chicken & bacon Cesar salad (3g)

Low carbohydrate croutons (3g)

200mls water

Dinner: Grilled sirloin steak & red pepper mayonnaise (1g)

60g/1/2cup green veg stir-fry (5g)

70g plain noodles (10g)

200mls water

***Tip : Muffin cases are great for cooking individual portions of bread—
less time to cook and they can then be frozen***

Day 13

Breakfast: 100g Bacon, 40g mushrooms & tomato (1g),
1 slice low carbohydrate bread (3g)
Tea/coffee

Lunch: 1 small apple (10g)
200mls Cauliflower & cheese soup
& 1 slice bread (8g)
200mls water

Dinner: 100g/1 cup chicken curry (11g)
& 15g/3 teaspoons uncooked BrownRice (12g)
200mls water

Day 14

Breakfast: 100g Bacon & egg & 30g cream cheese
Tea/Coffee

Lunch: 50g chicken tikka rolled in lettuce (3g)
1 sml orange (10g)
200mls water

Dinner: Lamb chops in white wine, tomato & olive (4g)
60g/1/2cup green beans (6g) 1 potato (10g)
100g Mixed berry crumble (11g)
200mls water

**Substitute one food for another as long as they have the same
carbohydrate content as each other**

Low Carbohydrate Breakfasts

- Tomato & Egg (5g): Cut beefsteak or large tomato into thick slices take out seed.-Beat egg, salt & pepper. -Heat a little oil in frying pan.-add the tomato to pan-pour the egg into centre.-top with cheese-cook until egg is set.(can cook in the oven if preferred)
- Mushrooms & bacon (trace): wash & slice 4 mushrooms.-heat oil in frying pan.-add mushrooms, salt, pepper & 2 bacon.-cook for 5 mins
- Scrambled/boiled/poached/fried Egg & bacon (0g)
- Mushroom, ham & cheese (1g): wash & stalk 4 mushrooms-grill on smooth side for 3 mins-turn over, put slice of ham, some worcheshire sauce and slice of cheese on each-cook for 3 mins
- Baked Egg, ham & cream (trace): heat oven 180oC-grease ramekin dish-dice ham, add to dish-add egg, cream, salt & pepper-put into roasting tin with water half way up the ramekin-cook for 15mins

Recipes

Cheese dip

Ingredients

- 30g cream cheese
- 1 garlic clove - crushed
- 1 scallion **OR** 1 diced roasted pepper
- salt & pepper

Method

1. Mix everything together to make a smooth dip

Tip : Garlic is a natural preservative therefore dressings that contain it raw can last 2/3 weeks if airtight, not opened too much and kept in the fridge

Pesto

Ingredients

- 30g basil **OR** Rocket-off stalks **OR** Sundried tomatoes
- 200ml Olive oil
- 10g pine nuts
- 10g parmesan cheese –grated
- 2 cloves of garlic

Method

1. Put all the ingredients into a tall measuring jug and blend well together, with a hand blender
2. Put into an airtight container and keep in the fridge.
(every time the container is opened and the air gets in the dressing deteriorates)

French dressing

Ingredients

- 100mls Olive oil
- 50mls Sunflower oil
- 50mls white wine vinegar
- 2 cloves of garlic
- 2teaspoons of mustard
- 2teaspoons of honey
- 10ml soy sauce

Method

1. Put all the ingredients into a tall measuring jug and blend well together, with a hand blender
2. Put into an airtight container and keep in the fridge.

***Tip : every time an airtight container is opened and the air gets into it
the food deteriorates***

Red Pepper Mayonnaise

Ingredients

- 200g Mayonnaise
- 2 red peppers
- 2 cloves garlic

Method

1. pre-heat the oven 200oC
2. Wash & cut the peppers in half to deseed them
3. Put them onto a lightly greased baking tray and put into the oven for 30mins—they will get quite black on the outside.
4. When immediately out of the oven put into a small bowl and cover with clingfilm. Leave to cool. Then take the skin off.
5. When peppers are cool but them and the mayonnaise and garlic into a measuring jug and blend well together.
6. Put in an airtight container in the fridge

Tip : Artificial sweeteners are good to help reduce sugar carbohydrates—but aspartame loses sweetness when heated in cooking

Tomato & basil Soup

(7g carbohydrate per 200mls)

