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The effect of a red meat meal, in conjunction with 12 weeks resistance training, on strength, body composition and skeletal muscle inflammation of 60-75 yr old women

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EXECUTIVE SUMMARY
To combat muscle loss with age, regular strength exercise and high quality dietary proteins are lifestyle interventions that stimulate muscle growth. This study evaluated the efficacy of red meat to enhance muscle mass and strength gains in older post-menopausal women, a population group at an increased risk of frailty and with a low intake of red meat. Additional measurement of muscle gene expression, zinc status, markers of disease risk and bacterial flora are reported.

Lean red meat, when consumed three times per week, immediately after a structured strength exercise session, resulted in small improvements in leg strength at the end of the 12 week intervention. Other measures of muscle size and function were unaltered when compared to a matched treatment group consuming a vegetarian meal. No changes in muscle genes, zinc status, whilst bowel health markers and colonic bacteria were unaltered by the diet and exercise.

Background
One major complication of growing old is muscle loss and frailty. Maintaining strong and healthy muscles will provide protection against falls, immobility and assisted-care. Even in old age, muscles respond to strength exercise, increasing both strength and mass. To enhance the gains in mass, one strategy is to increase the intake of quality protein, with recent evidence suggesting that the best time to consume this protein, in an older population, is immediately post exercise. This research aimed to evaluate the impact of supervised meals, either rich in lean red meat or vegetables (low protein), on muscle strength and mass in an older post-menopausal cohort.

Study Protocol
Over 12 weeks, 19 healthy women (67.1 ± 0.96 years) undertook a structured strength training program, three times per week over 12 weeks. The participants exercised individually and were supervised (at a one on one level) by a personal trainer. All exercise was completed in the late afternoon, with the participants receiving a complementary meal. Participants were randomised to receive either a lean red meat meal (80g protein from red meat) or vegetarian meal (25 g protein). Analysis was made of muscle size, strength, composition and gene expression. Further analysis was made of buccal cell zinc, faecal microflora and markers of bowel health.

Findings
The exercise program and dietary intervention was well tolerated by all participants. Despite the marked differences in meal composition, body composition, muscle mass, muscle size (cross-sectional area and fibre-size) and genes indicative of muscular change and inflammation did not differ between treatment groups. Analysis of strength gains demonstrate greater gains in muscular strength over the last 6 weeks of the training program in subjects randomised to the red-meat meals, although overall gains (0-12weeks) were not significantly. No significant treatment responses were evident with any other measured variable. Significantly, consuming meat 3 times per week for 3 months had little impact on markers of bowel cancer risk.

Conclusion
The consumption of lean red meat in post-menopausal women had no marked impact on muscular mass, however we did see minor differences between groups in terms of consistent strength improvement over the 12 week intervention. There was a tendency for greater gains in strength in the later half of the intervention. These alterations in strength, although modest, warrant further analysis in a larger population.

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1 BACKGROUND

The effects of resistance training, in conjunction with a high protein diet, on strength and body composition in older women.

Counteracting the symptoms of sarcopenia with resistance exercise.

Inactivity is one of many important contributors to Sarcopenia-an age related loss of muscle mass and strength. Sarcopenia has been associated with an increase in the risk of falls and health care costs in addition to a decline in physical function and independence. Although it is difficult to causally determine the relative importance of a sedentary lifestyle in the development of sarcopenia, it is well understood that even short-term physical inactivity causes severe declines in muscle mass and strength, in people of all ages. However, it has also been well documented that these muscle changes can be counteracted by exercise, with the greatest benefit achieved through resistance exercise [1, 2]. Numerous studies have reported that acute resistance exercise increases myofibrillar muscle protein synthesis both in young and old adults [3, 4]. Furthermore progressive resistance training has also been shown to induce muscle hypertrophy and increase strength in the elderly and even physically frail [5-7]. For example strength losses can occur at an average rate of 12-14% per decade after the age of 50. Yet with heavy resistance training, strength gains of up to 30-40% can occur within the first 3 months in older individuals [8-13]. Therefore approximately 2-3 months of resistance training has the capacity to reverse at least 2 decade of age-related strength loss [14].

Lower limb muscle mass and CSA decrease by at least 25% between the ages of 20 and 70 years [15]. However research has demonstrated that resistance training can significantly increase both muscle volume and muscle CSA in the elderly [16]. In contrast Welle et al (1996) reported that older people demonstrated significantly lower increases in muscle CSA in response to 3 months of strength training compared to the younger group [17]. However, there has been limited research which has investigated the effect of strength training on either muscle CSA or muscle mass in older adults.

Studies have also demonstrated that the size of type II fibers is diminished with age, in contrast to type I fibers which are much less affected [18-21]. Reported reductions in type II area range from 20-50%, compared to type I fiber loss ranging from 1-25%. Despite this fiber size variability, increases in muscle fiber CSA ranging from 5-40% have been reported following resistance training in elderly men and women [21-25].

Past research clearly indicates that despite the age-related differences in old compared to young muscle, older muscle still regains the plasticity to positively respond to an eccentric contraction stimuli practised in resistance exercise. Therefore resistance training may have the capacity to reverse and potentially prevent the onset of sarcopenia in the aging population.

Sarcopenia and diet.
Another potential contributor to the development of sarcopenia is poor diet, specifically insufficient energy and protein. Previous research has indicated that low dietary intake is common among the elderly population [26, 27]. In fact, over 60% of elderly individuals fail to ingest the recommended daily allowance for energy and protein [28]. The recommended dietary allowance (RDA) for adults for protein is 0.8 grams of protein per kilogram of body weight [29]. However as a result of the age-related drastic reductions in skeletal muscle mass and protein turnover rates some research has suggested that older adults may require more protein (~1g/kg/day) than the RDA in order to maintain nitrogen equilibrium [30]. Older adults with insufficient protein intake may be at risk of reduced functional capacity, losing muscle mass and compromising their immune status [29]. Therefore nutritional interventions (i.e. increasing protein intake) are an attractive potential method for preventing or treating sarcopenia.

There is increasing evidence to suggest that protein intakes above the current RDA may be beneficial to increase weight loss, and reduce the loss of lean tissue[31, 32] . More specifically, studies have shown that diets with higher protein and reduced carbohydrates appear to increase weight loss, increase loss of body fat and reduce loss of lean body mass [32]. The CSIRO (high protein, low carbohydrate) diet has been studied most extensively. Clifton et al (2003) suggests that a high red meat (34% protein, 46% carbohydrate, 20& fat) vs a low red meat (17% protein, 64% carbohydrate and 20% fat) diet demonstrated a greater weight loss in the high red meat group (-7.6 ± -3.3 kg) compared to the low red meat group (-6.9 ± -3.5 kg) [33]. Of this weight loss, the high protein group experienced greater fat loss (-5.7 ± -4.0 vs -4.6 ± -3.7 kg) and a decreased loss of lean mass (-1.6 ± -1.9 vs -1.8 ± -1.8 kg) [33]. Farnsworth et al (2003) demonstrated that following a 12 wk high protein diet (27% of energy as protein, 44% as carbohydrate and 29% as fat) vs a standard protein diet (16% of energy as protein, 57% as carbohydrate and 27% as fat) the females in the high protein group only lost 0.1 kg of lean mass in comparison to a 1.5kg loss in the standard protein group [34]. Clifton (2006) suggests that in general that by increasing protein as a percentage of energy from 15% to 30% has an effect of increasing weight loss by approximately 3kg compared with a high carbohydrate diet with further benefits on triglyceride levels [35].

While new emerging research dictates the potential benefits of high protein diets (including weight loss and the sparing of lean body mass), the metabolic mechanism behind these findings remains relatively unknown.

The effects of a high red meat diet, in conjunction with resistance exercise.
Very few studies have combined the potential joint benefits of red meat, as an excellent source of protein (20g/100g), with resistance exercise and investigate its effects on muscle

growth and strength [36]. McLennan et al. (2003) supplemented 60-80 yr old individuals with either a high red meat (800g/wk) or low red meat (400g/wk) diet, in conjunction with a 12 wk lower limb strength training program (twice per wk). A significant increase in leg strength was detected at 6 wks in the high meat consuming group. However, no treatment-induced increases in strength or muscle mass were detected following the red meat dietary intervention and 12 wks resistance training [37].

Campbell et al (1999) weight trained overweight to moderately obese men aged 51-69 yrs for 12 wks, in conjunction with an omnivorous (meat-containing, 14-16% of total protein came from beef) or a lactoovovegetarian diet (vegetarian plus dairy and eggs). This study reported a decrease in body fat percentage, an increase in FFM in the omnivorous group, however there was no significant intervention-related increases in muscular strength [38]. Haub et al (2002) also prescribed a12 wk resistance training program for men aged 65 ±5 yrs and fed one group a 'beef containing' diet (0.6g protein$\cdot kg^{-1} \cdot d^{-1}$ from beef) and the other group a lactoovovegetarian diet (0.6g protein$\cdot kg^{-1} \cdot d^{-1}$ from textured vegetable protein (soy) products.). No treatment-related changes were detected in strength or muscle mass [39].

Thus the very limited research which has focused on the effects of red meat consumption and resistance training on muscle mass and strength have reported contrasting results. In addition, these studies had several major flaws which may have contributed to the conflicting results. Timing is a crucial factor in protein supplementation in relation to the resistance exercise bout, as demonstrated by Esmarck et al (2001) in a 12 wk resistance training investigation. This study reported significant increases in muscle and fibre CSA and muscular strength in older men if the supplement (10 g protein, 7g carbohydrate and 3 g fat) was taken immediately after resistance exercise, as opposed to no change if taken 2 hrs after exercise [40]. No published studies to date, which have investigated the effect of red meat and resistance exercise, have taken this critical issue into consideration. Secondly, despite including a large quantity of red meat in one group's diet, dietary analysis in these studies revealed that in fact there was only a very small difference in terms of protein ingestion between the high red meat group and the vegetarian or low red meat groups. Therefore no accurate comparisons can be made between groups, in terms of protein-related effects. In addition, several of the studies did not match diets for fat or energy amounts and used measurements with large degrees of error such as skinfolds.

Further valid and accurate research is clearly warranted to determine if combining chronic resistance exercise and red meat consumption has a positive effect on muscle mass and strength in elderly people; i.e. as a tool to prevent or slow the development of sarcopenia. Therefore the current study aims to investigate the effect of 12 weeks of resistance training, in conjunction with a high red meat (vs a high carbohydrate) diet, on muscle strength and body composition in women aged 60-75 years.

The effects of resistance training, in conjunction with a high protein diet, on skeletal muscle inflammation in older women.

Exercise induced muscle damage and inflammation

Strenuous exercise not only increases protein synthesis and protein turnover, but it also induces a cascade of complex events leading to localised skeletal muscle inflammation. The importance of this post resistance exercise inflammatory response is not well understood. Eccentric (muscle lengthening) contractions cause minor muscle damage. This damage is associated with overloading the contractile elements and connective tissue of the muscle fibre- that is the force requirement of the fibre exceeds the normal day-to-day function. This micro trauma is repairable, however more specifically it is a necessary response that induces several remodelling processes that lead to muscle growth. This muscle damage induced adaptation process also includes a subsequent inflammatory response. It has been traditionally suggested that this inflammatory process is critical for optimal repair and recovery [41]. There has been limited research which has focused on the molecular mechanisms that mediate the inflammatory response within skeletal muscle.

Excessive muscle damage and inflammation has also been associated with muscle soreness (DOMS-a delayed onset of muscle soreness) and a decline in performance (i.e. a decline in force development, range of movement and proprioception etc). Therefore reducing exercise induced inflammation may help to improve recovery.

Aging and inflammation

Numerous studies have reported that aged muscle demonstrates a higher expression of genes involved in stress and inflammation at rest, which in turn have been associated with declines in physical function [42], disease and even mortality [43]. Further to this, research has also suggested that the actual inflammatory process and participating inflammatory mediators are altered in the elderly [44], thereby potentially altering recovery. A large range of various factors is likely to contribute to increased chronic inflammation in the elderly including, decreased production of sex steroids, subclinical disorders such as atherosclerosis and higher relative/absolute degree of adipose tissue [43, 45, 46].

Cytokines

The main mediators of the skeletal muscle inflammatory response are a family of small glycoproteins called Cytokines that serve as chemical messengers between cells [47]. There has been very limited research surrounding cytokines within skeletal muscle- i.e. the sources, actions of exercise-induced cytokines and their clinical significance. Cytokines are further divided into several different families, which include the interleukins (IL), tumor necrosis factors (TNFs), interferons, growth factors, colony stimulating factors (CSFs), cell adhesion molecules (CAMs) and chemokines [48]. Cytokines can be further characterised by their role-

i.e. as either pro- or anti- inflammatory. However the specific roles of many individual cytokines in the inflammatory response, has yet to be well established.

Chemokines

The attraction of leukocytes (white blood cells) to the injured tissue is controlled by a family of chemotactic cytokines called chemokines [49]. This family includes 8-10kd proteins of 70-90 amino acids that possess chemo-attractant properties and regulate cell traffic throughout the inflammatory process [50]. Over 40 chemokines have been identified to date. There are at least four families of chemokines (C, CC, CXC, CX3C) that are characterised by the position of one or two cysteine residues near the NH_2-terminal end [49, 50]. The α- and β-chemokines, which contain four cysteines, are the largest familiies. In the α –chemokines, one amino acid separates the first two cysteine residues (cysteine-X amino acid-cysteine, or CXC), in comparison the β-chemokines have two adjacent cysteine residues (cysteine-cysteine, or CC). Chemokines of the CC family are mainly involved in the migration and activation of monocytes, macrophages and lymphocytes and are of particular interest to this project.

The main stimuli for chemokine production are early pro-inflammatory cytokines such as IL-1, TNFα, interferon-γ and IL-4. Chemokines are secreted at sites of inflammation by resident tissue cells, resident and recruited leukocytes and cytokine-activated endothelial cells [49]. A concentration gradient of chemokines is then established surrounding the injured site [49]. Chemokines attract the surrounding leukocytes which are then activated by local pro-inflammatory cytokines to begin the removal of cellular debris [49] (See Figure 3.1).

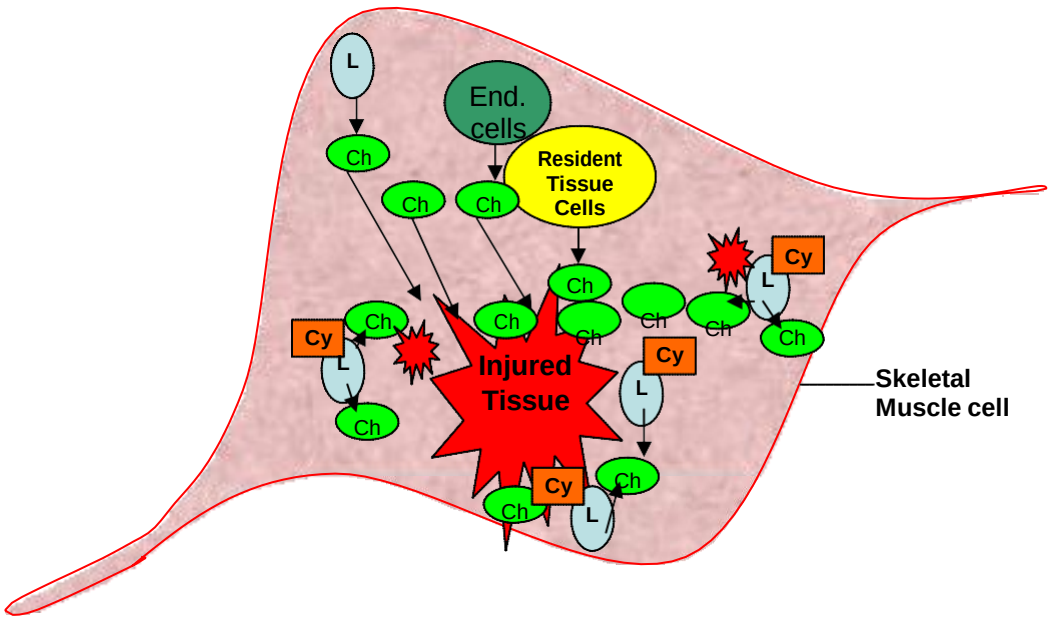

Figure 3.1: The Acute Phase Inflammatory Response.

Immediately following tissue injury, chemokines (Ch) (which are locally retained on the cell surface and cell matrix) are made by the skeletal muscle cell and are secreted by resident tissue cells, leukocytes and cytokine-activated endothelial cells. The chemokines then establish a concentration gradient around the injured tissue. Leukocytes (L) are drawn to the chemokines. The recruited leukocytes are then activated by cytokines (Cy) to clean up the injured area (i.e. dispose of cellular debris) and begin the remodelling process [49].

Exercise induced Cytokine release

Several studies have been conducted which have examined the effect of strenuous exercise on circulating levels of cytokines in plasma (in humans and rodents), however there has been very minimal research in human skeletal muscle. Numerous cytokines which play important roles in the inflammatory response have been shown to be elevated following strenuous exercise, such as; TNFα, IL-1, IL-4, IL-6, IL-8, IL-10, CCL2, CCL4 [48, 51-57].

Aging and the exercise-induced cytokine response

Previous research has suggested that aged muscle displays many stress related symptoms compared to young muscle (i.e. increased levels of inflammatory mediators). If the acute phase inflammatory response, induced by intense exercise, does stimulate the early stages of muscle remodelling, aging muscle that is chronically 'stressed' (i.e. displaying low grade chronic inflammation) may not have the capacity to respond appropriately to induce increases in muscle protein synthesis in order for hypertrophy to occur [58]. Limited research has shown that the elderly display a blunted response to eccentric exercise, in terms of turning on the genes which are involved in the early inflammatory and muscle remodelling processes [58-

61]. However it is not well understood why older muscle demonstrates different inflammatory responses at rest, and in response to exercise, compared to young muscle.

Chemokines role in exercise induced inflammation

Despite numerous studies demonstrating that freeze injury, cardiotoxin injection and many diseases (i.e. atherosclerosis) induce drastic increases in chemokine levels (i.e. 80-fold), few studies to date have examined the effect of exercise on the chemokine response. The only investigation to focus on the effect of exercise on CCL2 (i.e. MCP-1-a major inflammatory mediator) was conducted by Chen and colleagues (2003), who demonstrated a 24-fold increase in CCL2 in human skeletal muscle following a bout of strenuous eccentric exercise. Aoi et al (2005) demonstrated an increase in CCL2 in skeletal muscle at 24 hours after 1 hour of treadmill running in rats.

Ostrowski et al (2001) reported a 3.5- and 4.1- fold increase in human plasma CCL3 and CCL4 levels, respectively, 30 minutes following a marathon run. In contrast, Risoy et al (2003) found no increase in CCL4 plasma concentration following 60 minutes of leg strength exercises. Clearly, the mechanisms surrounding the exercise induced chemokine response in skeletal muscle is largely unknown and requires further investigation.

The effect of supplementation on the exercise induced inflammatory response.

