Resistance-exercise training: The effects on muscle function, body composition and risk factors for chronic disease with ageing.

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RESISTANCE-EXERCISE TRAINING: THE EFFECTS ON BODY COMPOSITION, MUSCLE FUNCTION AND RISK FACTORS FOR CHRONIC DISEASE WITH AGEING.

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IV. ABBREVIATIONS

α-KIC	alpha-ketoisocaproate
1-RM	One-repetition maximum
4EBP-#	4E binding protein-
AA	Amino acid
A-band	Anisotropic band
Acrp30	Adiponectin
AdipoQ	Adiponectin
ALBM	Appendicular lean body mass
Apo	Apolipoprotein
ATP	Adenosine tri-phosphate
BC	Body composition
BCAA	Branched-chain amino acid
β-cells	Beta-cells
BF	Body fat
BMC	Bone mineral content
BMD	Bone mineral density
BMI	Body mass index
BMR	Basal metabolic rate
BP	Blood pressure
BPM	Beats per minute
CD4	Cluster of differentiation 4
CETP	Cholesterol-ester transfer protein
CEUS	Contrast-enhanced ultrasound
CHD	Coronary heart disease
CHO	Carbohydrate
COPD	Chronic obstructive pulmonary disease
CRP	C-reactive protein
CT	Computerized tomography
CV	Cardiovascular
CVD	Cardiovascular disease
Db	Body density
DBP	Diastolic blood pressure
DEXA	Dual-energy X-ray absorptiometry

DNA	Deoxyribonucleic acid
DRI	Dietary recommended intake
EAA	Essential amino acid
ECG	Electrocardiogram
EDTA	Edetic acid
EIA	Enzyme immunoassay
ELISA	Enzyme-linked immuno-sorbent assay
eNOS	Endothelial nitric oxide
FFA	Free fatty-acids
FFM	Fat-free mass
FM	Fat mass
FOXO-#	Forkhead box-
FSR	Fractional synthetic rate
G6P	Glucose-6-phosphate
HCL	Hydrochloric acid
HDL	High-density lipoprotein
HGH	Human growth hormone
HL	Hepatic lipase
HOMA	Homeostasis model assessment
HR	Heart rate
HRP	Horseradish peroxidase
I-band	Isotropic band
ICAM-#	Intracellular adhesion molecule-
IGF-#	Insulin-like growth factor-
IGT	Impaired glucose tolerance
IL-#	Interleukin-
IMGU	Insulin-mediated glucose uptake
iNOS	Inducible nitric oxide synthases
InR	Insulin receptor
IPAQ	International physical activity questionnaire
IRS-#	Insulin receptor substrate
KO	Knock-out
LBF	Leg blood flow
LBM	Lean body mass

LDL	Low density lipoprotein
LDLR	Low density lipoprotein receptor
LiHep	Lithium heparin
LPR	Leg peripheral resistance
LRC	Lipid Research Centre
LTM	Lean tissue mass
LVC	Leg vascular conductance
MBV	Mean blood velocity
MCP-#	Monocyte chemo-attractant protein
MET	Unit of metabolic equivalent
MHC	Myosin heavy chain
MI	Myocardial infarction
mmHg	Millilitres of mercury
MPB	Muscle protein breakdown
MPS	Muscle protein synthesis
MRI	Magnetic resonance imageing
mRNA	Messenger ribonucleic acid
mTOR	Mammalian target of rapamycin
NADH	Nicotinamide adenine dinucleotide
NEFA	Non-estrified fatty acids
NF-κB	Nuclear factor-kappaB
NO	Nitric oxide
PA	Postabsorptive
PAI-#	Plasminogen activator inhibitor-
PAL	Physical activity level
PBS	Phosphate buffered saline
PDCAAS	Protein digestibility corrected amino acid score
PDK-#	Phosphoinositide dependent protein kinase-
PECAM	Platelet endothelial cell adhesion molecule
PGE2	Prostaglandin E2
PI3K	Phosphoinositide 3-kinases
QUICKI	Quantitative insulin sensitivity check index
RBP-#	Retinol binding protein-
REE	Resting energy expenditure