Ingredients

- 15ml/1tbsp olive oil, salt & pepper
- 1 onion
- 1 clove of garlic
- 450g tinned tomatoes
- 250ml chicken/veg stock
- Handful of fresh basil

Method

1. Heat the oil, then add onions, garlic salt and pepper. sauté until soft
2. Add the tin of tomatoes and stock and bring to the boil. Lower the heat and simmer for 20minutes
3. Add the basil just before blending the soup

Tip : Avoid shop bought sauces & soups they may have carbohydrate to thicken & sugar to preserve

Chicken & Sweetcorn Chowder

Ingredients

- 1 onion fine dice, 1 clove crushed garlic
- A little olive oil, dill, stock cube, Salt & pepper
- 2 chicken breasts—cut in small dice
- 1 small tin sweetcorn
- 1 tin coconut milk—full fat

Method

1. Heat the oil in a saucepan, add the onion and garlic, lower heat to a simmer for 2 mins
2. Add the chicken pieces and dill and sautee for 5 mins.
3. Add the coconut milk & two tins of water and the stock cube
4. Bring to the boil for 2 mins then lower and simmer for 15mins
- 5.

Tip : Weigh out the ingredients then put them into a cup or mug to know quantities quickly

Chicken & Bacon Caesar salad

Ingredients

- Chicken (can be left over slices or half a breast)
- 2 x rashers
- 80g Cos lettuce
- 50g mayonnaise
- 10mls white wine vinegar
- 1 clove garlic
- 30g parmesan cheese
- 1 slice low carbohydrate bread
- 50mls olive oil

Method

1. Cut the bread into cubes and toss in oil, put on a baking tray and bake at 200oC until golden
2. Cut the bacon & chicken into bite size pieces and dry fry on a pan
3. Wash and cut the lettuce
4. Grate the cheese
5. Put the mayo, vinegar & garlic into a jug and hand blend well
6. Put all the prepared ingredients into a bowl and combine well.

Tip : You will be able to gauge portions by eye after weighing out a few times

Celeriac / Parsnip Chips

Ingredients

- 90g /1 cup Celeriac or Parsnips
- 30mls olive oil Salt & Pepper
- Paprika (or chili powder if you like it spicy)

Method

1. Pre-heat the oven to 200oC
2. Cut the veg into thin slices, put into a bowl with the oil and seasoning, mix well
3. Lay out flat on a baking tray and cook for approx 20mins or until golden and crisp.

Lamb Casserole

Ingredients

- 2 x lamb chops, salt & pepper
- 100g tomatoes - chopped
- 50g olives
- 60mls white wine

Method

1. Preheat the oven to 180oC
2. Place all the ingredients into a casserole dish and cook for 30mins

Tip : Except for spirits Alcoholic drinks contain a lot of carbohydrate—1/2 pint beer = 4.6g; 1 glass sparkling white wine = 6.4g; 1 glass port = 6g; 1 glass sherry = 3g.

Tip : Avoid shopping when you are hungry or get someone else in the house to buy foods for others that you can not eat

Moussaka

Ingredients

- 250g minced lamb
- 1 onion & 2 cloves of garlic & stock cube
- 2 teaspoons of oregano
- 2 cups/200g aubergine
- 1 tin of tomatoes
- Olive oil & salt & pepper
- 1 egg
- 300mls crème fraiche
- 100g cheddar cheese

Method

1. Cut onion into small dice, crush garlic
2. Heat oil in saucepan, add onion, garlic & oregano
3. Add lamb, salt & pepper stir well
4. Add tomatoes and stock cube, bring to the boil then lower to a simmer. Cook for 10mins
5. Cut the aubergine into thin slices.
6. Beat the egg, add the crème fraiche, mix well
7. Grate the cheese
8. Put a layer of meat sauce into a casserole dish, then a layer of aubergine. Repeat until all meat and aubergine are used up.
9. Put the egg & crème fraiche on top and top with cheese
10. Put into oven 190oC for 20mins

Tip : A hand blender is an essential tool for making soups, sauces & dips

Roasted Red Pepper Soup

Ingredients

- 2 Red Peppers
- 1 onion
- 2 cloves garlic
- 1 tin of tomatoes
- Olive oil & chicken stock cube