Several studies have demonstrated that a carbohydrate supplement is able to attenuate the exercise induced rise in several cytokines in skeletal muscle, however other studies have demonstrated contrasting results. There has been very limited research into the effect of protein supplementation on the exercise-induced inflammatory response. Flakoll et al (2003) supplemented soldiers with protein (10 g protein, 8 g carbohydrate and 3 g lipid) during a 54 day training camp which consisted of hiking, running push up, sit ups etc. Those in the protein group had 33% fewer medical visits, 28% fewer bacterial/viral infections, 37% fewer visits due to muscle/joint problems and 83% fewer visits due to heat exhaustion [62]. Most importantly, Flakoll et al (2005) also reported that muscle soreness was also reduced via protein supplementation. This indirectly infers that protein had an effect on the skeletal muscle inflammatory response.

Furthermore, a study conducted by Saunders and colleagues (2004) supplemented trained cyclists with a carbohydrate + protein beverage (7.3% carbohydrate, 1.8% whey protein) or a carbohydrate beverage (7.3% carbohydrate). The cyclists completed 2 rides to exhaustion (at 75% then 85% VO2 max) 12-15 hrs apart. The group taking the carbohydrate + protein drink rode 29% longer on the first ride and 40% longer on the second ride compared to the carbohydrate group. More importantly, the creatine phosphokinase (CPK) (a common indicator of muscle damage) levels measured 12-15 hours after the ride were significantly reduced 83% in the carbohydrate + protein group compared to the carbohydrate group [63]. This indicates that the protein in the supplemental beverage decreased muscle damage

which infers that muscle inflammation was also reduced. This further confirms the regulatory or blunting role that protein has on the exercise-induced inflammatory response.

Clearly more research is required to truly understand the mechanisms that govern these altered supplement- and exercise -induced inflammatory processes. However these results do lend to potential therapeutic interventions for the diseased, the elderly or athletes that suffer from inflammatory symptoms.

The effects of a high red meat diet on bowel health.

Past research has suggested that high intakes of red meat may be associated with increased risks of colorectal cancer (CRC)[64-66], however more research is necessary to understand the potential changes in faecal markers. A number of faecal markers related to protein and carbohydrate metabolism and believed to be relevant to CRC risk were assessed[67-69]. These included by-products of protein fermentation (phenol, cresol and ammonia) that can accumulate in the colon and may be potentially harmful. Other considerations in this research should include faecal short chain fatty acids (SCFA)-acetate, propionate and butyrate, faecal pH, undigested fibre residues (resistant starch and non-starch polysaccharides) and faecal output.

Feacal lactoferrin- luminal marker of inflammation: It is now well established that the process of inflammation is involved in the process of carcinogenesis [70-72].Therefore the exposure of the colonic epithelium to carcinogens, oxidative damage and other damaging dietary factors may play an important role in the initiation and progression of carcinogenesis in the colon. During the process of inflammation, leukocytes infiltrate the intestinal mucosa and increase the level of fecal lactoferrin. Fecal lactoferrin, therefore, can be used as a specific marker for inflammation in the colon. Furthermore, as lactoferrin can be measured in fecal samples - there is no need to take invasive rectal biopsy specimens to assess inflammation.

Aims

The specific aims of this research project are to;

1. *Investigate the effects of a high red meat diet, in conjunction with resistance training on muscular strength in women aged 60-75 yrs.*

2. *Investigate the effects of a high red meat diet, in conjunction with resistance training on body composition (i.e. muscle mass, fat mass, muscle CSA, fiber CSA and fiber type) in women aged 60-75 yrs.*

3. *Investigate the effects of a single eccentric exercise bout and a post exercise red meat meal on the expression of several skeletal muscle inflammatory genes in women aged 60-75 yrs.*

4. *Investigate the effects of 12 weeks of resistance training, in conjunction with a high red meat diet, on several skeletal muscle inflammatory genes in women aged 60-75 yrs.*

5. *Analysis of the changes in buccal and plasma zinc (in collaboration with Asoc. Prof. Leigh Ackland, School of Science and Technology, Deakin University).*

6. *Analysis of the faecal microflora to determine whether either diet impact on the population density of known beneficial (pro-biotic) or detrimental bacterial species (in collaboration with Dr Stuart Smith, School of Exercise and Nutrition Sciences, Deakin University).*

7. *Measurement of faecal biomarkers of bowel health and colonic cancer risk (in collaboration with Dr Jane Muir, Department of Gastroenterology, Box Hill Hospital).*

3 METHODOLOGY

Study design

Twenty healthy untrained female volunteers aged 60-75 yrs were recruited for the present study (Refer to Table 1 for Subject Characteristics). Informed written consent was obtained from each subject after the nature, purpose and risks of the study were explained. A Deakin University medical history questionnaire was used to exclude subjects at risk of heart disease or subjects who were on any medications that may confound the results of the study (i.e. anti-inflammatory drugs). Exclusion criteria included resistance training within the past six months, medications, or previous history of a diagnosed condition or illness that would endanger the subjects during strenuous resistance exercise. All experimental procedures involved in this study were formally approved by the Deakin University Human Research Ethics Committee. All participants passed a medical screening at Mckinnon Sports Medicine Centre prior to beginning the study. This study was funded by Meat and Livestock Australia (MLA).

Table 1 Subject Characteristics

	Red Meat Group	Carbohydrate Group
Age (yrs)	67.5 ± 1.65	66.7 ± 1.07
Weight (kg)	75.29 ± 4.05	70.23 ± 4.27
Height (cm)	163.3 ± 1.88	161.9 ± 0.91
BMI ($kg \cdot m^{-2}$)	28.23 ± 1.45	26.75 ± 1.52
Estimated 1RM leg press (kg)	113.89 ± 7.39	117.32 ± 9.38
Estimated 1RM leg extension (kg)	30.50 ± 1.68	29.27 ± 2.37

Values are expressed as means ± SEM.

Familiarisation

Approximately one week prior to beginning the study, subjects attended Deakin University for a leg strength familiarisation session. Subjects completed 2 sets of 8 repetitions on the Cybex NORM Dynamometer (Cybex International Inc. UK) involving concentric and eccentric contractions of the knee extensors at a set speed of 60°/sec at approximately 50% maximum effort.

Acute Experimental Design

For the 24 h preceding the trial, subjects were instructed to abstain from alcohol, caffeine, tobacco and additional exercise. Subjects reported to the Deakin University exercise physiology laboratory, following an overnight fast. After resting for 10 minutes a 5ml blood sample and muscle biopsy were collected. The *vastus lateralis* muscle of the dominant leg was sampled by percutaneous needle biopsy technique, modified to include suction [73, 74]. Subjects then completed 3 sets of 8 repetitions at maximum effort on the Cybex NORM Dynamometer (Cybex International Inc. UK). Concentric and eccentric contractions of the knee extensors at a set speed of 60°/sec was performed to determine maximal knee extensor strength (Peak Torque, Nm). Subjects were randomly allocated to a protein or carbohydrate

group. Immediately following exercise subjects consumed either a high protein or high carbohydrate meal and were instructed to remain in the laboratory for a further 2 hours. After 2 hrs rest an additional biopsy was taken from the right leg. Excised muscle tissue from each biopsy was immediately frozen and stored in liquid nitrogen for subsequent analysis. A small amount of muscle tissue was preserved using tissue tek and isopropanol, then frozen in liquid nitrogen for subsequent fibretyping and CSA analysis.

Diet

The majority of protein intake in the protein group consisted of red meat (approx 80g). Each meal in the protein group included an average of 70-90g protein and <30g carbohydrate. The high carbohydrate meals were vegetarian and included >85g carbohydrate and <30g protein. Both groups were energy (3000kj) and fat (30g) matched. The meals were prepared on campus by a certified nutritionist. Meals were weighted before and after consumption to monitor food intake. Subjects were counseled by a nutritionist to fast for 2 hours prior and 1 hour after each session and also to maintain their normal diet throughout the entire study period. Three day food diaries were completed by the subject at week 1, 6 and 12 (including 2 weekdays and 1 weekend day). Dietary information was analysed using Foodworks (version 3.02, Xyris Software). An Anti-cancer Council (Victoria) Food Frequency Questionnaire was also completed at the start and end of the 12 weeks to establish dietary protein, carbohydrate and fat intake.

Chronic Experimental Design

Following the completion of the acute study day, subjects had a DEXA scan (Lunar Prodigy (GE Lunar Corp., Madison, WI, USA, software version 8.10.027, Deakin University, Burwood) to assess full body composition and QCT scan (Siemens Emotion Duo scanner; Siemens AG, Erlangen, Germany Hoppers Crossing- Taft Imaging) to assess cross sectional area and attenuation of the thigh muscles (see below for details). DEXA and CT scans were analysed by Dr Rob Daly, Deakin University. Subjects then completed 12 weeks of resistance training (3 times per week). Each 45 minute exercise session was be immediately followed by a supervised meal (i.e. high protein or high carbohydrate meal). Following the 12 week training program subjects completed the acute study again and also had a final DEXA scan and QCT scan. Therefore, muscle biopsies were collected in four different states: 1) untrained rested, 2) untrained acutely exercised, 3) trained rested and 4) trained acutely exercised.

Body Composition

Dual energy X-ray absorptiometry (DXA)

Total body and regional lean mass (kg), fat mass (kg) and percentage of body fat were estimated at baseline and 12 weeks by dual-energy X-ray absorptiometry (Lunar Prodigy, Lunar Corp, Madison, WI). Positioning of patients was made according to standard procedures, and all follow-up scans were analyzed using the manufacturer's scan comparison mode. Appendicular skeletal muscle mass (ASM) was calculated from the sum of lean tissue

mass in both the right and left arms and legs derived from the total body scan. The arm region included the hand, forearm and upper arm which was separated from the trunk by a line positioned through the scapulo-humeral joint. The leg region included the foot, lower leg and thigh, which was separated from the pelvis by two separate lines which were angled through the femoral neck. Since absolute muscle mass is correlated with height, ASM was divided by height squared to derive an index of relative appendicular skeletal muscle mass (RASM), as previously described (Baumgartner et al Am J Epid 1998).

Quantitative Computed Tomography (QCT)

Skeletal muscle cross-sectional area (CSA) and attenuation at the mid-thigh were assessed at baseline and 12 weeks using quantitative computed tomography (QCT) (Siemens Emotion Duo scanner, Siemens AG, Erlangen, Germany). The CT scan parameters were 130 kVp, 100 mAs, and 3 mm slice thickness. For each participant, an anterior-posterior scout scan was obtained of the entire left and right femur. The mid-point of the left femur was determined by drawing a line from the femoral head to the lateral femoral condyle using the QCT ruler function. A single slice was then taken through the mid-femur of both legs, but measurements were made only on the left thigh. All cross-sectional CT images were analysed using the Geanie 2.1 software program (BonAlyse Oy, Jyvakyla, Finland). Mid-thigh muscle CSA area was measured for each participant from muscle pixels with attenuation values ranging between 0 to 200 Hounsfield units (HU). To measure skeletal muscle attenuation, which provides an estimate of muscle fat infiltration (Goodpaster et al. J Appl Physiol 2000), a line was first manually drawn around the outermost edge of the thigh muscle (excluding subcutaneous fat). Muscle attenuation was then measured as the mean attenuation value for all pixels within the range of 0 to 100 HU, as described previously (Goodpaster et al. Am J Clin Nutr 2000).

Resistance Training Program

The subjects completed a full body 12 week resistance training program, 3 times per week, instructed by certified personal trainers. The program was devised with reference to; the ACSM current recommendations for resistance training in the elderly, and also previous resistance training studies in older people [40, 75, 76]. Under individual supervision the progressive bilateral resistance training program consisted of 3 sessions per week for 12 weeks. The first three training sessions were conducted using light resistance to familiarize the subjects with the equipment, training protocol, and correct execution of the exercises. After the familiarization sessions strength testing was performed to determine appropriate starting weights for all subjects (See Table 4.1). One-repetition maximum (RM) strength was estimated from their 5-RM results for all exercises. 5-RM was retested at week 6 and week 12, and the training load was adjusted accordingly to ensure that the training was progressive. Each training session was preceded by a 5min warm-up on a stationary cycle followed by a full set of exercises with light (warm-up) weights. The exercises consisted of leg press, bench press, lat pull down, leg extension, dumb-bell bicep curl, and some abdominal exercises (See

Table 4.2). Following the warm-up weights, subjects completed 2 sets of the same exercises

always performed in the prescribed order with 2 min rests between each set and 3-5 min rest between each exercise. Initially, the exercises were set to 50% of subjects 1-RM for one week followed by a progressive increase in the weights lifted each week until 80% of 1-RM was attained at week 6. The exercise intensity was set at 80% of 1-RM for the remaining 6 weeks. During this time, the load for all exercises was adjusted, if necessary, at every third or fourth training session to ensure the participant was working within the required rep range and prescribed intensity. The 'GymAware Performance Analysis' (Kinetic Performance Technology, ACT, Australia) infra-red sensor equipment was used on the leg extension and leg press exercises to monitor each repetitions peak and mean force in the eccentric and concentric phase.

Table 4.1: Resistance Exercise Program

Order	Exercise
-	Warm-up (bike & stretches)
1	Leg Press
2	Bench Press
3	Lat Pull Down
4	Leg Extension
5	Dumb-bell bicep curl
6	Abdominal Exercise
-	Cool Down Stretches

Table 4.2: 12 Week Resistance Exercise Intensity and Overload Program

Week	Intensity (of estimated 1RM)	Reps	Sets
1	Familiarisation	12	2
2	50%	12	2
3-4	60%	10	2
5-6	70%	8-10	2
7-12	80%	8	2

RM =repetition maximum

Analysis

RNA Extraction & cDNA synthesis

Total RNA from ~20 mg of wet muscle was isolated using the ToTALLY RNA^{TM} Kit (Ambion Inc., Austin, TX) protocol and reagents. RNA quality and quantity was analysed using the RNA 6000 Nano LabChip kit on the Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA) following the protocols described by the manufacturer. Extracted RNA (1µg) was heated at 65°C for 10 minutes immediately prior to first strand cDNA being generated using AMV reverse transcriptase (kit A3500; Promega, Madison, WI) with Oligo(dT)$_{15}$ primer, in the presence of 1mM of each dNTP and 20 U of recombinant RNasin ribonuclease inhibitor. The reaction was incubated at 42°C for 60 min and then terminated at 99°C for 10 min and 4°C for 5 min. The cDNA was stored at -20 ^{o}C for subsequent analysis.

Primer Design

PCR primers were designed using Primer Express software version 2.0 (Applied Biosystems, Foster City, CA.) from gene sequences obtained from GenBank. Primer specificity was confirmed using BLAST. Primers were purchased from GeneWorks (Adelaide, SA, Australia). Efficiency of PCR primers was confirmed by examining the dynamic range of responses for a series of dilutions of cDNA. Using the slopes of the lines, the efficiency (E) of each target amplification was calculated using the equation $E = (10^{-1/slope})-1$. All primers used in this study demonstrated efficient amplification.

Real-time PCR

Real-time PCR was performed using the GeneAmp® 5700 Sequence Detection System (Applied Biosystems). For the PCR step, reaction volumes of 20µl contained SYBR Green PCR Master Mix (Applied Biosystems), forward and reverse primers and cDNA template (diluted 1:40). All samples were run in duplicate. The real-time PCR reaction was run for 1 cycle (50°C 2 min, 95°C 10 min) followed by 40 cycles (95°C 15 s, 60°C 60 s) and fluorescence emissions was measured after each of the repetitive cycles. Because SYBR green indiscriminately binds to double-stranded DNA, a melting point dissociation curve was generated to confirm the only a single product was amplified. Heat dissociation of oligonucleotides detects differences in melting temperature and produces a single dissociation peak for each nucleotide within a 2 ^{o}C difference in melting temperature [77].

mRNA quantification

Data was analysed using the comparative critical threshold (Ct) method where the amount of target was normalised to the amount of an endogenous control is given by the equation $2^{-\Delta Ct}$. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) has been previously identified as an appropriate endogenous control for resistance exercise studies [78, 79] and as such was selected for the endogenous control for this study. The appropriateness of this gene as an endogenous control was confirmed by examining the $2^{-\Delta Ct}$ normalised to control values (data not shown).

Skeletal muscle fibre-typing

Muscle tissue previously preserved and frozen in tissue-tek (company) was cut into 5-10µm slices using a Cryostat (Leica CM1850-8-1, German) and stored at -80°C until ready to use. Samples were fixed in 4% PFA/PBS for 10 min, permeated with 5% TX-100 (Triton) for 5 min and blocked with 20% BSA in Tris Buffered Sailne (TBS) (pH7.6) for 10min. Samples were incubated for 30 min with anti-skeletal myosin (Slow) (clone NOQ7.5.4D) (1/2000) in 4%BSA (Sigma Aldrich, St Louis, MO). Antibody was washed off with TBS and samples were incubated in anti-mouse HRP (Quantum Scientific, Melbourne, Australia) (1/50) in 4% BSA for 60 min. After the antibody was washed off with TBS, the Vector SG peroxidase substrate solution kit (VECTOR SK-4700, Vector Laboratories, Burlingame, CA) was prepared according to kit instructions and added to the samples with colour development monitored for 5-15 min via microscopic examination. Samples were washed using sterile distilled water and incubated in TBS for 5 min. Samples were blocked a second time using 20% BSA for 10 min then incubated for 60 min with an alkaline phosphatase conjugated monoclonal anti-skeletal myosin (fast) antibody (MY-32) (Sigma-Aldrich, St Louis, MO) (1/50) in 4% BSA. The vector red alkaline phosphatase substrate solution kit I (VECTOR SK-5100, Vector Laboratories, Burlingame, CA) was prepared according to kit instructions and added to the samples, following a rinse with TBS, and monitored for 10-20 min via microscopic examination. Samples were washed using sterile distilled water and mounted in TBS. Samples were examined using an Olympus IX70 Microscope (Japan) using Optotronics MagnaFire Camera Imaging and Control Software, Version 1.1.

Cross-Sectional Area of muscle fibers

Muscle tissue previously stained for fibre-type analysis were examined using an Olympus IX70 Microscope (Japan) using Optotronics MagnaFire Camera Imaging and Control Software, (Version 1.1) and Image-Pro Express Software (Version 4).

Buccal cell Zinc Content

Buccal cells were collected at the beginning and end of the 12 week resistance study period for the analysis of zinc levels. Buccal cell samples were collected by gentle oral abrasion and subsequent 50ml rinsing with water into a collection tube. Cells were then washed twice and the cell number determined by trypan blue exclusion. Cells were digested overnight in nitric acid (SupraPure, Merck) and then diluted to a concentration of 1x106 cells/ml. The diluted digest was subsequently analysed spectrophotometrically for zinc on a Varian SpectrAA-800 at a wavelength of 213.9nm by microinjection. Results are presented as ug of Zn per million cells. All zinc free equipment and consumables, by either manufacture or by acid washing, was confirmed by Atomic Absorption Spectrophotometer (Varian SpectrAA-800) analysis.