RET	Resistance-exercise training
RHR	Resting heart rate
ROI	Region of interest
ROS	Reactive oxygen species
SBP	Systolic blood pressure
SKF	Skinfold
SR	Sarcoplasmic recticulum
STM	Soft-tissue mass
TBBM	Total body bone mineral
t-BDMS	Tert-butyldimethylsilyl
TC	Total cholesterol
TEE	Total energy expenditure (daily)
Th-#	T-helper cells
TLR	Toll-like receptor
TNF-α	Tumour necrosis factor-α
TNFR	Tumour necrosis factor receptor
TRL	Triglyceride-rich lipoprotein
tRNA	Transfer ribonucleic acid
T-Tubules	Transverse tubules
VLDL	Very low-density lipoprotein
WHO	World Health Organisation

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CHAPTER 1- STUDY DESIGN

1.1 Setting the scene: Physical activity, health and ageing

Physical activity and physical fitness are said to have long been linked with health; the earliest records of exercise for health promotion stem from ancient China in 2'500 BC (Hardman & Stensel, 2003;Grunfeld & Feingold, 1991). A modern trend with chronic health and economic implications is that of the worldwide demographic change, of increased life expectancy and an ageing population. Although this trend cannot be altered by physical activity, being more active may go some way to alleviate a number of age-associated problems. More than 580 million people worldwide are older than 60 years and this number is expected to rise to 1000 million by 2020 (Knoops *et al.*, 2004). The UK, like many other developed countries has an ageing population, with the median age of the UK population having risen from 34.1 years to 38.6 years in the last three decades (http://www.statistics.gov.uk).

There is much controversy regarding the cost of both clinical and social care for elderly individuals when they can no longer maintain the necessary functions to survive independently or need care as they age. Ageing is one of the most important social and financial problems facing western society- it is both intransient and associated with considerable morbidity (Greiwe *et al.*, 2001). In the UK, hip fractures alone, in the elderly, cost approximately £280 million per annum (Hollingworth *et al.*, 1995). Consensus has been reached supporting any method which may prevent this decline in independence maintenance (a state of health and well-being that allows individuals to sustain normal daily activity and functional independence (Sumukadas *et al.*, 2006)), thus having economic, social and health benefits both for the elderly individuals involved and for society as a whole.

Regardless of other predisposing factors, diet and lifestyle influence mortality and morbidity during the course of life. Because of the

cumulative effect of factors throughout life, it is particularly important for older persons to adapt to and adopt diet and lifestyle practices that will minimize their risk of premature death and maximize their chances of healthy, active ageing (Knoops *et al.*, 2004).

One of the most significant and visually apparent changes with ageing is that of reduced muscle mass and historical observations of this loss are common (Evans, 1995b; Narici & Maffulli, 2010). This undesirable loss of muscle mass with ageing is called sarcopenia (Rosenberg, 1997) with the term derived from the Greek words "sarcos" (flesh) and "penia" (lack of). Interest in sarcopenia has been widely apparent since the 1980's but more recently evidence is emerging that dynapenia; the lack of strength with ageing, is a more consistent factor in compromised well-being with age and in some cases the term sarcopenia is now being used to describe losses of both size and strength (Morley *et al.*, 2001). Both the US NIH and the European Working Group on Sarcopenia now recognize sarcopenia as changes in body composition *and* function, with the latter suggesting that three stages of sarcopenia should be recognized: 1. Presarcopenia – the loss of muscle mass, 2. Sarcopenia – the loss of muscle mass accompanied by a loss of either strength or physical performance and 3. Severe Sarcopenia – when all three of the above features are present (Cruz-Jentoft *et al.*, 2010). Not all are in agreement that one term should be used to describe both losses though as it implies proportionality between losses of mass and function which are not always apparent (Narici & Maffulli, 2010) and the term dynapenia as a separate concept has been proposed to refer to functional compromise of the entire neuromuscular apparatus (Clark & Manini, 2008).