Method

1. Cut the peppers in half & deseed, put on a baking tray and cook dry in oven 200oC for 20mins
2. Cut the onion & garlic into small dice,
3. Heat the oil in a saucepan and add onion & garlic lower heat and cook until soft
4. Add tin of tomatoes & one tin of water & stock cube
5. Bring to the boil, then lower and simmer for 15 mins.
6. Take the skin off the peppers and add to the soup
7. Liquidize with a hand blender

Cauliflower & cheese soup

Ingredients

- 1 onion & 1 garlic clove
- 1 small cauliflower
- Olive oil & chicken stock cube
- 50g cheddar cheese

Method

1. Cut onion & garlic into small dice
2. Heat the oil and add the onion & garlic, lower heat and cook until soft
3. Cut the cauliflower into small pieces and add to the saucepan
4. Add enough water to cover all the vegetables and add the stock cube, bring to the boil, then lower.
5. Cook until everything is soft, approx 10mins
6. Grate the cheese, turn off the heat and add, stir well.
7. Liquidize with a hand blender

Tip : Soups & other foods can be frozen easily in one portion bags

Coconut Chicken

Ingredients

- 2 chicken breasts –cut into dice
- 40g/1 small onion –cut into small dice
- 1 crushed clove garlic, 1 finely chopped chili
- 40g/5 slices of red pepper
- 60g/1 cup of mangetout
- 1 tin full fat coconut milk
- 1 tbls lemon juice, 1 stock cube, handful coriander, Salt & pepper

Method

1. Heat the oil in a saucepan, add the onion & garlic sautee for 5 mins
2. Add the pepper, mangetout, chicken, coriander, salt & pepper sautee for 10mins.
3. Add the lemon juice, coconut milk, stock cube, and enough water to cover all ingredients, bring to the boil and lower to simmer for 15mins

Tip : Mixers in Alcoholic spirits usually contain high carbohydrates.

100ml lemonade = 6g: 100ml cola = 11g: 100ml tonic water = 9g:

100ml cream soda = 13g

Low-carbohydrate croutons

Ingredients

- 1 slice low carbohydrate bread (does not have to be fresh)
- 50mls oil
- 2 crushed cloves of garlic
- 30g parmesan cheese

Method

1. Cut the bread into cubes and put into a bowl
2. Add crushed garlic, oil, cheese, salt & pepper. Mix well.
3. Place in a single layer on a baking tray
4. Cook at 200oC for 20mins or until golden brown

Tip : Avoid carbohydrates in concentrated fruit drinks—

45ml Lime juice cordial = 13.4g

45ml Blackcurrant = 27g

45ml Barley Water = 8.3g

Asian Vegetable stir-fry

Ingredients

- 50g mushrooms
- 50g Bok choy
- Half a red pepper
- 20mls soya sauce
- Salt & pepper
- Sesame seed oil & sesame seeds

Method

1. Wash & cut mushrooms into slices
2. Wash the bok choy and cut large slices in half
3. Deseed the pepper and cut into slices
4. Heat the oil in a pan, add the veg and stir fry until soft
5. Add the sesame seeds & soya sauce at the end.

Lemon & garlic Chicken legs (3g)

Ingredients

- 3 x Chicken legs
- Juice of 1 lemon
- 1 onion, salt & pepper
- 2 cloves of garlic
- 50mls olive oil

Method

1. Preheat the oven 200oC
2. Crush the garlic and cut onion into rings
3. Juice the lemon
4. Place the chicken, garlic, onions, lemon & oil into a casserole dish.
(you can leave this overnight in the fridge)
5. Cook in the oven for 30mins

Tip : Pesto or flavored mayonnaises can make meat, fish, eggs or salads taste completely different, so you do not get bored

Tuna & paprika salad

Ingredients

- 92g/1/2 tin tuna - drained
- 4 slices of cucumber - diced
- Half a tomato - diced
- 5 slices of pepper - diced
- 30g mayonnaise
- 20g lettuce

Method

1. Combine all the ingredients well together

Chicken tikka lettuce rolls

Ingredients

- 1 tablespoon of tikka paste, salt & pepper
- 1 yogurt & juice of half a lemon
- Half a cucumber & pepper finely diced
- Half a breast of cooked chicken - diced
- 6 large lettuce leaves

Method

1. Combine everything except the lettuce leaves
2. Put 2 spoons of chicken mix into each lettuce leaf and roll up

Tip : Quantities of recipes can be adapted depending on how many will be eating them—either multiplied or divided

Small Salad (3g)