Gut Microflora Analysis

Three-day faecal sample collections were performed at the beginning and end of the 12 week study period for the examination of gut microflora. Faecal bacterial populations were assessed by FISH analysis. Briefly, 1g of each faecal sample was suspended in PBS, homogenised and then centrifuged to pellet cellular debris. An aliquot of the resulting supernatant was mixed with paraformaldehyde solution and fixed overnight at 4°C. Fixed samples were stored at -80°C until further use.

Fixed faecal samples were diluted in PBS to a suspension containing approximately 30-100 colony forming units (cfu) per field of view. A 10 µl aliquot of the diluted sample was spread over the entire area of a one centimetre grid square drawn onto a Vectabond treated slide. Slides were dried at room temperature and then fixed with 96% (v/v) ethanol at room temperature. Immediately prior to use, the suspensions were further diluted with hybridisation buffer (0.9 M NaCl, 20 mM Tris-Hcl [pH 7.2], 0.1% SDS [wt/v]) at 50°C to a concentration of 10 ng/µl. A 20 µl aliquot of the FITC-labelled oligonucleotide probe (final concentration 50 ng/µl) probe was applied to the fixed cells. A coverslip was then placed on the slide(s) and for all probes except Bac 303, the slides were incubated at 50°C for 16 hours in a dark moist chamber. For the Bac 303 probe the slide(s) were incubated for 2 hours at 45°C. Once hybridisation was complete, the slides were rinsed with wash buffer (0.9 M NaCl, 20 mM Tris-HCl [pH 7.2]) at 50°C for 20 minutes and then rinsed briefly in sterile milliQ water. The slides were then air dried rapidly and mounted with 6 µl of Vectashield® hardset mounting medium with DAPI and a coverslip was placed on top. Nail polish was used to adhere the coverslip to the slide and the slide(s) were left to sit for one hour at 4°C prior to visual counting, to allow the Vectashield® to harden. Slides were viewed with an Olympus IX Inverted System Microscope using a 100 × oil objective, illumination from a mercury lamp, ultraviolet (UV) filter, Indo-1 filter and bandpass filter. Digital images were taken with a Magnafire camera. The images produced were analysed by counting the fluorescent cells with a counting chamber in the software program Image-Pro®Express. Depending on the number of fluorescent cells, approximately 25 microscopic fields of view were taken, with at least 30-100 positive objects counted in one field of view. The following calculation was used to determine the total number of positive objects (target microorganisms) per gram of wet faeces:

Positive objects per gram of wet faeces = average number of positive objects in 25 microscopic fields × area of microscopic field (1600) × depth of microscopic field (100) × dilution factor.

The wet weight figures were then converted to dry weight for a more accurate analysis. The percentage (%) solids figure was obtained by drying the faecal samples with a rotary evaporator and this procedure was conducted by Dr. J. Muir from the Department of Gastroenterology, Monash University, Box Hill Hospital. The number of positive objects per gram of dry faeces was then calculated by the equation:

Positive objects per gram of dry faeces = wet weight / % solids × 100.

Statistical Analysis

Statistical analysis was performed using GraphPad Prism 4 for windows (Version 4.03, GraphPad Software, San Diego, CA). Unless otherwise stated, means were compared using a one-way ANOVA with repeated measures (for normally distributed data) and any significant differences analysed using a Post Hoc Newman-Keuls Multiple Comparison Test. Data is presented as mean ± standard error of the mean (SEM) unless otherwise stated. $P<0.05$ was considered statistically significant.

3 RESULTS

3.1 BODY COMPOSITION

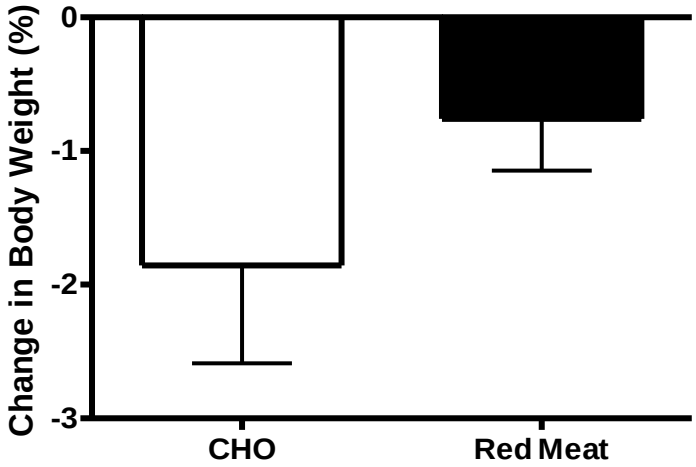

Figure 1. Change in percent body weight of older ladies following 12 weeks of strength training using DEXA Scan Analysis.

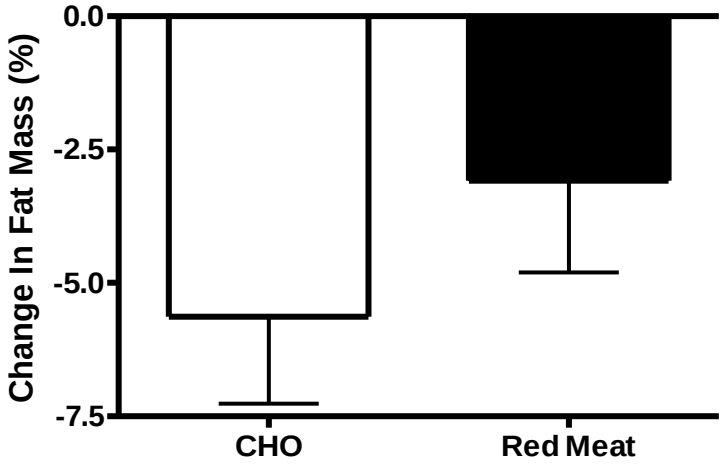

Figure 2. Change in percentage fat mass of older ladies following 12 weeks of strength training using DEXA Scan Analysis.

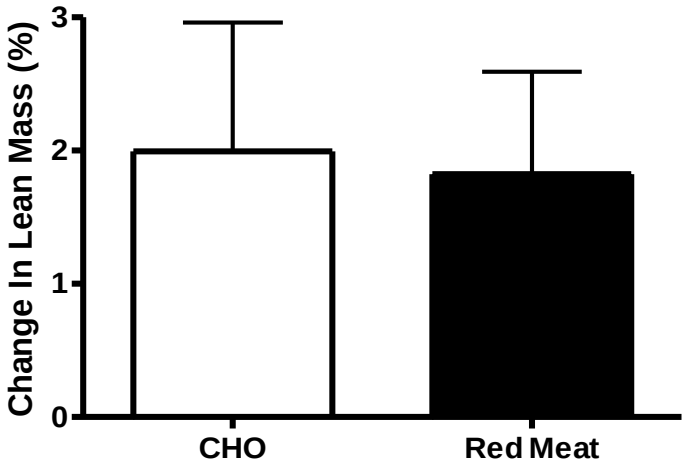

Figure 3. Change in percentage lean mass of older ladies following 12 weeks of strength training using DEXA Scan Analysis.

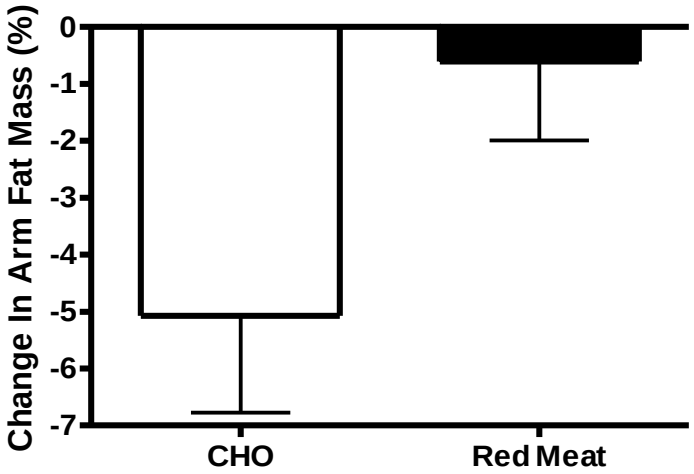

Figure 4. Change in percentage arm fat mass of older ladies following 12 weeks of strength training using DEXA Scan Analysis.

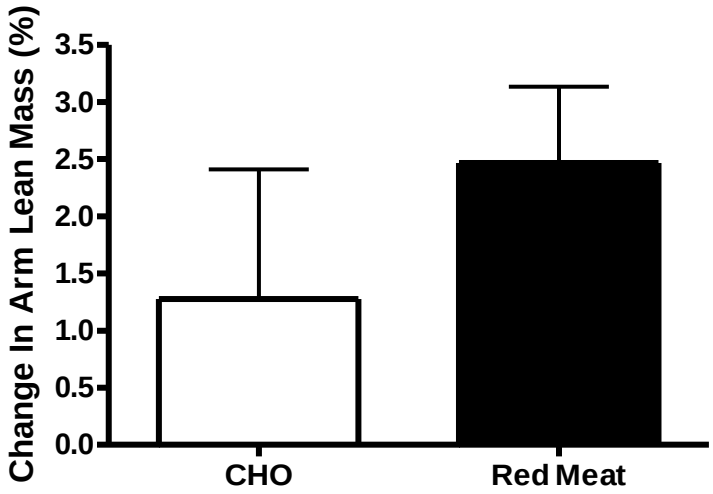

Figure 5. Change in percentage arm lean mass of older ladies following 12 weeks of strength training using DEXA Scan Analysis.

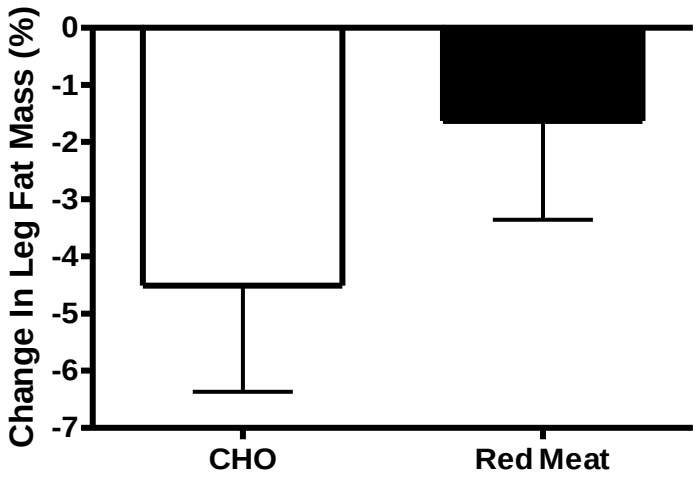

Figure 6. Change in percentage leg fat mass of older ladies following 12 weeks of strength training using DEXA Scan Analysis.

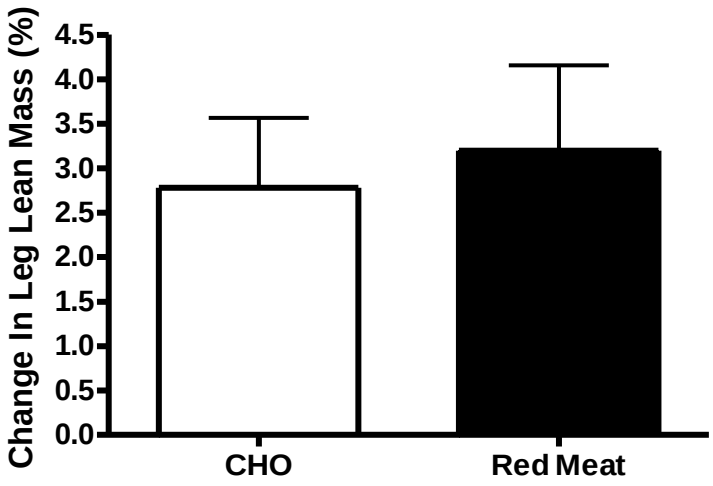

Figure 7. Change in percentage leg lean mass of older ladies following 12 weeks of strength training using DEXA Scan Analysis.

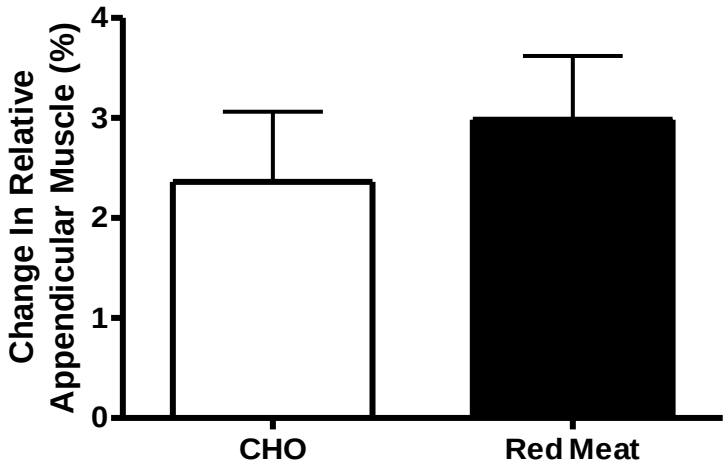

Figure 8. Change in relative appendicular muscle of older ladies following 12 weeks of strength training using DEXA Scan Analysis.

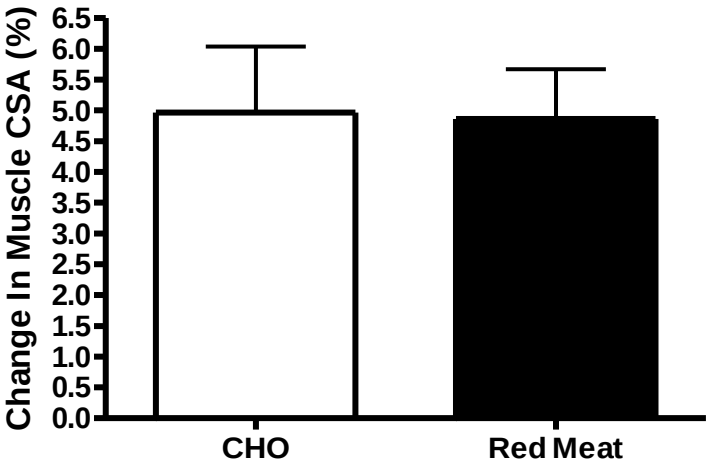

Figure 9. Change in muscle CSA of older ladies following 12 weeks of strength training using CT Scan Analysis.

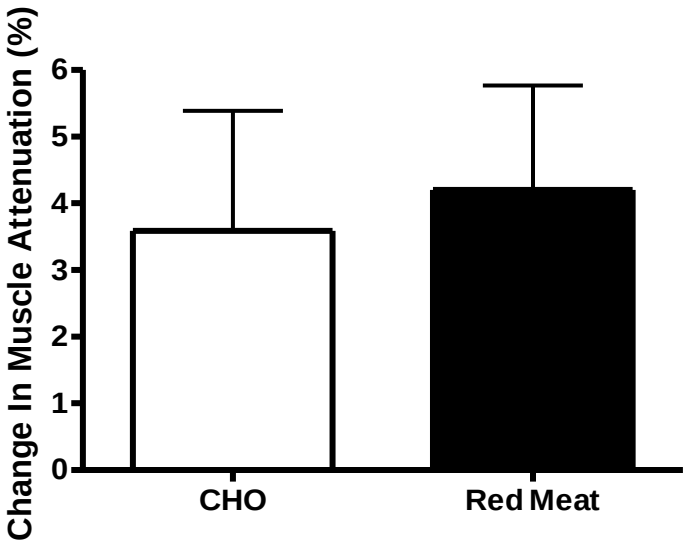

Figure 10. Change in muscle attenuation of older ladies following 12 weeks of strength training using CT Scan Analysis.

Immunohistochemistry-Muscle Fiber Analysis

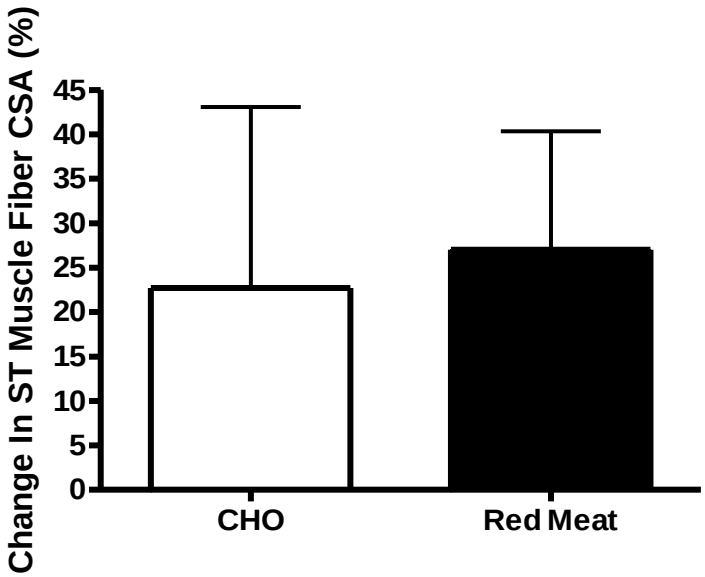

Figure 11. Change in percentage slow twitch (ST) muscle fibre CSA in the *vastus lateralis m*. of older ladies following 12 weeks of strength training.

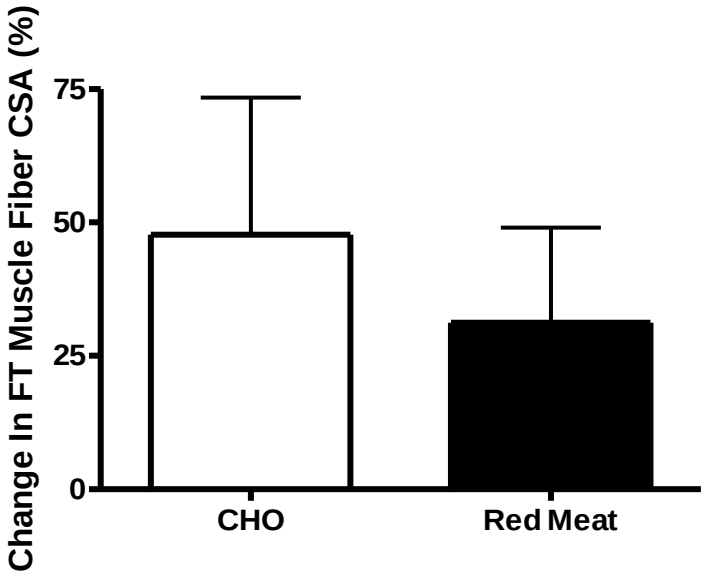

Figure 12. Change in percentage fast twitch (FT) muscle fibre CSA in the *vastus lateralis m*. of older ladies following 12 weeks of strength training.

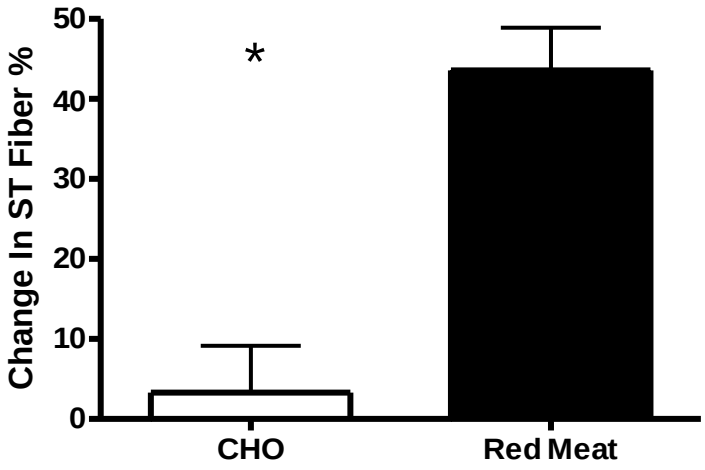

Figure 13. Change in percentage slow twitch (ST) muscle fibres in the *vastus lateralis m*. of older ladies following 12 weeks of strength training.