If the concepts of dynapenia or staged sarcopenia are to be believed then it is muscle quality, and not just quantity that impacts on quality of life with ageing and force generating capacity per unit cross-sectional area must be key to these declines. With this belief comes even more uncertainty as to the underlying mechanisms of sarcopenia with a raft of mechanisms proposed to be at play, although which if any of these is the most

significant is still to be determined. One suggestion is that there may be genetic determinants of sarcopenia (Gonzalez-Freire *et al.*, 2010), although current reviews suggest that "sarcopenic gene" knowledge is tentative and inconsistent (Tan *et al.*, 2012; Garatachea & Lucia, 2011). A second, and much more accepted mechanism is that of alterations in muscle protein turnover. This has been shown by numerous research groups (Rennie, 2009; Kumar *et al.*, 2009; Rasmussen *et al.*, 2006; Cuthbertson *et al.*, 2005; Guillet *et al.*, 2004) and it is not a question of whether this mechanism contributes to sarcopenia and/ or dynapenia, but more a question of the underlying changes causing this dysregultion. A third proposed mechanism is that of changes in hormone (Lamberts *et al.*, 1997; Haren *et al.*, 2008) and cytokine (Yende *et al.*, 2006; Roubenoff *et al.*, 1998) milieu, especially involving androgens, growth hormone, insulin-like growth factor 1 (IGF-1) and interleukin (IL)-6. Loss of innervation is another proposed mechanism and this in itself can be attributed to a number of age-related changes including: 1. "grouped dennervation atrophy" (Tomlinson *et al.*, 1969), 2. a progressive loss of motor units (Brown, 1972) and 3. a compromised potential for terminal sprouting and reinnervation of muscle fibres (Einsiedel & Luff, 1992). The fifth proposed potential mechanism is that of compromised vascularisation (DeSouza *et al.*, 2002) which may expose ageing muscles to hypoxia and impaired nutrient delivery. The sixth proposed mechanism is increased oxidative stress. With age there is a reduction in cellular anti-oxidant buffering and an increase in free radicals due to dysfunction in the mitochondrial respiratory chain (Barreiro *et al.*, 2006). This increased exposure to oxidative stress may damage the muscles DNA, myofibrillar proteins, myocellular proteins, the neuromuscular junction, elements of the sarcoplasmic reticulum which are responsible for Ca^{+} release and also contribute to motor unit atrophy and the reduced number and function of satellite cells seen in old age (Bornemann *et al.*, 1999). The penultimate proposed mechanism is that of poor nutrition, where despite the increases in body fat seen in old age, there is a decrease in food intake with age. The reasons for the anorexia of ageing are complex but are thought to include visceral, hormonal, neurological, pharmacological and psychological

factors (Morley, 1997), with many older adults failing to meet recommended protein requirements (Roubenoff & Hughes, 2000), which along with Vitamin D deficiency (Visser *et al.*, 2003) has been shown to significantly increase the risk of sarcopenia (Solerte *et al.*, 2008). The final proposed mechanism is that of physical inactivity. There is much evidence to support various modalities of exercise in older people, with improvements reported in muscle volume and torque (Morse *et al.*, 2005), physical performance of activities of daily living (Liu & Latham, 2009) and in the promotion of muscle protein synthesis (MPS) and suppression of the expression of genes related to muscle protein breakdown (MPB) (Harber *et al.*, 2009). Masters athletes have also been found to have protection against the age-related loss of motor unit number (Power *et al.*, 2012) and vascular responsiveness to vasodilatory stimuli (Ebeling *et al.*, 1993), the implications of which are outlined above.

Continuing the theme of physical activity, evidence that resistance-exercise training (RET) is of benefit to older adults has been long documented, with different training types and methods instigating various responses and adaptations. It is well established that RET interventions can partially reverse losses in 1. strength (Fiatarone *et al.*, 1990; Vandervoort, 2002; Porter *et al.*, 1995; Frontera *et al.*, 1988), 2. muscle mass (Hurley & Roth, 2000; Tracy *et al.*, 1999) and 3. power, thus reducing the difficulty in performing daily tasks, enhancing energy expenditure, improving body composition and promoting participation in spontaneous physical activity (Hunter *et al.*, 2004).