Ingredients

- 40g lettuce
- 1 scallion - diced
- Half a tomato - diced
- 3 slices of cucumber - diced
- 5 olives
- 15mls French dressing

Method

1. Combine everything together

Double this for a Large Salad (6g)

Remember : There is no limit on quantities of meat, fish and oil based dressings, these can be increased if you feel hungry

Meatballs in Spicy sauce

Ingredients

- 2 onions, Salt & Pepper
- 1 clove of garlic - crushed
- 1 red pepper
- 1 Tin tomatoes
- 1 teaspoon Chili powder / flakes
- 200g Minced beef
- 1 egg
- Mixed herbs & olive oil & A little flour

Methods

1. Cut the onions into small dice, Heat a little oil in a saucepan, add half the onion, all the garlic
2. Deseed the pepper, cut into small dice and add
3. Add the tomatoes, bring to the boil, then lower and simmer for 10mins
4. Beat the egg a little, put this into a bowl with the beef, other half of the onions, salt & pepper, mix well
5. Shape a tablespoon into a meatball and roll in a little flour
6. Heat a little oil and add the meatballs turn until browned
7. Put them in a casserole dish, cover with the tomato sauce. Cook at 180oC for 30 mins

Lamb Stew

(makes 860g/4 portions: 212g/1 cup =1 portion : 1 portion =12g carbohydrates)

Ingredients

- 1 cup onion, 1 clove garlic, Salt & pepper
- 1 cup carrot, 1 cup/3 sticks celery
- 2 cups/4 small potatoes
- 300g diced lamb
- Stock cube, mixed herbs, a little oil

Method

1. Dice the onions, crush the garlic
2. Heat oil in saucepan add the onions, garlic, herbs salt & pepper,
3. Then add the lamb and stir until all is browned
4. Wash peel and cut the potatoes in large cubes, add to the pot.
5. Peel the carrot and slice it and the celery, add to the pot.
6. Add enough water so that everything in the pot is covered, add the stock cube. Bring to the boil then lower to simmer for 1 hour.

Fruit Crumble

(Makes 700g—half the recipe to make less : 100g =11g carbohydrates)

Ingredients

- 50g soya flour
- 50g oats
- 50g chopped nuts
- 100g butter
- 75g/3 tablespoons of artificial sweetener granules (not aspartame)
- Cinnamon
- 400g Mixed berries

Method

1. Put the flour, oat, nuts, sugar and butter into a bowl and rub together until like fine breadcrumbs
2. Wash and peel the fruit and cut into bite size pieces
3. Place the berries into an ovenproof dish and sprinkle the crumble over the top
4. Put into a pre- heated oven 180oC for 30mins

Tip : Ice-cream is high in sugar carbohydrates—substitute it with whipped cream / yoghurt / crème fraiche

Low-Carbohydrate soya bread

(3g carbohydrate per slice)

Ingredients

- 50g/2oz/half cup full fat soya flour
- 15ml/1 tbsp baking pdr
- Pinch salt
- 4 eggs separated
- 40g/1 and half oz/3 tbsp melted butter
- 45ml/3 tbsp crème fraiche

Method

1. Sieve flour, salt and baking pdr together
2. Beat egg yolks with the butter and crème fraiche until well blended
3. Whisk the egg whites until stiff, beat 30ml/2 tbsp into the flour first then fold the remainder in gradually and gently.
4. Put into a well greased and base lined 450g/1lb loaf tin.
5. Bake in a pre-heated oven 180oC/gas 4, for 40 min
6. Turn onto a wire rack to cool.

Mocha cream cheese

Ingredients

- 50g Cream cheese
- 1 teaspoon cocoa powder
- 1 teaspoon instant coffee
- 1 tablespoon artificial sweetener granules

Method

1. Combine all the ingredients well until the coffee & cocoa are dissolved
2. Can be served with cream

***Beverages that contain zero carbohydrates = Diet Cola, Club Soda,
Water, Tea infusions without milk,
Alcoholic spirits.***

Stewed Rhubarb

Ingredients

- 140g Rhubarb
- 2 tbs artificial sweetener (not aspartame)
- 20mls water

Method

1. Wash and peel the outside layer of skin off the rhubarb
2. Cut into small slices
3. Put the rhubarb, sugar & water into a saucepan, bring to the boil, lower the heat and simmer until the fruit is soft.

Can be served with sugar free jelly or cream