* indicates a significant difference in change in Slow Twitch fiber percent between groups (*P*=0.001).

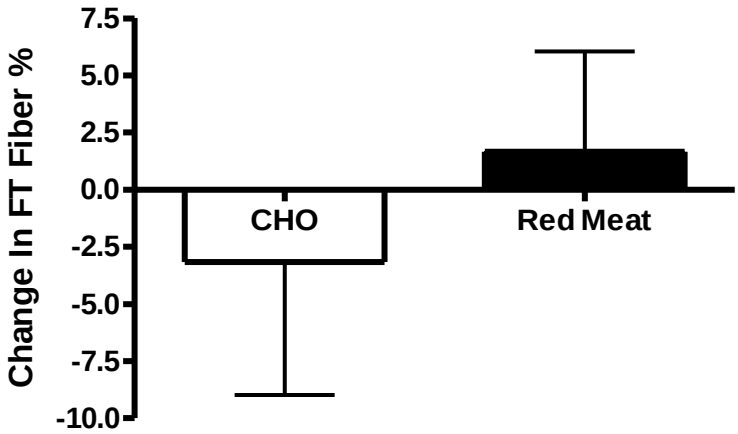

Figure 14. Change in percentage fast twitch (FT) muscle fibres in the *vastus lateralis m*. of older ladies following 12 weeks of strength training.

3.2 STRENGTH

GymAware Analysis

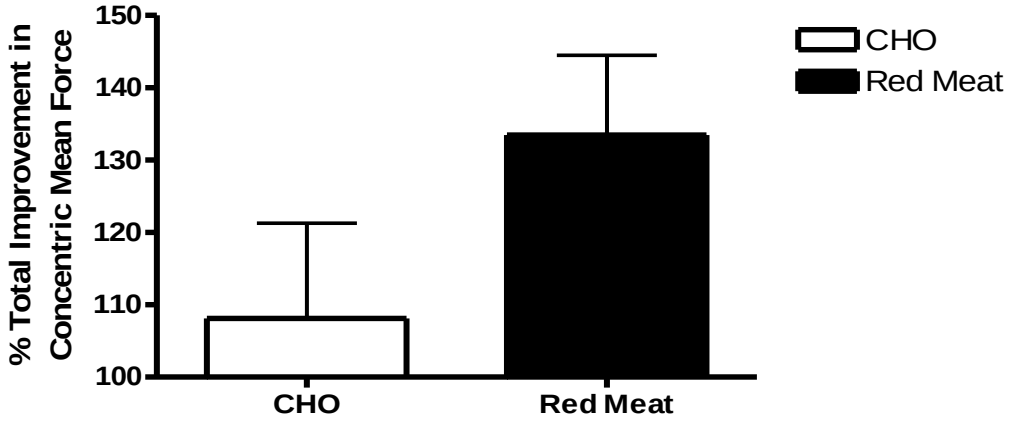

Figure 15. Total improvement in concentric mean force measured on the leg press following 12 wks of resistance training, in conjunction with consuming a high carbohydrate diet (CHO) or high red meat diet (MEAT), in older women.

Percent Improvement in concentric mean force between groups (*P*=0.08)

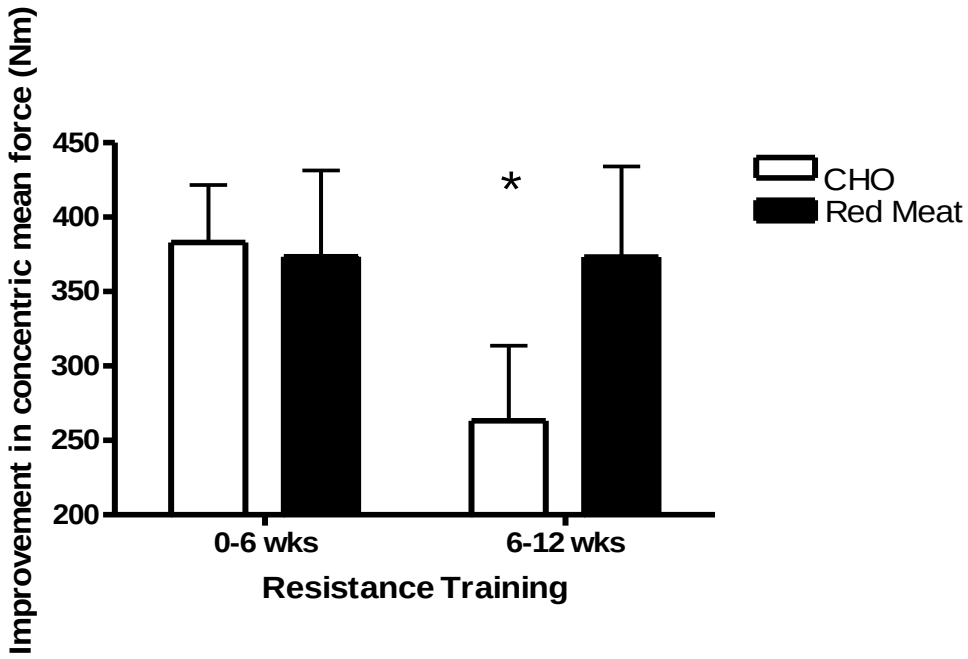

Figure 16. Improvement in concentric mean force measured on the leg press over 0-6 weeks and 6-12wks in older women consuming a high carbohydrate diet (CHO) or high red meat diet (MEAT) in conjunction with 12wks resistance training.

* indicates a significant difference in improvement in concentric mean force in the carbohydrate group between time points (*P*=0.03).

Improvement in concentric mean force 6-12 wks between groups (*P*=0.09).

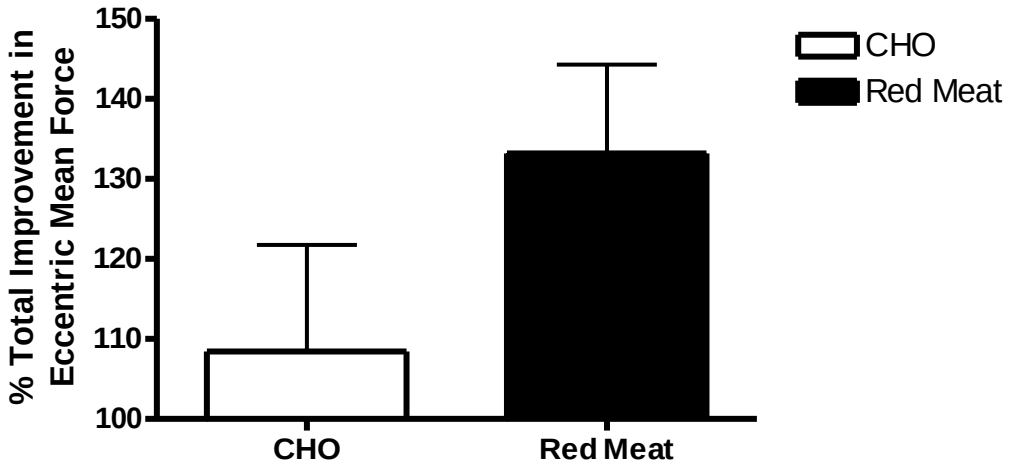

Figure 17. Total improvement in eccentric mean force measured on the leg press following 12 wks of resistance training, in conjunction with consuming a high carbohydrate diet (CHO) or high red meat diet (MEAT), in older women.
Percent Improvement in eccentric mean force between groups (*P*=0.08)

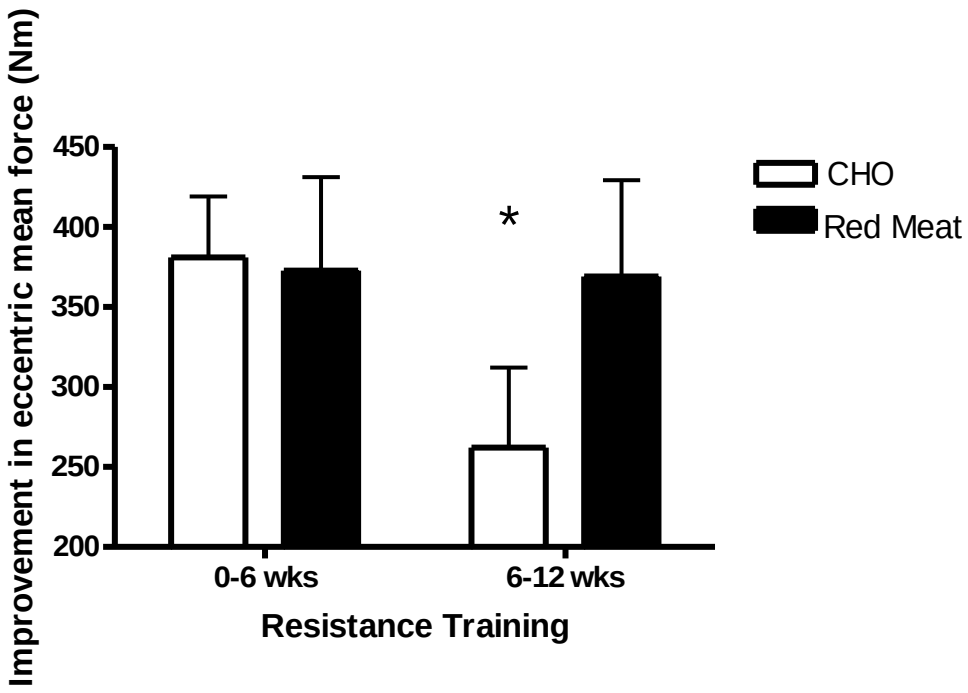

Figure 18. Improvement in eccentric mean force measured on the leg press over 0-6 weeks and 6-12wks in older women consuming a high carbohydrate diet (CHO) or high red meat diet (MEAT) in conjunction with 12wks resistance training.
* indicates a significant difference in improvement in eccentric mean force in the carbohydrate group between time points (*P*=0.03).
Improvement in eccentric mean force 6-12 wks between groups (*P*=0.09)

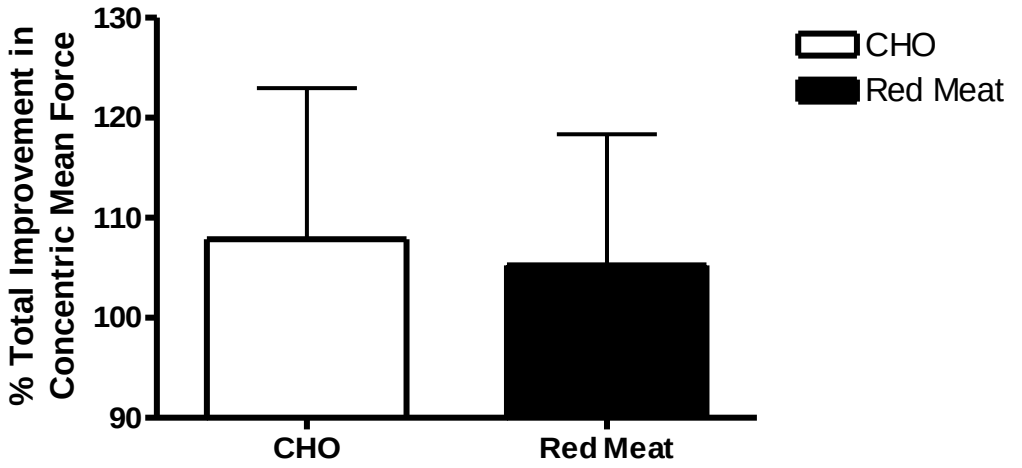

Figure 19. Total improvement in concentric mean force measured on the leg extension following 12 wks of resistance training, in conjunction with consuming a high carbohydrate diet (CHO) or high red meat diet (MEAT), in older women.

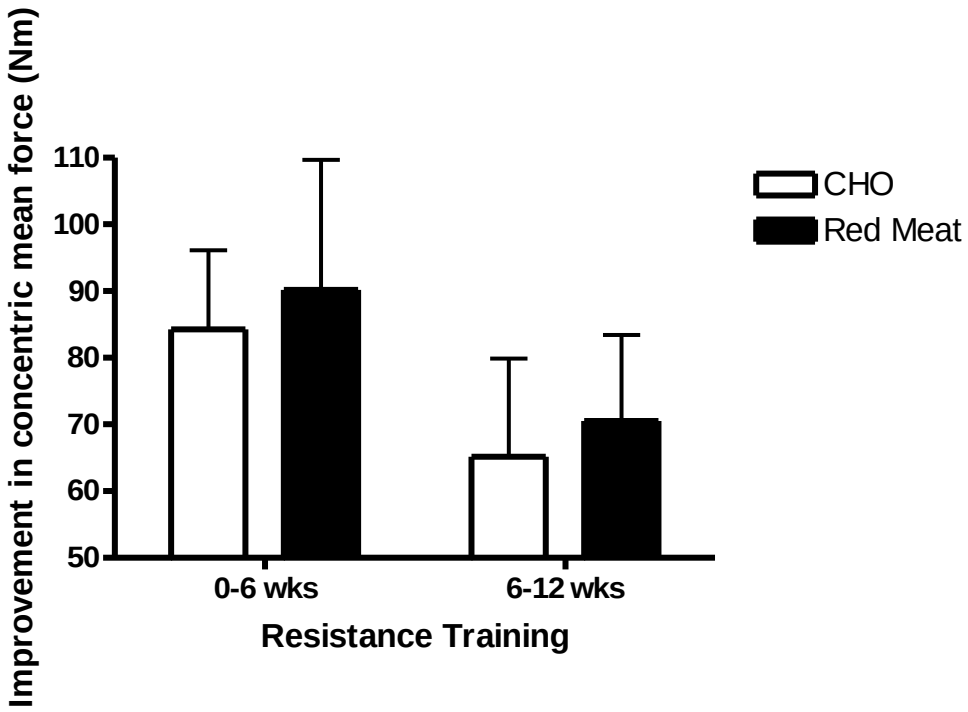

Figure 20. Improvement in concentric mean force measured on the leg extension over 0-6 weeks and 6-12wks in older women consuming a high carbohydrate diet (CHO) or high red meat diet (MEAT) in conjunction with 12wks resistance training.

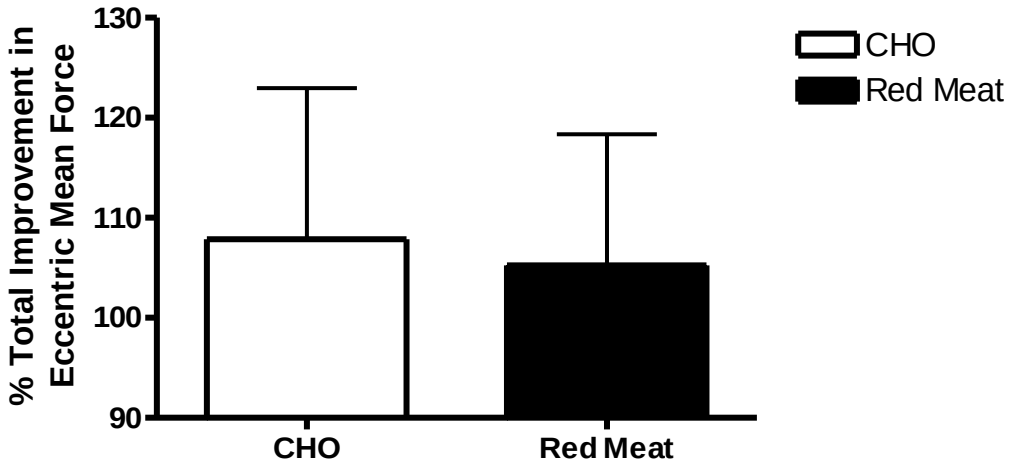

Figure 21. Total improvement in eccentric mean force measured on the leg extension following 12 wks of resistance training, in conjunction with consuming a high carbohydrate diet (CHO) or high red meat diet (MEAT), in older women.

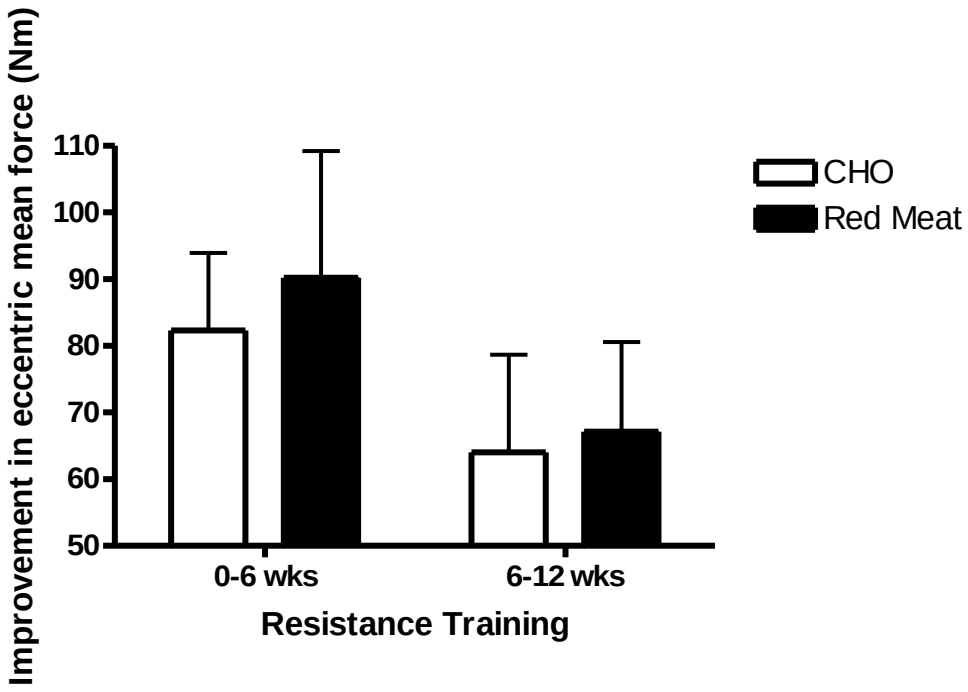

Figure 22. Improvement in eccentric mean force measured on the leg extension over 0-6 weeks and 6-12wks in older women consuming a high carbohydrate diet (CHO) or high red meat diet (MEAT) in conjunction with 12wks resistance training.