RET can be defined as movements performed against a specific external force and has benefits not only for the elderly but for people of all ages, be they performance, health-related or relative to desired changes in body composition. Using RET, both muscular strength and endurance are developed on the principle of overload; this is increasing the frequency, intensity or duration, to above that normally experienced. Every activity, including activities performed in everyday life, requires some degree of strength, power and endurance. The enhancement of muscular strength

and/ or endurance will enable individuals to perform these tasks with less physiological stress and will aid in maintaining functional independence (Franklin, 2000). For the elderly, it is believed that maintenance of muscle mass and strength as we age leads to much improved functional capacity and quality of life (Tipton, 2001), with exercise seeming more effective at preventing muscle loss than restoring lost muscle mass (Wolfe, 2006).

With the exclusion of H_2O, protein is the major component of muscle, so over a given period of time muscle loss will only occur if muscle protein breakdown (MBP) exceeds muscle protein synthesis (MPS). Acute RET has been shown to stimulate MPS, both absolutely and relative to MPB (Phillips *et al.*, 1997); following a resistance training bout or session, with changes in MPS of a much greater magnitude than changes in MPB (Phillips *et al.*, 1999). It has also been frequently suggested in the literature that muscle hypertrophy following chronic RET results from an increase in the basal level of MPS, such that during much of the day following training, muscle is in a more positive net protein balance. Studies have concluded that RET, for as little as two weeks results in an increase in the basal levels of MPS in both the young and the elderly (Yarasheski *et al.*, 1992; Hasten *et al.*, 2000).

Research has shown that short term RET may reverse 2-3 decades of muscle loss (Hikida *et al.*, 2000; Yarasheski *et al.*, 1999; Welle *et al.*, 1995) and in the elderly RET improved their performance of activities required for daily living (Fiatarone *et al.*, 1990; Hunter *et al.*, 2001; Skelton *et al.*, 1995), both those based around strength and aerobic capabilities; as less activation is required to stimulate a stronger muscle, hence delaying fatigue (Frontera *et al.*, 1990). Even in the frail elderly, progressive RET has been shown to increase muscle strength, gait velocity and power and although these improvements in muscular performance were paired with only a modest 2.7% increase in muscle cross-sectional area, the frail elderly did retain the ability to increase their rate of MPS in response to RET, with increases of up to 48% following a 16 week RET program (Yarasheski *et al.*, 1999).

The smaller than expected increase in muscle cross-sectional area observed by Yarasheski and colleagues (Yarasheski *et al.*, 1999) suggests that contractile proteins within the muscle cells of physically frail elderly people have a reduced capacity to hypertrophy in response to RET. This suggestion in combination with the work of others, who observed that RET increased the MPS in healthy elderly to a similar magnitude as it did in healthy younger subjects (50-180%) (Hasten *et al.*, 2000), leads one to suggest that for optimal benefits and to promote healthy ageing, RET should where possible, begin before frailty occurs (Greig *et al.*, 2011).

In this work we aimed to collectively assess the impact of 20 weeks RET on muscle structure and function (focus of chapter 2), body composition (focus of chapter 3) and risk factors for chronic disease (focus of chapter 4) in young, middle-aged and older individuals.

1.2 Study design

1.2.1 Screening and participant inclusion

Recruitment for this study resulted in the inclusion of 51 subjects who formed 3 groups and underwent the complete study protocol (Table 1.1). We recruited 9 males and 5 females aged 18-28 y who are referred to as 'young'. We recruited 10 'middle-aged' males and 10 'middle-aged' females aged 45-55 y and we also recruited 10 males and 7 females aged 65-75 y, the 'old' age group. Recruitment targeted healthy volunteers, who in the opinion of a clinician were capable of participating. Healthy volunteers were recruited from the community by poster advertisements, radio and newspaper coverage, internet advertisements and recruitment talks at targeted interest groups.

Table 1.1 Subject characteristics

Age-Group	*Males: Females*	*Age (y) ±SEM*	*BMI ($kg \cdot m^2$) ±SEM*
Young	9:5	24.9±3.5	23.5±2.3
Middle-aged	10:10	49.5±3.7	26.7±3.0
Older	10:7	69.6±3.2	26.9±1.9

Subjects were excluded from study recruitment if they showed evidence of:

- Overt muscle wasting .i.e. more than one standard deviation below normal muscle or fat-free mass (FFM) for age (Fuller et al, 1992).
- A body mass index (BMI) <18 or >28, or a BMI >30 with a waist circumference >102cm (males) or >88cm (females).
- Active cardiovascular disease: uncontrolled hypertension (blood pressure (BP) >160/100), angina, heart failure or arrhythmia.
- Cerebrovascular disease: previous stroke, large vessel or intracranial aneurysm.
- Respiratory disease including chronic obstructive pulmonary disease (COPD) and severe asthma.
- Metabolic disease: hyper and hypo parathyroidism, untreated hyper and hypothyroidism, Cushing's disease, types I or II diabetes.
- Active inflammatory bowel disease, renal disease or malignancy
- Recent (within 6 months) steroid treatment or hormone replacement therapy.
- Clotting dysfunction e.g. DVT, pulmonary embolus, warfarin therapy and/ or haemophilia.
- Pregnancy: all pre-menopausal women were tested prior to both acute studies.
- Musculoskeletal or neurological disorders.

Ethical approval for all aspects of this study was granted by The University of Nottingham Medical School Ethical Advisory Committee and was received on the 21/03/2006 (Appendix 1).

Prior to their baseline acute study and after reading the volunteer information sheet (Appendix 2) subjects underwent a screening session in which they were asked to provide written consent for participation in the study (Appendix 3). They underwent a medical examination; including medical history, a physical examination, physical strength tests, an electrocardiogram (ECG) and blood tests (FBC, U&E, Glucose, LFT and TFT) to ensure they fulfilled all inclusion criteria. Body composition was measured by dual energy X-ray absorptiometry (DEXA) (Lunar Prodigy II, GE Medical Systems).

At their screening session subjects performed a 1-RM assessment on a leg-extension RET machine (ISO Leg Extension, Leisure Lines (GB) Ltd) to be used for the calculation of the exercise to be performed during the subject's acute studies.

1.2.2 Baseline acute study prior to RET

Following their screening visit subjects underwent a baseline acute study. They were required to fast for 12 hours prior to this study commencing.

Under local anesthetic (1% lignocaine) a cannula was introduced into a forearm vein for infusion of stable tracer amino acids, a hand vein for arterialized blood sampling and the femoral vein of each leg for venous blood sampling. The "hot-hand" technique was used to obtain arterialized-venous blood samples, as a less invasive alternative to direct arterial sampling. A well-established technique developed in the 1980's, this method involved placing the hand in a heated box, at ~55°C to create an arterio-venous shunt of blood by vasodilation of veins in the fingers, allowing arterialized-venous blood sampling from a retrograde catheter in the dorsum of the hand (Abumrad *et al.*, 1981).

Primed constant infusions of stable isotope amino acids were continued for the duration of the study and blood samples taken as illustrated (Figure 1.1). Plasma samples were collected and processed for the quantification of

insulin, cytokines, serum cholesterol and glucose and for use in measures of muscle protein synthesis.