CYBEX Analysis

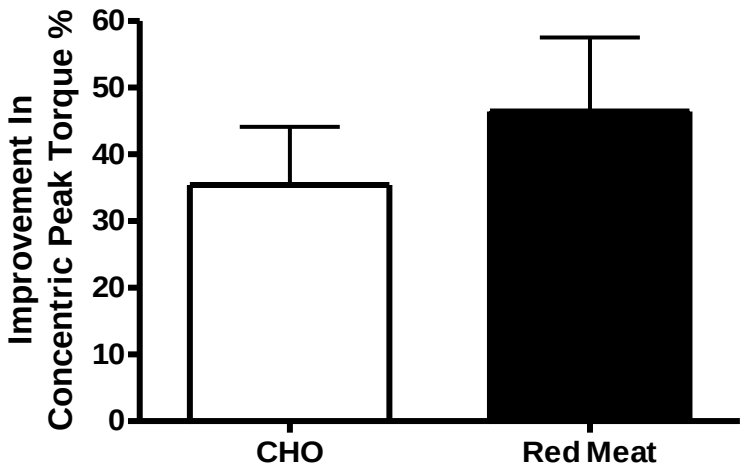

Figure 23. Total improvement in Concentric Peak Torque measured on the CYBEX following 12 wks of resistance training, in conjunction with consuming a high carbohydrate diet (CHO) or high red meat diet (MEAT), in older women.

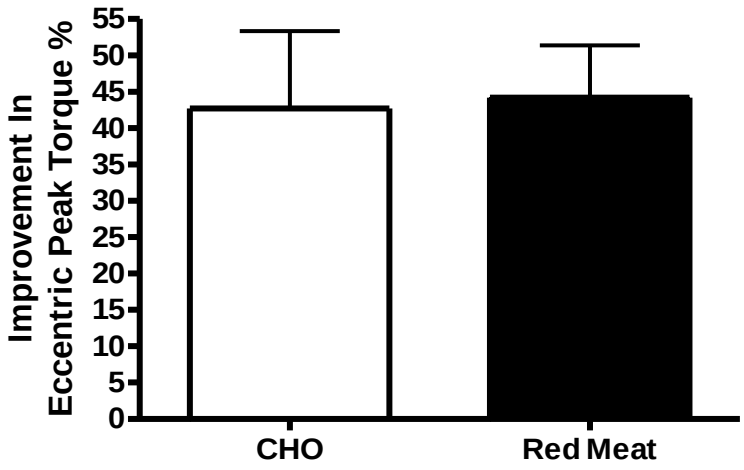

Figure 24. Total improvement in Eccentric Peak Torque measured on the CYBEX following 12 wks of resistance training, in conjunction with consuming a high carbohydrate diet (CHO) or high red meat diet (MEAT), in older women.

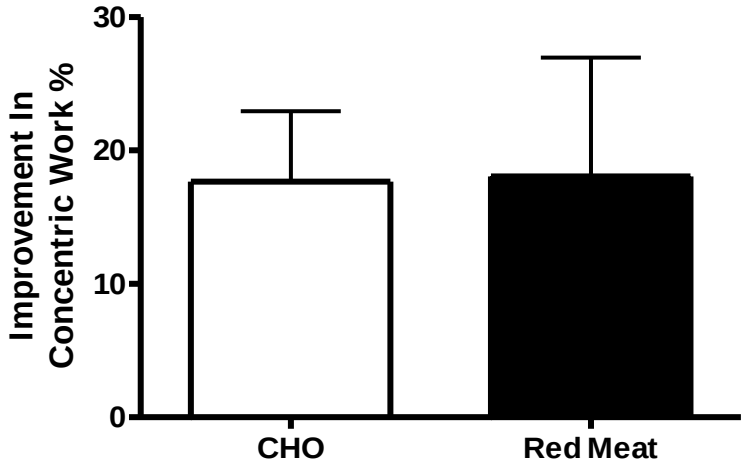

Figure 25. Total improvement in Concentric Work measured on the CYBEX following 12 wks of resistance training, in conjunction with consuming a high carbohydrate diet (CHO) or high red meat diet (MEAT), in older women.

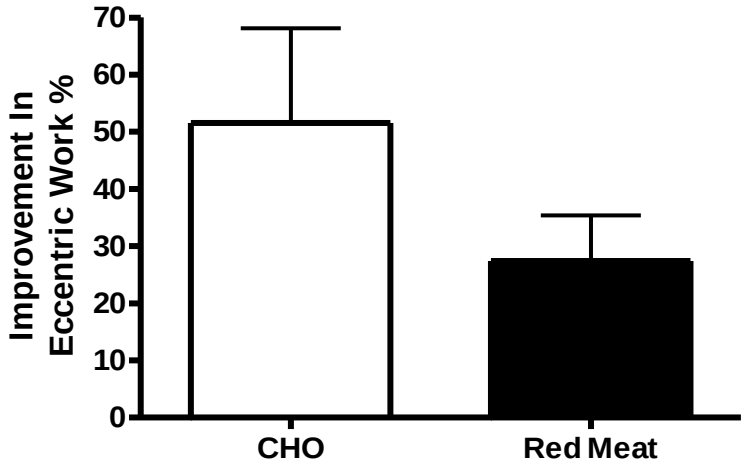

Figure 26. Total improvement in Eccentric Work measured on the CYBEX following 12 wks of resistance training, in conjunction with consuming a high carbohydrate diet (CHO) or high red meat diet (MEAT), in older women.

Repetition Maximum Analysis

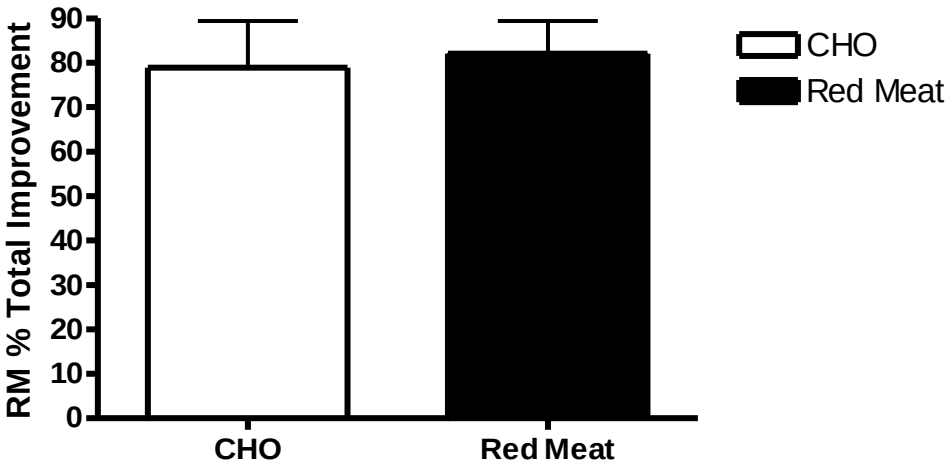

Figure 27. Total improvement in Repetition Maximum (RM) testing measured on the Leg Press following 12 wks of resistance training, in conjunction with consuming a high carbohydrate diet (CHO) or high red meat diet (MEAT), in older women.

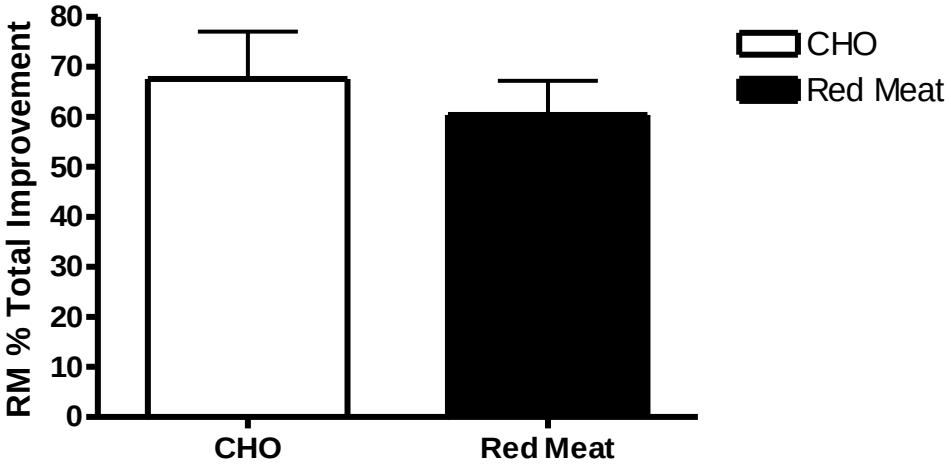

Figure 28. Total improvement in Repetition Maximum (RM) testing measured on the Leg Extension following 12 wks of resistance training, in conjunction with consuming a high carbohydrate diet (CHO) or high red meat diet (MEAT), in older women.

3.3 DIET

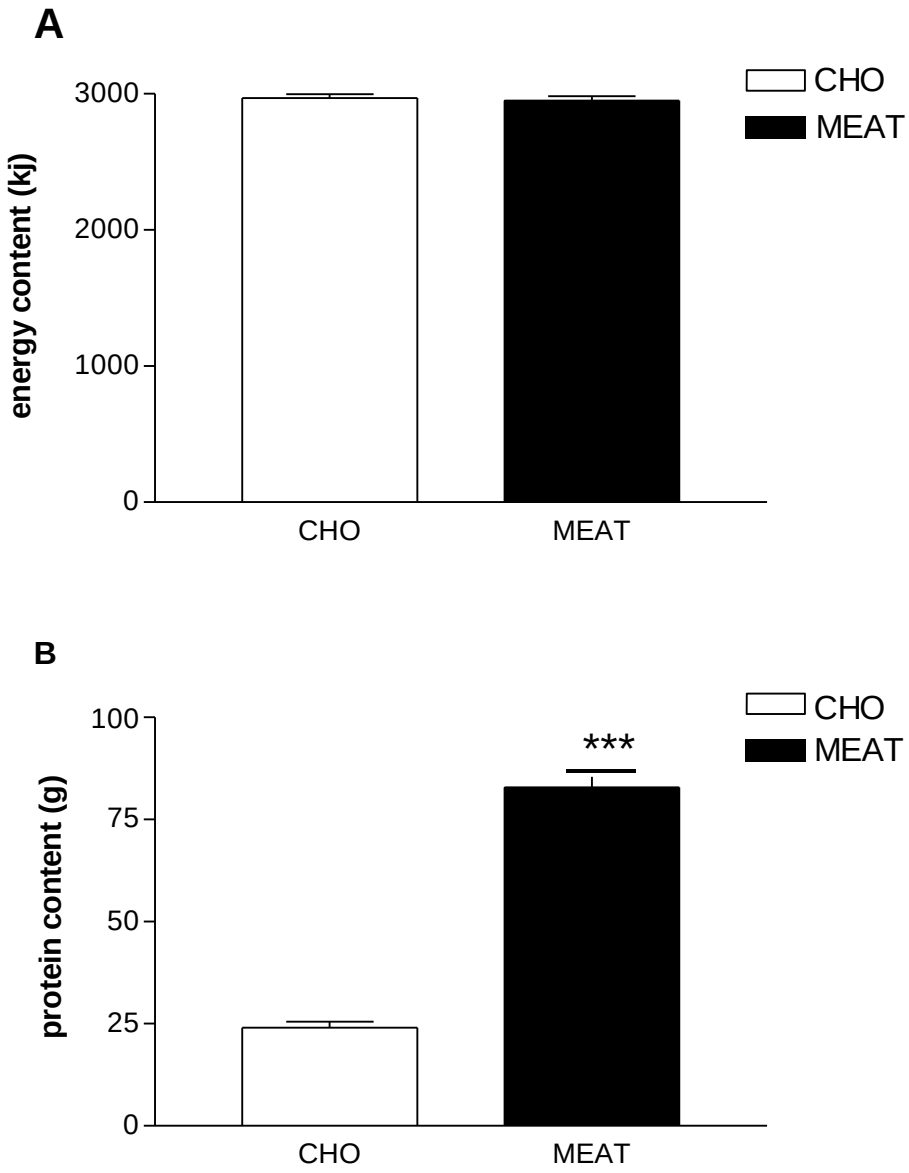

Figure 29. Average energy (A) and protein (B) content of the prescribed meals.

*** Significantly different from CHO (P<0.0001)

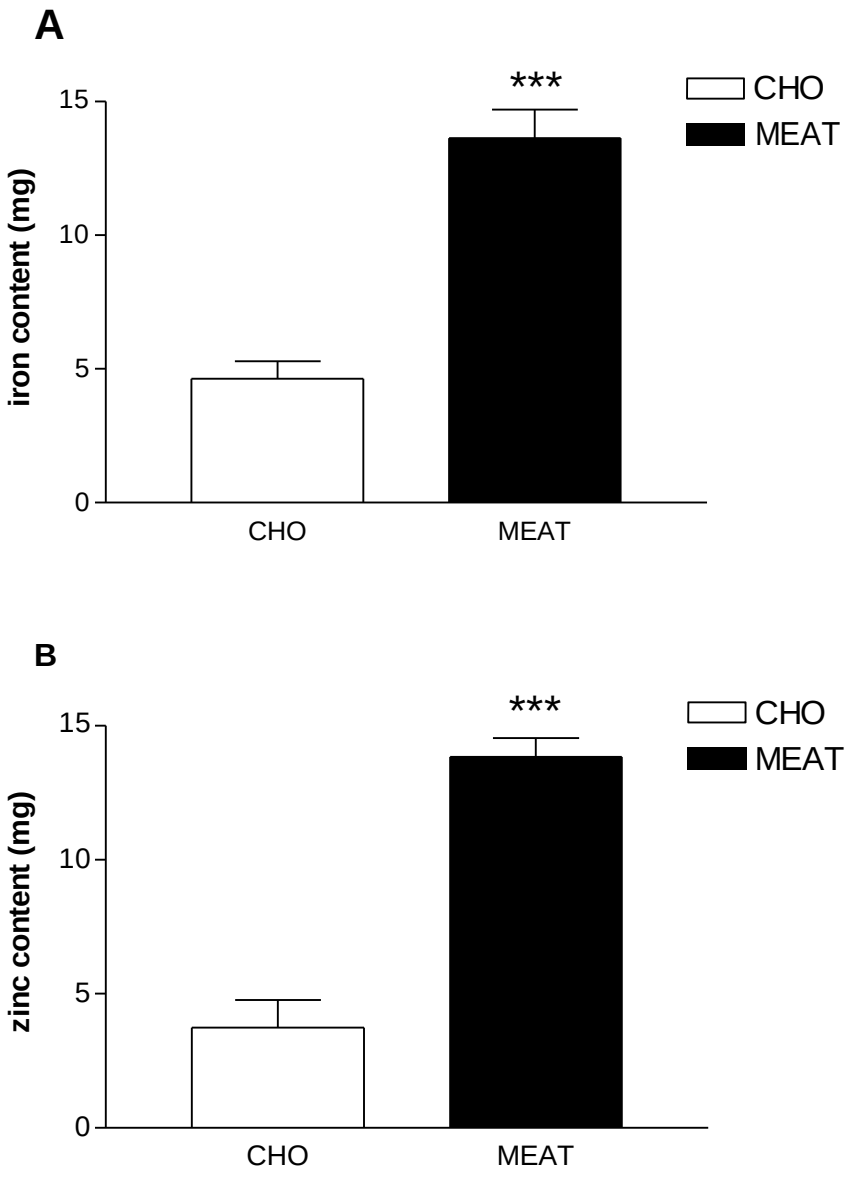

Figure 30. Average iron (A) and zinc (B) content of the prescribed meals.

*** Significantly different from CHO (P<0.0001)

The baseline diet and the impact of the intervention of overall diet was assessed using both a 3-day dietary diary and a Food Frequency Questionnaire.

Table 3.1: ANALYSIS OF THREE DAY FOOD DIARIES. Assessment was made on 1 weekend day and 2 week days, including one exercise (intervention) day.

CARBOHYDRATE GROUP			
	Pre Intervention	**Mid Intervention**	**Post Intervention**
Energy (kj)	7047.9 ± 559.7	7339.0 ± 345.9	7288.6 ± 685.6
Protein (g)	75.7 ± 3.7	78.3 ± 4.5	79.0 ± 7.0
Protein (%)	*19.1 ± 1.1*	*18.6 ± 1.0*	*19.0 ± 1.1*
Carbohydrate (g)	192.5 ± 22.6	196.6 ± 14.3	201.75 ± 21.5
Carbohydrate (%)	*46 ± 2.1*	*45.8 ± 2.0*	*48.0 ± 2.7*
Fat (g)	61.1 ± 4.2	66.7 ± 3.9	61.9 ± 9.2
Fat (%)	*33.1 ±1.3*	*34.6 ± 1.6*	*30.8 ± 2.4*
Iron (mg)	9.97 ± 0.9	9.9 ± 0.6	10.8 ± 0.8
Zinc (mg)	10.03 ± 0.7	10.4 ± 0.6	10.4 ± 1.1
Fibre (g)	22.9 ± 2.3	24.7 ± 2.4	21.7 ± 1.7
MEAT GROUP			
	Pre Intervention	**Mid Intervention**	**Post Intervention**
Energy (kj)	7268.6 ± 592.1	8506 ± 1203.6	7746.8 ± 574.9
Protein (g)	80.1 ± 4.3	105.7 ± 15.7 [a b]	89.4 ± 11.4
Protein (%)	*19.6 ± 1.0*	*21.5 ± 3.1*	*19.9 ± 1.6*
Carbohydrate (g)	174.8 ± 14.2	204 ± 29.8	181.5 ± 12.3
Carbohydrate (%)	*41.5 ± 1.4*	*40.8 ± 5.8*	*40.75 ± 2.2*
Fat (g)	66.9 ± 5.8	78 ± 10.8	73.9 ± 7.3
Fat (%)	*34.8 ± 1.8*	*34.3 ± 4.7*	*35.6 ± 2.0*
Iron (mg)	11.0 ± 1.0	11.6 ± 1.9	11.3 ± 0.9
Zinc (mg)	12.4 ± 1.2	12.5 ± 2.0	13.0 ± 2.1
Fibre (g)	21.2 ± 2.1	20.2 ± 3.1	18.2 ± 1.6

[a] significantly different from carbohydrate group (mid intervention), $p<0.05$
[b] significantly different from meat group (pre intervention), $p<0.05$

Table 3.2: ANALYSIS OF FOOD FREQUENCY QUESTIONNAIRES DIARIES.

CARBOHYDRATE GROUP		
	Pre Intervention	***Post Intervention***
Energy (kJ/day)	6879 ± 471	6668 ± 490
All Fat (g/day)	61.7 ± 4.02	62.9 ± 4.81
Saturated Fat (g/day)	21.6 ± 1.54	22.8 ± 2.51
Polyunsaturated Fat (g/day)	12.3 ± 1.53	12.2 ± 0.96
Monounsaturated Fat (g/day)	22.4 ± 1.65	22.3 ± 1.78
Protein (g/day)	83.4 ± 5.62	85.3 ± 8.28
Carbohydrate (g/day)	190 ± 16.78	174 ± 12.97
Fibre (g/day)	24.1 ± 2.79	21.0 ±1.61
Calcium (mg/day)	1070 ± 99	1054 ± 125
Folate (µg/day)	255 ± 12.8	245 ± 14.0
Iron (mg/day)	11.6 ± 0.95	11.3 ± 0.81
Sodium (mg/day)	2194 ± 149	2114 ± 156
Zinc mg/day	11.0 ± 0.70	11.6 ± 1.17
MEAT GROUP		
	Pre Intervention	***Post Intervention***
Energy (kJ/day)	7360 ± 583	7295 ± 504
All Fat (g/day)	72.9 ± 6.53	70.6 ± 4.92
Saturated Fat (g/day)	29.1 ± 2.52	27.6 ± 1.81
Polyunsaturated Fat (g/day)	10.9 ± 1.17	10.8 ± 1.25
Monounsaturated Fat (g/day)	26.4 ± 2.57	25.9 ± 2.34
Protein (g/day)	89.9 ± 6.06	86.0 ± 5.08
Carbohydrate (g/day)	187 ± 17.0	192 ± 17.4
Fibre (g/day)	23.9 ± 2.02	22.2 ± 1.86
Calcium (mg/day)	933 ± 119	973 ± 102
Folate (µg/day)	301 ± 27.8	274 ± 20.4
Iron (mg/day)	14.5 ± 1.89	12.5 ± 1.41
Sodium (mg/day)	2368 ± 246	2085 ± 135
Zinc mg/day	11.7 ± 0.83	11.1 ± 0.79

3.4 BOWEL HEALTH

Table 3.3: Inclusion of moderate amounts of red meat in the diet of exercising older women: Impact on faecal output, frequency, pH and faecal carbohydrate excretion (mean±SD).

	Carbohydrate Control Group		Meat Group	
	Baseline (n=10)	**Post-diet (n=8)**	**Baseline (n=10)**	**Post-diet (n=10)**
Fecal Output:				
Wet wt g/d	136±46	115±54	181±111	127±84
Dry wt g/d	39±13	30±10	46±22	33±19
%moisture	71±2	72±7	72±5	72±4
%dry matter	29±2	28±7	28±5	28±4
Frequency	1.5±0.2	1.2±0.3	1.4±0.3	1.2±0.4
Fecal pH	6.90±0.36	6.78±0.38	6.75±0.21	6.72±0.28
Fecal Starch				
g/d	0.9±1.2	0.7±0.5	0.6±0.4	0.6±0.7
%	1.4±0.7	2.3±1.3	1.4±0.9	1.8±1.9
Fecal NSP				
g/d	5.6±2.5	3.6±1.9	9.2±9.0	5.9±5.7
%	15.8±5.9	12.5±6.4	16.9±9.9	15.9±5.7

Table 3.4: Inclusion of moderate amounts of red meat in the diet of exercising older women: Impact on faecal short chain fatty acids (mean±SD).

Faecal Parameter	Carbohydrate Control Group		Meat Group	
	Baseline (n=10)	Post-diet (n=8)	Baseline (n=10)	Post-diet (n=10)
SCFA mmol/L				
acetate	46.0±16.6	54.7±15.6	48.8±22	53.4±20.6
propionate	13.6±4.7	15.2±5.5	15.1±5.8	16.8±5.5
butyrate	12.3±5.7	13.1±5.2	13.3±5.9	14.5±8.1
valerate	1.52±0.7	1.8±0.7	1.81±0.6	1.9±0.4
caproic	0.61±0.6	0.8±0.7*	0.4±0.5	0.3±0.3*
isobutyrate	1.7±0.5	1.9±0.7	1.8±0.5	2.2±0.5
isovalerate	2.3±0.7	2.5±1.1	2.3±0.7	2.9±0.8
Total SCFA	78.0±25.9	90.0±25.6	83.5±33	92.4±33.3
SCFA mmol/d				
acetate	6.5±3.4	6.8±4.1	10.2±8.7	7.4±6.4
propionate	1.9±0.8	1.8±0.9	3.2±2.9	2.3±2.1
butyrate	1.8±1.1	1.6±1.0	2.8±2.5	2.2±2.3
valerate	0.2±0.1	0.2±0.1	0.4±0.3	0.25±0.20
caproic	0.1±0.1	0.09±0.08*	0.06±0.05	0.04±0.03 *
isobutyrate	0.2±0.1	0.2±0.1	0.33±0.25	0.27±0.17
isovalerate	0.3±0.1	0.3±0.1	0.43±0.31	0.35±0.21
Total SCFA	10.9±5.4	10.9±6.1	17.4±14.7	12.9±11.2
SCFA % Total				
acetate	58.6±3.9	60.9±4.5	57.7±6	57.61±3.8
propionate	17.6±3.1	16.9±3.6	18.4±3.7	18.5±2.8
butyrate	15.4±4.0	14.1±2.8	15.4±2.0	14.4±3.9
valerate	1.9±0.7	2.1±0.7	2.3±0.6	2.2±0.5
caproic	0.7±0.6	0.8±0.7	0.6±0.6	0.48±0.47
isobutyrate	2.4±1.1	2.2±1.0	2.38±0.9	2.7±0.9
isovalerate	3.4±1.8	3.1±1.7	3.3±1.4	3.7±1.5
Total SCFA	100	100	100	100

Table 3.5: Inclusion of moderate amounts of red meat in the diet of exercising older women: Impact on Products of Protein Fermentation (mean±SD).

	Carbohydrate Control Group		**Meat Group**	
	Baseline (n=10)	**Post-diet (n=8)**	**Baseline (n=10)**	**Post-diet (n=10)**
Phenol µg/g				
Phenol	2.8±3.2	2.2±1.1	6.28±10.6	2.92±2.9
p-cresol	102±42	135±84	99±67	140±61
Total	105±43	137±84	106±65	143±59
Phenol output mg/d				
Phenol	0.32±0.25	0.27±0.2	1.9±3.8	0.53±0.83
p-cresol	13.3±6.8	13.9±9.9	16.3±13	15.5±8.8
Total	13.7±6.8	14.2±9.9	18.2±14	16.1±8.9
Ammonia				
µg/g	622±158	699±288	571±166	841±201
mg/d	82±31	60±45	103±60	97±46
SCFA (branch chain)				
iso-butyrate mmol/l	1.68±0.5	1.86±0.7	1.75±0.5	2.2±0.5
iso-valerate mmol/l	2.28±0.7	2.5±1.1	2.3±0.7	2.9±0.8
iso-butyrate mmol/d	0.22±0.09	0.20±0.1	0.33±0.3	0.27±0.2
iso-valerate mmol/d	0.30±0.13	0.27±0.1	0.43±0.3	0.35±0.2
iso-butyrate %	2.4±1.1	2.2±1.0	2.4±0.9	2.7±0.9
iso-valerate %	3.4±1.8	3.1±1.7	3.3±1.4	3.7±1.5

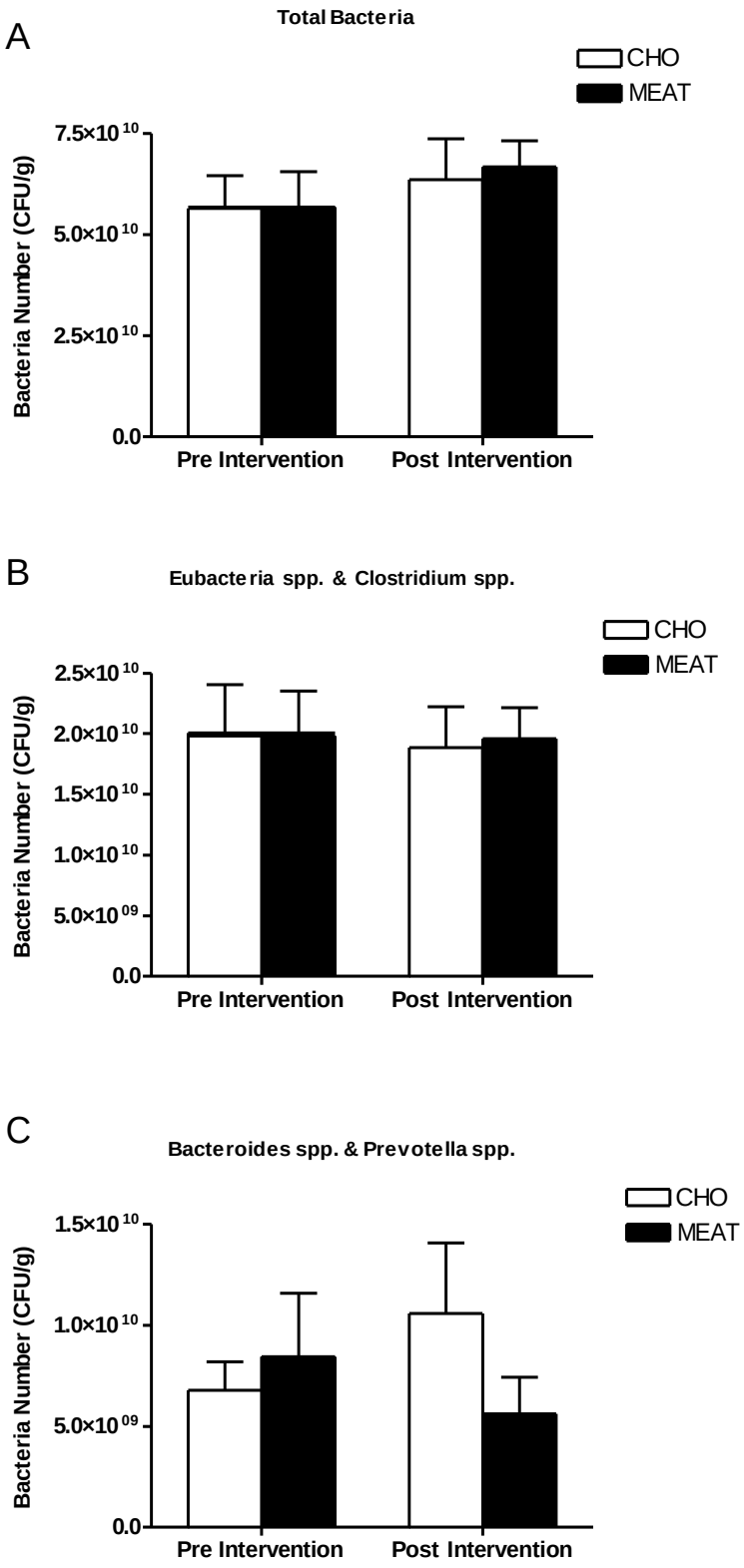

Figure 31. Number of total bacterial colonies (A), Eubacteria spp. & Clostridium spp. (B) and Bacteroides spp. & Prevotella spp. (C) in the faecal samples of older women consuming a high carbohydrate diet (CHO) or high red meat diet (MEAT) before and after a 12 week of resistance exercise training intervention.

3.5 ZINC STATUS

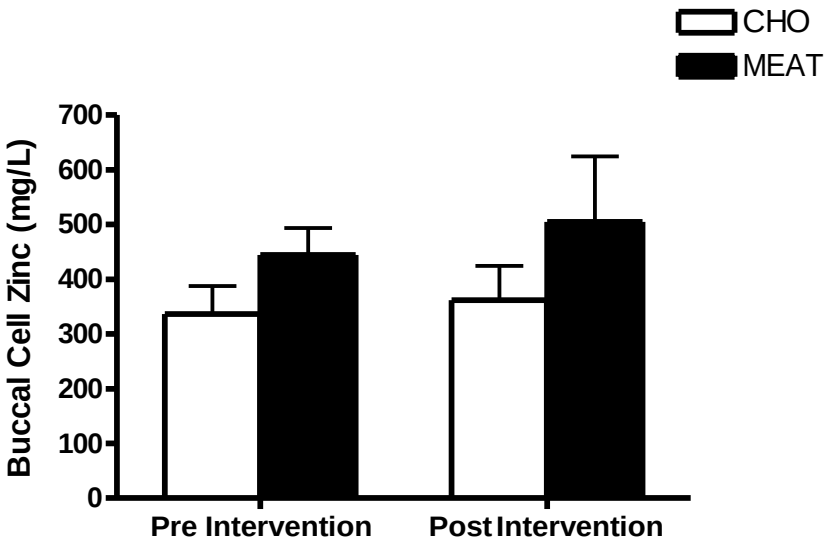

Figure 32. Buccal cell zinc content in older women consuming a high carbohydrate diet (CHO) or high red meat diet (MEAT) before and after a 12 week of resistance exercise training intervention.

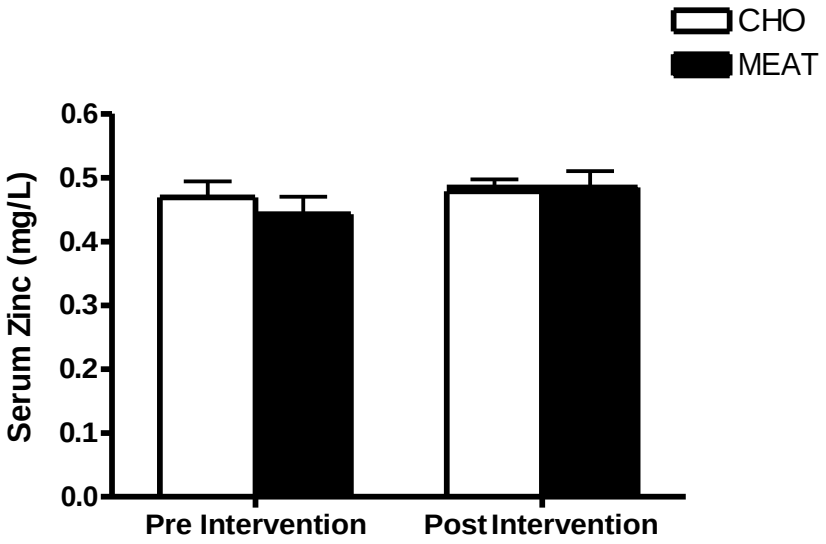

Figure 33. Serum zinc concentrations in older women consuming a high carbohydrate diet (CHO) or high red meat diet (MEAT) before and after a 12 week of resistance exercise training intervention.

3.6 INFLAMMATION

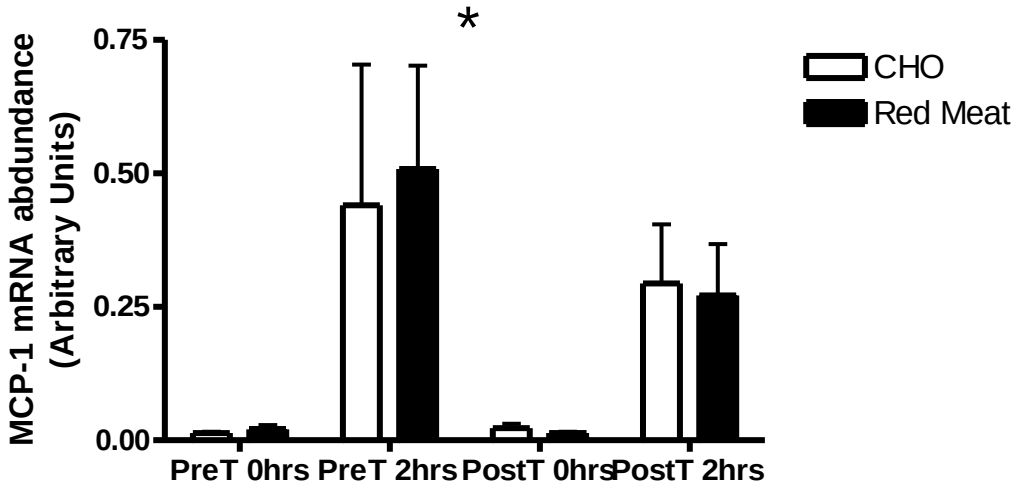

Figure 34. Pre Training (T):MCP-1 mRNA expression pre and 2 hrs post leg extension exercise (CYBEX) and following a red meat or CHO meal. Post Training (T): MCP-1 mRNA expression following12 wks of resistance training, in conjunction with consuming a high carbohydrate diet (CHO) or high red meat diet (MEAT), in older women- pre and 2 hrs post leg extension exercise (CYBEX) and following a red meat or CHO meal.
* indicates a significant time difference between 0hrs and 2 hrs both pre and post training (*P*=0.0017).

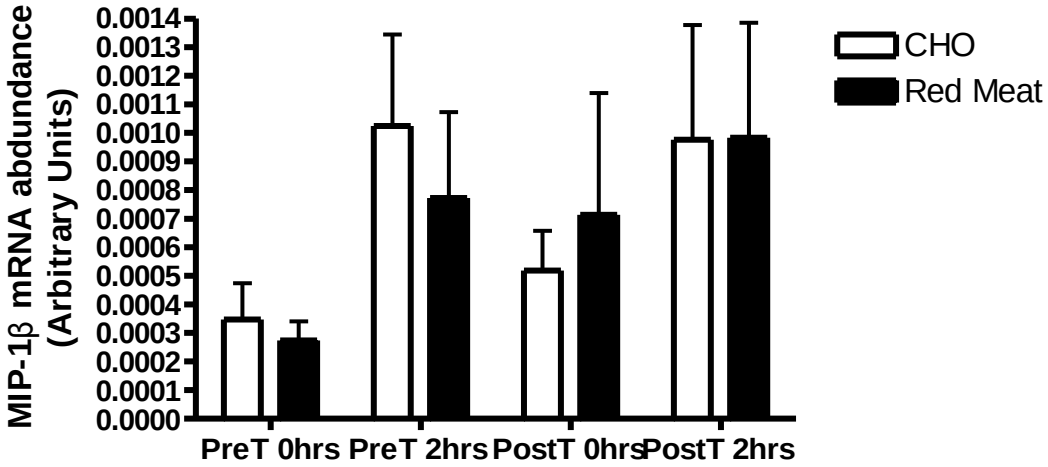

Figure 35. Pre Training (T):MIP-1β mRNA expression pre and 2 hrs post leg extension exercise (CYBEX) and following a red meat or CHO meal. Post Training (T): MIP-1β mRNA expression following12 wks of resistance training, in conjunction with consuming a high carbohydrate diet (CHO) or high red meat diet (MEAT), in older women- pre and 2 hrs post leg extension exercise (CYBEX) and following a red meat or CHO meal.
endency for a time effect between 0 hrs and 2 hrs (*P*=0.07).

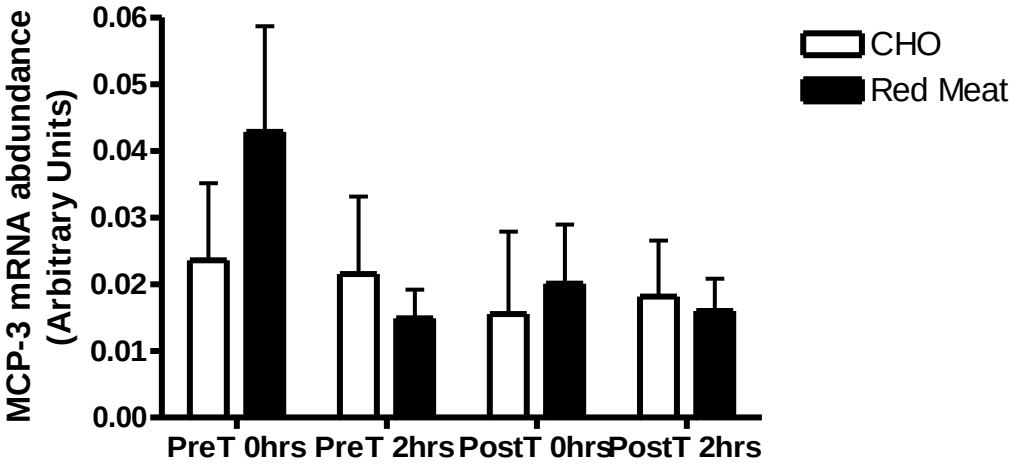

Figure 36. Pre Training (T):MCP-3 mRNA expression pre and 2 hrs post leg extension exercise (CYBEX) and following a red meat or CHO meal. Post Training (T): MCP-3 mRNA expression following12 wks of resistance training, in conjunction with consuming a high carbohydrate diet (CHO) or high red meat diet (MEAT), in older women- pre and 2 hrs post leg extension exercise (CYBEX) and following a red meat or CHO meal.