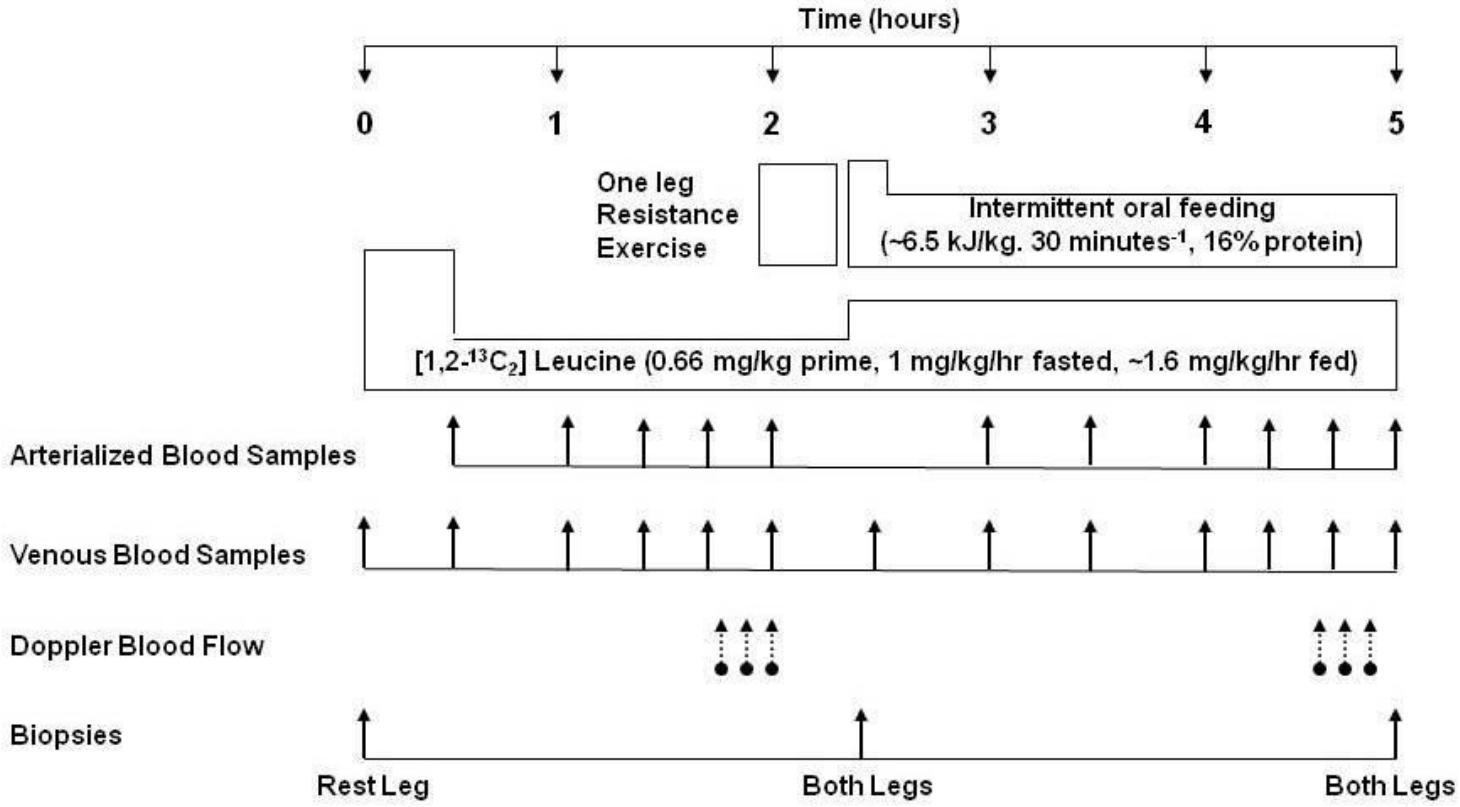


Figure 1.1 Schematic representation of baseline acute study protocol.

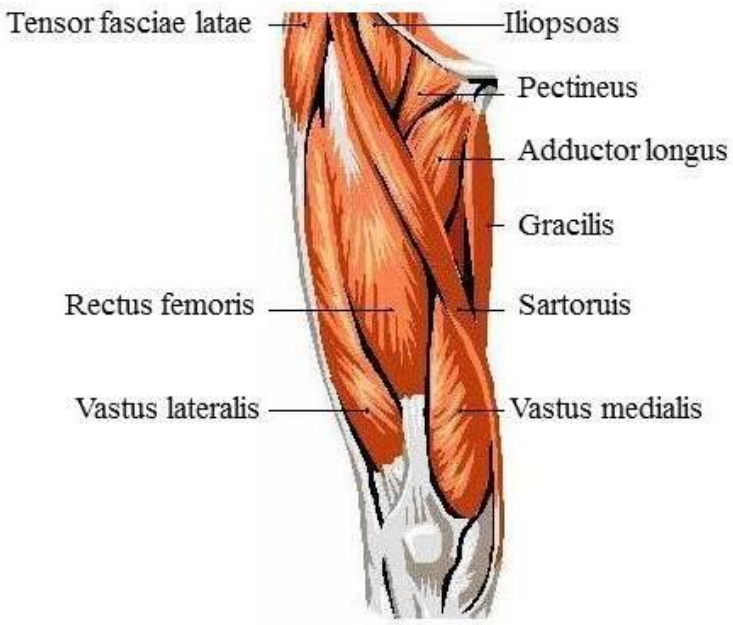
During the first two hours of the acute study, subjects were given tracer infusions only and studied in the postabsorptive state.