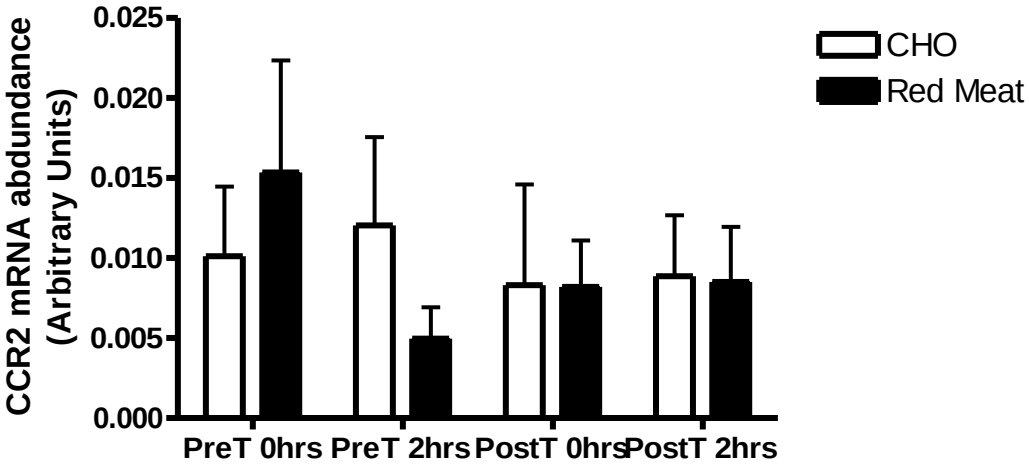

Figure 37. Pre Training (T):CCR2 mRNA expression pre and 2 hrs post leg extension exercise (CYBEX) and following a red meat or CHO meal. Post Training (T): CCR2 mRNA expression following12 wks of resistance training, in conjunction with consuming a high carbohydrate diet (CHO) or high red meat diet (MEAT), in older women- pre and 2 hrs post leg extension exercise (CYBEX) and following a red meat or CHO meal.

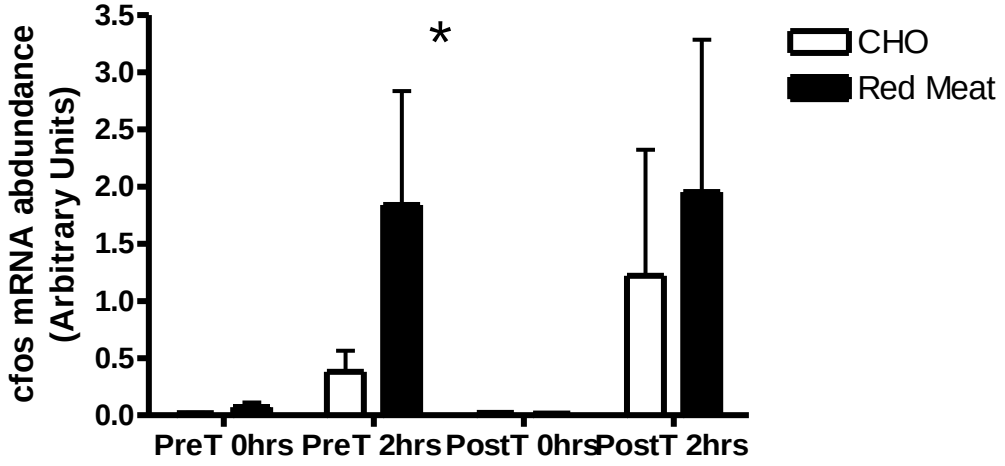

Figure 38. Pre Training (T):cfos mRNA expression pre and 2 hrs post leg extension exercise (CYBEX) and following a red meat or CHO meal. Post Training (T): cfos mRNA expression following12 wks of resistance training, in conjunction with consuming a high carbohydrate diet (CHO) or high red meat diet (MEAT), in older women- pre and 2 hrs post leg extension exercise (CYBEX) and following a red meat or CHO meal.

* indicates a significant time difference between 0hrs and 2 hrs both pre and post training (*P*=0.0376).

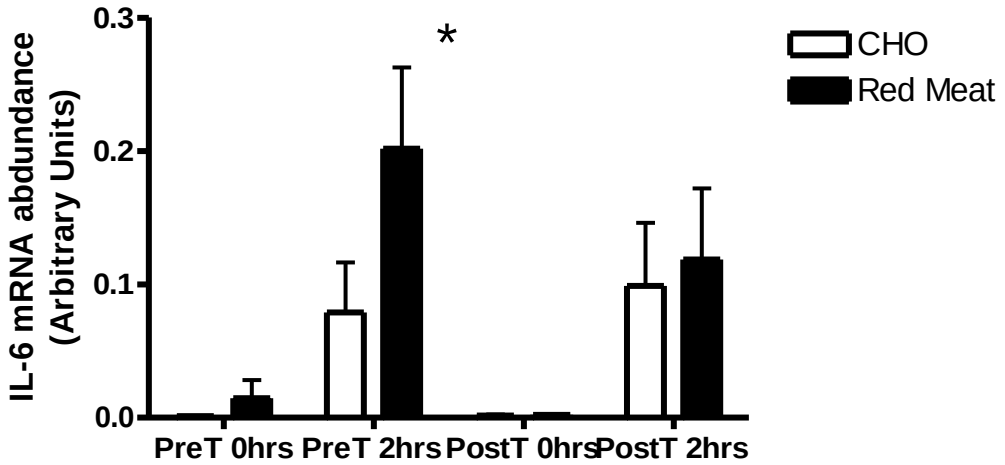

Figure 39. Pre Training (T):IL-6 mRNA expression pre and 2 hrs post leg extension exercise (CYBEX) and following a red meat or CHO meal. Post Training (T): IL-6 mRNA expression following12 wks of resistance training, in conjunction with consuming a high carbohydrate diet (CHO) or high red meat diet (MEAT), in older women- pre and 2 hrs post leg extension exercise (CYBEX) and following a red meat or CHO meal.

* indicates a significant time difference between 0hrs and 2 hrs both pre and post training (*P*=0.0004).

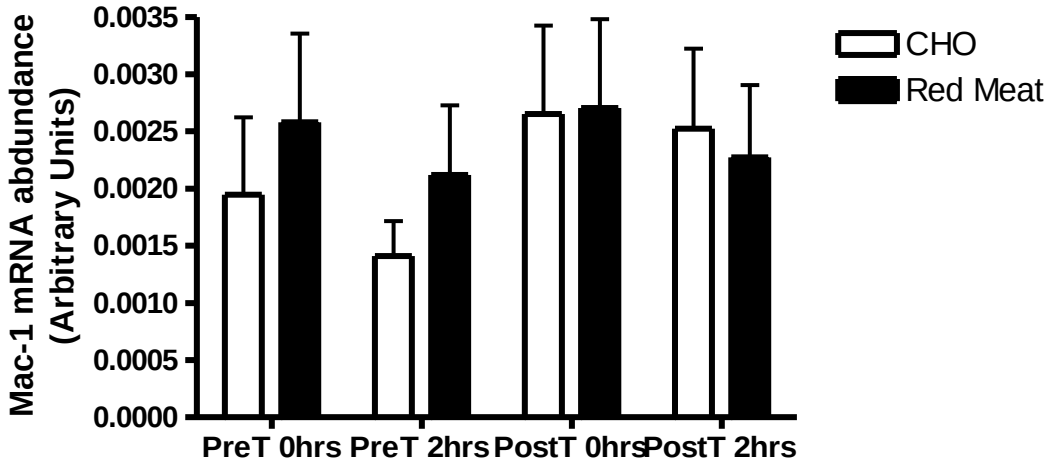

Figure 40. Pre Training (T):Mac-1 mRNA expression pre and 2 hrs post leg extension exercise (CYBEX) and following a red meat or CHO meal. Post Training (T): Mac-1 mRNA expression following12 wks of resistance training, in conjunction with consuming a high carbohydrate diet (CHO) or high red meat diet (MEAT), in older women- pre and 2 hrs post leg extension exercise (CYBEX) and following a red meat or CHO meal.

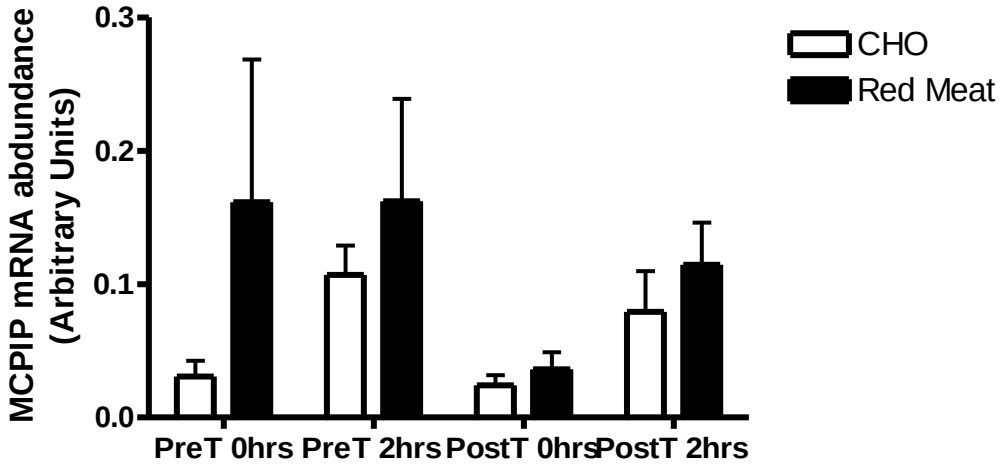

Figure 41. Pre Training (T):MCPIP mRNA expression pre and 2 hrs post leg extension exercise (CYBEX) and following a red meat or CHO meal. Post Training (T): MCPIP mRNA expression following12 wks of resistance training, in conjunction with consuming a high carbohydrate diet (CHO) or high red meat diet (MEAT), in older women- pre and 2 hrs post leg extension exercise (CYBEX) and following a red meat or CHO meal.

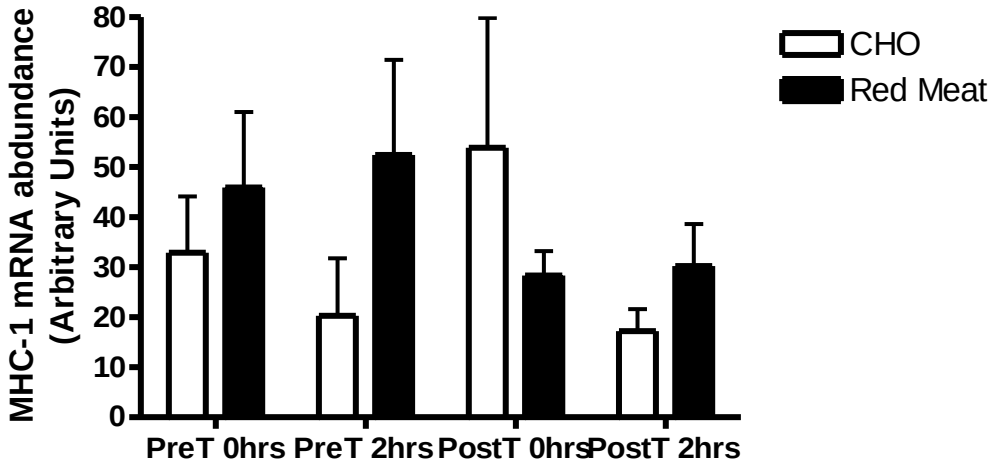

Figure 42. Pre Training (T):MHC-1 mRNA expression pre and 2 hrs post leg extension exercise (CYBEX) and following a red meat or CHO meal. Post Training (T): MHC-1 mRNA expression following12 wks of resistance training, in conjunction with consuming a high carbohydrate diet (CHO) or high red meat diet (MEAT), in older women- pre and 2 hrs post leg extension exercise (CYBEX) and following a red meat or CHO meal.

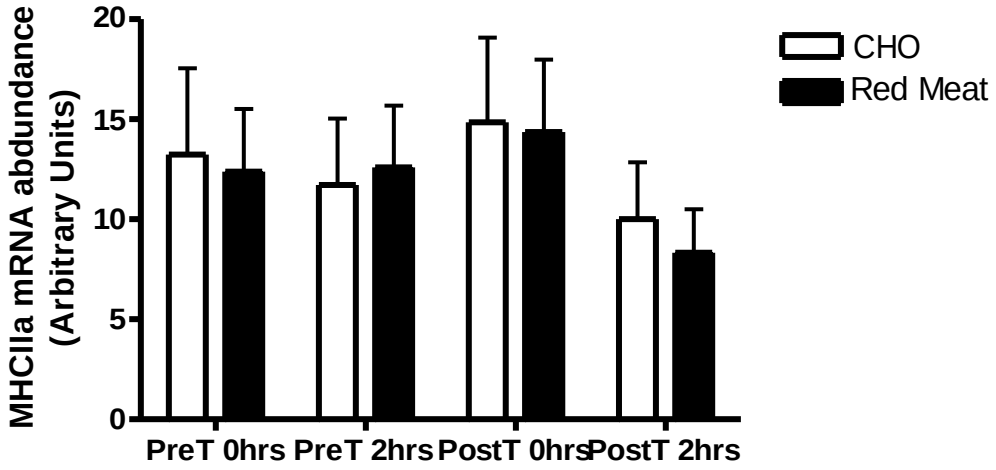

Figure 43. Pre Training (T):MHCIIa mRNA expression pre and 2 hrs post leg extension exercise (CYBEX) and following a red meat or CHO meal. Post Training (T): MHCIIa mRNA expression following12 wks of resistance training, in conjunction with consuming a high carbohydrate diet (CHO) or high red meat diet (MEAT), in older women- pre and 2 hrs post leg extension exercise (CYBEX) and following a red meat or CHO meal.

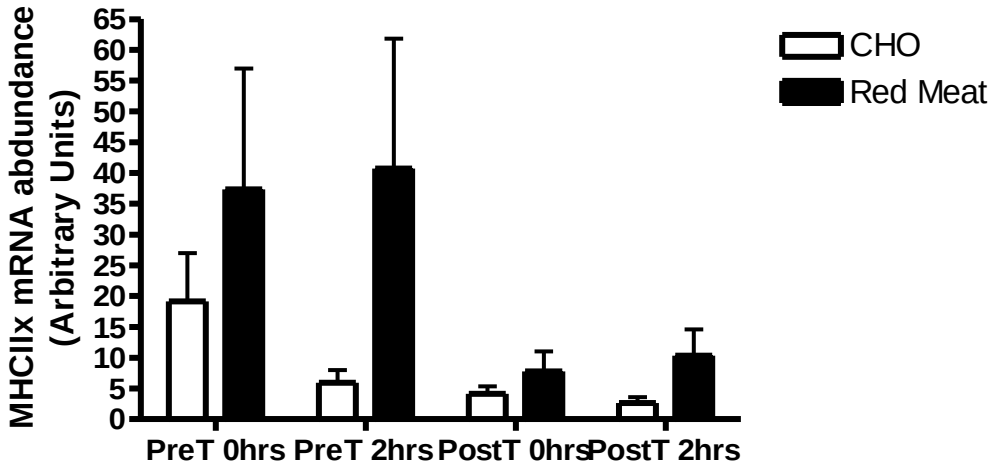

Figure 44. Pre Training (T):MHCIIx mRNA expression pre and 2 hrs post leg extension exercise (CYBEX) and following a red meat or CHO meal. Post Training (T): MHCIIx mRNA expression following12 wks of resistance training, in conjunction with consuming a high carbohydrate diet (CHO) or high red meat diet (MEAT), in older women- pre and 2 hrs post leg extension exercise (CYBEX) and following a red meat or CHO meal.

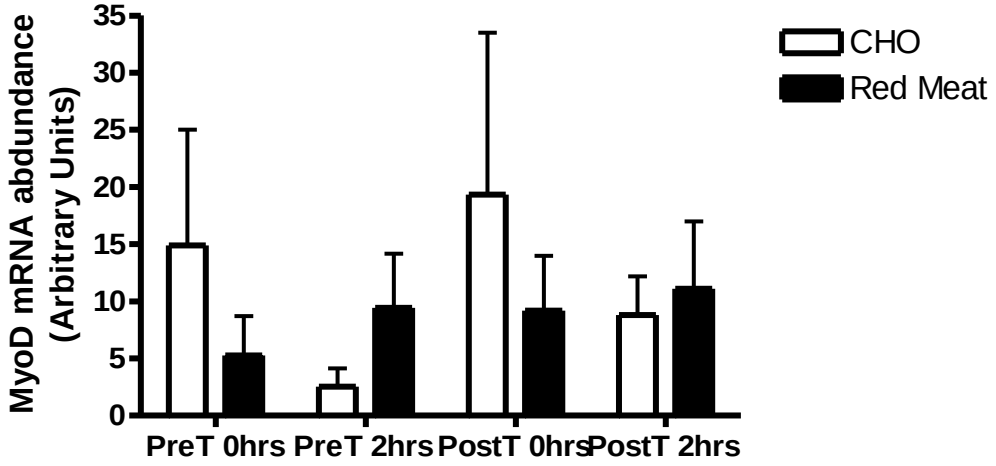

Figure 45. Pre Training (T):MyoD mRNA expression pre and 2 hrs post leg extension exercise (CYBEX) and following a red meat or CHO meal. Post Training (T): MyoD mRNA expression following12 wks of resistance training, in conjunction with consuming a high carbohydrate diet (CHO) or high red meat diet (MEAT), in older women- pre and 2 hrs post leg extension exercise (CYBEX) and following a red meat or CHO meal.

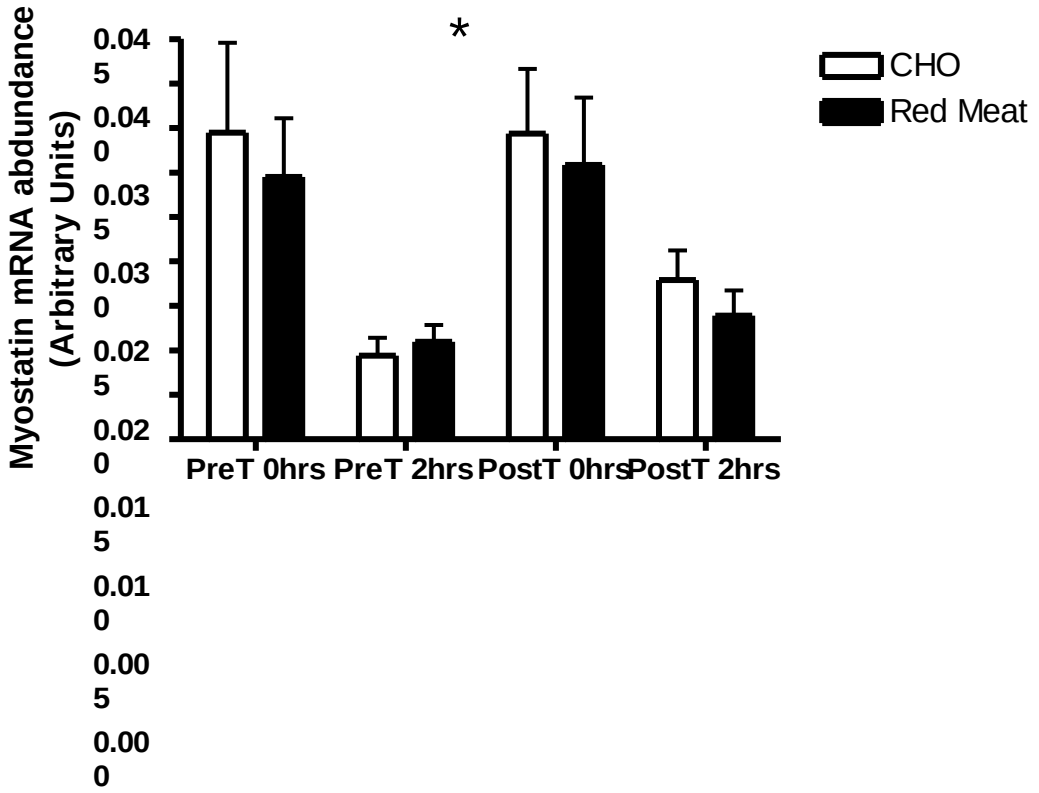

Figure 46. Pre Training (T):Myostatin mRNA expression pre and 2 hrs post leg extension exercise (CYBEX) and following a red meat or CHO meal. Post Training (T): Myostatin mRNA expression following12 wks of resistance training, in conjunction with consuming a high carbohydrate diet (CHO) or high red meat diet (MEAT), in older women- pre and 2 hrs post leg extension exercise (CYBEX) and following a red meat or CHO meal.

* indicates a significant time difference between 0hrs and 2 hrs both pre and post training (*P*=0.0003).

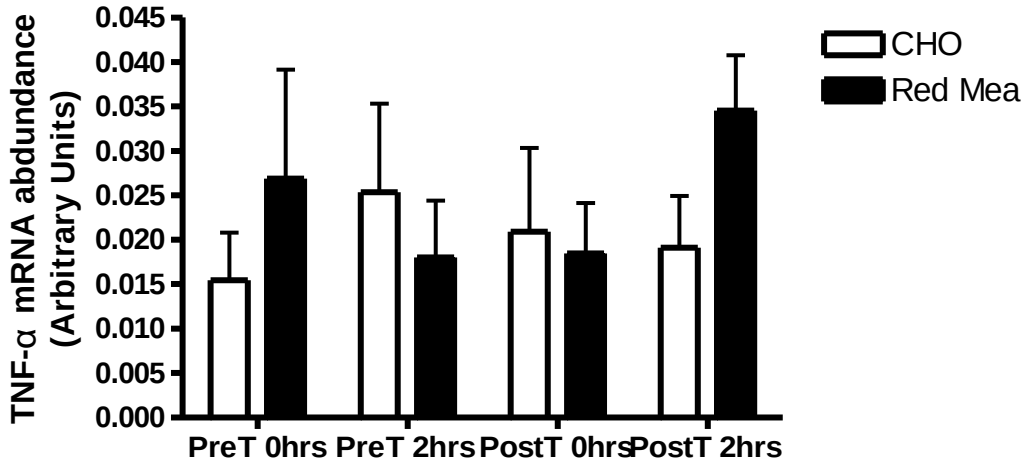
TNF-α mRNA abdundance (Arbitrary Units)
0.045
0.040
0.035
0.030
0.025
0.020
0.015
0.010
0.005
0.000
PreT 0hrs
PreT 2hrs
PostT 0hrs
PostT 2hrs
CHO
Red Mea

Figure 47. Pre Training (T):TNF-α mRNA expression pre and 2 hrs post leg extension exercise (CYBEX) and following a red meat or CHO meal. Post Training (T): TNF-α mRNA expression following12 wks of resistance training, in conjunction with consuming a high carbohydrate diet (CHO) or high red meat diet (MEAT), in older women- pre and 2 hrs post leg extension exercise (CYBEX) and following a red meat or CHO meal.

Summary of Results

BODY COMPOSITION

Both groups lost a small percentage of body weight, however no differences were detected between groups (Figure 1). DEXA scan analysis demonstrated a decrease in overall fat mass (Figure 2) and approximately a 2% increase in total lean mass (Figure 3), however no differences were observed between groups. Both groups demonstrated a loss in arm fat mass (Figure 4) and an increase in arm lean mass (Figure 5) (no differences were detected). Similarly an overall decline in leg fat mass (Figure 6) and approximately a 3% increase in leg lean mass (Figure 7). Figure 8 illustrates an overall 2-3% increase in relative (i.e. to height) appendicular muscle in both groups. CT scan analysis revealed a 4.5-5% increase in thigh muscle CSA following the intervention (Figure 9). Muscle attenuation (i.e. muscle quality) increased by 3.5% and 4.2% in the CHO and Red Meat group respectively (Figure 10). Both groups increased their slow twitch and fast twitch muscle fiber CSA following 12 wks resistance training (Figures 11-12), no differences were detected between groups. Figure 13 shows that the Red Meat group significantly increased their percentage of ST muscle fibers (P=0.0016) in comparison to the CHO group. Fiber analysis also revealed that the CHO group decreased and the red meat group increased their percentage of FT fibers (Figure 14).

STRENGTH

GymAware analysis revealed that the red meat group increased both their concentric and eccentric mean force on the leg press machine by approximately 25% more than the CHO group (P=0.08) (Figures 15 & 17, respectively). Further analysis revealed that the CHO group had significantly reduced improvement in mean force (concentric and eccentric) in the final 6 weeks on the leg press machine (P=0.03) (Figures 16 and 18, respectively).

Both groups increased their concentric and eccentric mean force on the leg extension machine by approximately 106%, no differences were detected between groups (Figures 19-22).

Total improvement in concentric peak torque (i.e. rotational force) measured on the CYBEX increased by approximately 35% in the CHO group and 46.5% in the red meat group (Figure 23). Overall CYBEX testing indicated that the women increased their eccentric peak torque by approximately 43% however no differences were detected between groups (Figure 24). Post training CYBEX investigation also demonstrated an 18% and 38% approximate improvement in concentric and eccentric work, respectively (Figure 25 & 26).

Repetition maximum (RM) tests demonstrated an overall 80.42% and 64.96% increase in the weight lifted on the leg press and leg extension machine, respectively (Figure 27 & 28).

DIET

Figure 29A illustrates that the energy content of the meals were matched for each group. The Red Meat group consumed significantly more protein, iron and zinc than the CHO group on each of the testing days throughout the study (P<0.0001) (Figures 29B, 30A, 30B). The impact the three intervention days on overall diet was assessed using both 3-day diet diaries and FFQ's. The diary specifically included one test day, whilst the FFQ's were administered to reflect habitual eating patterns and possible impact of the intervention. The total energy intakes were not significantly different between measures, nor did the intervention demonstrate any impact. The Food Diary analysis demonstrated a tendency for greater 30day protein ingestion, iron and zinc. This however only reached statistical significance for protein 6 weeks into the study. Analysis of habitual diet by FFQ demonstrated no changes.

BOWEL HEALTH

Post intervention analysis demonstrated that the there were no alterations in wet and dry faecal weight (g/d) with exercise or dietary modification (Table 3.1). Similarly defecation frequency was unaltered. Further analysis of faecal short-chain fatty acids, biomarkers of bowel carbohydrate fermentation, again demonstrated no change (Table 3.2). Specific markers of protein fermentation, most commonly linked to bowel cancer risk, again failed to demonstrated changes with exercise or diet (table 3.3).

Bacterial species in the faecal matter were assessed using specific fluorescent probes (stains) that bind to specific bacterial species. Following specific staining and counting, no significant differences were observed for total bacteria number, *Eubacteria* spp & *Clostridium* spp and *Bacteriodes* spp & *Prevotella* spp. throughout the intervention (Figure 31 A-C).

ZINC STATUS

Figures 32 and 33 illustrate that zinc levels were not different between groups or altered as a result of the intervention. Interestingly, the plasma zinc concentrations demonstrated in this population were low (typical definition of low serum Zinc; $\leq$ 10.7 mmol/L; 0.7mg/L (80)).

INFLAMMATION

RT-PCR analysis indicated a significant increase in the expression of MCP-1 (P=0.0017), cfos (P=0.0376) and IL-6 (P=0.0004) at 2 hours post exercise before and after training (Figures 34, 38, 39 respectively). MIP-1β also demonstrated a strong trend for increased expression post exercise (P=0.07) (Figure 35). Myostatin mRNA concentration significantly decreased 2hrs post exercise before and after training (P=0.0003) (Figure 46). MCP-3, CCR2, Mac-1, MCPIP, MHC-I, MHCIIa, MHCIIx, MyoD and TNF-α did not demonstrate any time or treatment effects as a result of the intervention (Figures 36, 37, 40, 41, 42, 43, 44, 45, 47, respectively).

4 DISCUSSION & CONCLUSIONS

The Australian population is rapidly ageing, increasing the burden on the existing medical infrastructure. Interventions aimed at counteracting frailty in an ageing will be significant for the health of the individual and may impact significantly in the population burden of disease. Combined resistance exercise training and enhanced protein ingestion at the time of this exercise can maximise the rate of protein synthesis. This may translate to greater muscle mass and strength gains. Furthermore, experimental research suggests that proteins ingested at the time of exercise may ameliorate the inflammatory (or painful) response to this exercise. This research therefore aimed to provide only three meals per week of high-quality protein (lean red meat) to post-menopausal women as part of an individually supervised strength training program.

The intervention was exceptionally well tolerated, with only 2 women withdrawing, due to influenza and arthritic pain. Compliance was outstanding with only 2 sessions being missed by the entire cohort over the 12 week intervention (36 sessions per person). The women relished the company, the exercise, the attention, and the noticeable gains in strength, 'trimness' and 'energy-levels 'associated with strength training. An important component of the intervention was the meals, with women going to a staff dining area and enjoying table service. The women thoroughly enjoyed each meal, whether randomised to the red-meat or the vegetarian group. This is thanks to the skill and energy of Charlotte Miller (APD) who generated an enjoyable and nutritious schedule of energy-matched meals. One key aspect of this study was the donation of muscle biopsies on commencement and completion of the study. No adverse events were reported and compliance was again 100%.

OUTCOME 1 - *Investigate the effects of a high red meat diet, in conjunction with resistance training on muscular strength in women aged 60-75 yrs.*

Analysis of strength gains is complex, thus the present study utilised isodynamic testing (Cybex dynanometer), analysis of force generated within the training session using force-analysis technology (GymAware) and maximal weight lifted under controlled conditions (repetition maximum; RM) were all utilised in this study. Using these technologies demonstrated a trend for greater leg strength (leg press) in those subjects randomised to the red meat meal (Figures 15-18). Concentric peak torque (rotational force whilst pushing away from the body) was 11% greater in the red meat group than the CHO group as measured on the CYBEX dynamometer (Figure23). Over the 12 week intervention there was an average gain in leg strength of 106.5% and 120% when the sophisticated GymAware equipment was employed on the leg extension and leg press equipment (Figures 19, 21, 15, 17). This equipment uses infra-red detection of movement which is downloaded in real-time to a palm pilot PDA. Based on the pre-entered parameters, including the weight to be lifted, accurate data on the power, acceleration and velocity achieved is recorded. Interestingly the current

study displayed a 25% greater total improvement in concentric and eccentric mean force, as measured using GymAware equipment on the leg press machine in the red meat group (*P*=0.08) (Figures 15 & 17).

OUTCOME 2 - *Investigate the effects of a high red meat diet, in conjunction with resistance training on body composition (i.e. muscle mass, fat mass, muscle CSA, fiber CSA and fiber type) in women aged 60-75 yrs.*

Analysis of muscular mass and strength were the major outcome variables of this intervention. Whilst weight loss was not specifically expected, all women tended to lose a small amount of body weight (Figure 1). All women reported favourably about 'toning'. Weight loss didn't differ between groups, although the reduction in fat mass was 4.3% (See Figure 2). DEXA scan analysis suggested a tendency for the CHO group to have a larger decrease in arm fat mass (P=0.0563) however the red meat group had a slightly greater increase in arm lean mass (2.4% increase vs 1.2% increase) (Figure 4 and 5). The gains in leg muscle mass did not differ between groups, but was a marked 4.9% average gain in muscle cross-sectional area (CSA) (Figure 7 and 9). More detailed analysis of the muscle quality (CT scan attenuation, fibre-type number and CSA) failed to demonstrate any significant dietary effects (Figure 10).

OUTCOME 3 - *Investigate the effects of a single eccentric exercise bout and a post exercise red meat meal on the expression of several skeletal muscle inflammatory genes in women aged 60-75 yrs.*

OUTCOME 4 - *Investigate the effects of 12 weeks of resistance training, in conjunction with a high red meat diet, on several skeletal muscle inflammatory genes in women aged 60-75 yrs.*

Previous research has suggested that repeated eccentric contractions cause small micro tears to the muscle fibre resulting in an inflammatory response. This study analysed the main mediators of muscle inflammation and regeneration. It has been demonstrated that protein ingestion attenuates muscular inflammation and accelerates the expression of genes necessary for muscle repair. Despite extensive analysis of a wide spectrum of candidate genes for inflammation, hypertrophy and muscular structural adaptation, no differences were evident (Figures 34-47). Thus we are unable to provide a molecular basis for the tendency to see greater gains in muscle strength, nor were we able to demonstrate any dietary effects plausible regulators of muscle inflammation.

OUTCOME 5 - *Analysis of the changes in buccal and plasma zinc (in collaboration with Asoc. Prof. Leigh Ackland, School of Science and Technology, Deakin University)*

Red meat makes a substantial contribution to zinc requirements (81). In the present study, the inclusion of a single high protein meal led to a 3-fold increase in Zinc intake on the day of exercise, however 3-day diet diary analysis demonstrated only marginal (NS) changes in total zinc intake (approx 12.5mg per day). With this level of zinc ingestion the plasma zinc concentrations of both the carbohydrate and red-meat group were low. Analysis of tissue abundance, by measuring buccal zinc failed to demonstrate any changes with exercise or the dietary intervention. Given the importance of zinc for optimal physiological and psychological functions (82), demonstrating the value of lean red meat in the maintenance of optimal zinc in an aged cohort is of public health significance and warrants further analysis (83) .

OUTCOME 6 - *Analysis of the faecal microflora to determine whether either diet impact on the population density of known beneficial (pro-biotic) or detrimental bacterial species (in collaboration with Dr Stuart Smith, School of Exercise and Nutrition Sciences, Deakin University).*

OUTCOME 7 - *Measurement of faecal biomarkers of bowel health and colonic cancer risk (in collaboration with Dr Jane Muir, Department of Gastroenterology, Box Hill Hospital).*

A major consideration of increased meat intake has the speculation of greater risk of bowel cancer but until now there has not been enough significant studies undertaken on changes in the gut microbiota that may be involved in influencing bowel health. This study utilised quantitative FISH analyses of the predominant bacterial species in faecal samples collected from subjects at pre-diet (baseline) and post diets (after either on of the two diets). Neither the meat-based diet nor the CHO diet significantly modified many of the predominant bacterial sub-populations most commonly associated with bowel ill-health (clostridium species). However, there was an important trend for greater Bacteroides and Prevotella spp. with the carbohydrate diet, whilst these species fell in the meat group. Bacteroides species are predominantly described as beneficial for vitamin B production but they contain many medically important pathogens and are associated with formula-fed infants, production of intestinal carcinogens and intestinal putrefaction. ‘Healthy’ bacterial populations are commonly associated with their reduction in diets high in fibre which increases beneficial bifidobacteria at there expense but they may also be influenced by the meat-rich diet. These analyses were not significant due to the small study group but such results warrant further investigation in larger population cohorts in crossover studies.

A number of faecal markers related to protein and carbohydrate metabolism and believed to be relevant to CRC risk were assessed. These included by-products of protein fermentation (phenol, cresol and ammonia) that can accumulate in the colon and may be potentially harmful. Also measured were; faecal short chain fatty acids (SCFA)-acetate, propionate and butyrate, faecal pH, undigested fibre residues (resistant starch and non-starch

polysaccharides) and faecal output. There were no significant differences between the dietary treatment groups.

5 RECOMMENDATIONS

Meals containing in excess of 80 grams of lean red meat was tolerated by women greater than 60 years of aged, three times weekly. When consumed within 30 minutes of exercise, there was a tendency for greater gains in leg strength, although these outcomes failed to reach significance over the entire study. Analysis of strength gains over just the last 6 weeks suggested a trend for continued gains in strength in the red meat group, whilst the carbohydrate group had diminished gains.

Future studies should be designed to address the actions of consuming lean red meat on muscular mass, strength and body composition over a period in excess of 12 weeks. These studies ideally should be performed in both an actively exercising and sedentary cohort. Based on the data described in the current study it is possible to accurately predict study intervention size that may demonstrate statistically significant gains in strength. It is premature to conclude that red meat, when consumed after exercise is beneficial for muscular strength gains, although interesting trends were evident.

Studies with prebiotic dietary fibres have already shown changes in beneficial microbiota such as bifidobacteria in Dr Smith's laboratory at Deakin University and he is now examining proprietary dietary fibres to that end. Dr Smith also considers that other dietary changes between vegetarians and meat eaters is required to examine the value of meat–rich diets on gut microbiota which can heavily influence gut and bowel health. Certainly, there has been a long-held assumption that vegetarian diets are more beneficial compared with meat diets but this is dubious as meat protein-rich diets are important in weight loss (CSIRO-total protein diet). However, we know little of the beneficial changes in gut microbiota linked to these diets and programs and proper studies including placebo-controlled crossover dietary-interventions have yet to be undertaken to examine the full benefits of meat-based diets.

Certainly the preliminary studies here suggest that the CHO diet (rich in readily-degraded polysaccharides) is enhancing the growth of potentially pathogenic organisms such as *Bacteroides* spp. and *Prevotella* spp. whilst the meat-rich diet caused a reduction in these same bacteria although a crossover study with the same subjects is required to strengthen this data. Similarly, the data is missing the opportunity to analyse the influence of meat-rich diets on the probiotic bacteria such as Bifidobacteria and Lactobacillus. These studies are important and we believe we can assist the MLA in addressing these questions.

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