At two hours the subjects were asked to perform one leg resistance exercise; unilateral leg extensions at 75% 1-RM on a free weight leg extension machine (ISO Leg Extension, Leisure Lines (GB) Ltd). All subjects performed 6 sets of 8 repetitions followed by 2 sets of 10 calf-raises on the exercising leg to ensure blood flow to the lower leg muscles (i.e. *Soleus and Gastrocnemius*).

After the exercise the subjects received small intermittent meals, every 30 minutes, consisting of a clinical liquid nutritional supplement (Fortisip, Nutricia Clinical Care), supplying energy at 4.25 times the subjects basal metabolic rate (BMR), calculated by standard equations (Schofield, 1985) and with 15% of the total energy being supplied as protein:

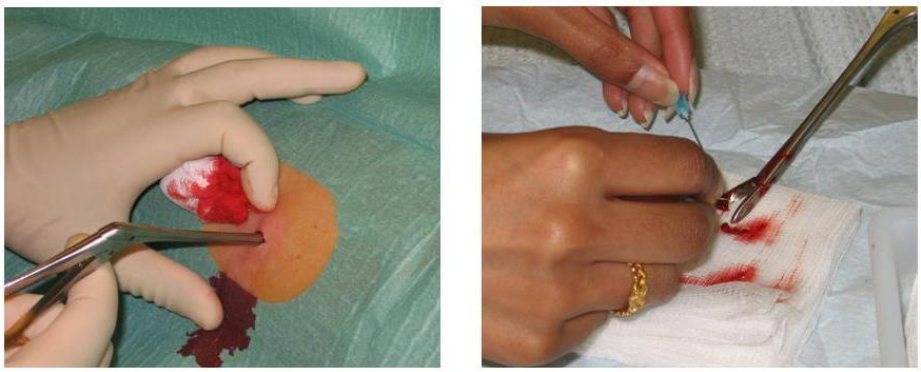
$$\textbf{Total dose} = [(4.25 \times \textbf{BMR kcal.24 h}^{-1}) / 24] \times 2.5$$

Biopsies (~150 mg) of *Vastus Lateralis* muscle (Figure 1.2) were taken from the 'rest leg' at 0 hours, from both legs immediately following the exercise bout, and from both legs 2.5 hours after the exercise. Biopsies were taken using the conchotome technique (Dietrichson *et al.*, 1987) after skin and fascia incisions were made under local anesthesia (1% lignocaine). Subsequent biopsies after the initial biopsy at 0 hours were taken at least 2cm proximal to the last. At each time point three ~50 mg tissue samples were collected from the same biopsy site; a sample for RNA extraction was immediately snap-frozen in liquid nitrogen and the other two, for tracer analysis and protein extraction were washed in ice-cold phosphate buffered saline (PBS) before snap freezing in liquid nitrogen and then being stored at -80^{o}C prior to analysis (Figure 1.3). The biopsy sites were closed with synthetic sutures and covered with shower-proof dressings. Sutures were removed and biopsy sites checked at 7 days following the acute study.



Taken from: www.orgs.jmu.edu/strength/images/male_muscles_quadriceps.htm

Figure 1.2 Superficial muscles of the upper right leg, anterior surface.



A. **B.**

Figure 1.3 Muscle biopsy extraction and collection.
A. Muscle biopsy extraction by clinician
B. Muscle biopsy preparation by technician prior to storage

Measurements of leg blood flow using Doppler ultrasound were obtained from both legs at three time points nearing the end of both the fasted and fed phases.

1.2.3 Repeat acute study after RET

More than three but less than seven days after the final exercise session of RET, subjects underwent a repeat acute study. Protocol for this was identical to the baseline acute study with the exception of it being preceded by a DEXA (GE LUNAR II) scan, as previously performed at the screening session.

1.2.4 Diet diaries

At the first acute study, at least one week before RET began all subjects were asked to complete a 3-day diet diary recording all food and drink taken during that three day period. Subjects were advised how to record their intake accurately and diet diaries based on those used by The Royal Derby Hospital dietetic department were issued, including instructions for completion of the diary (Appendix 4). Subjects were asked to complete a second diet diary mid-way through their RET program (week ten or eleven).

1.2.5 Resistance-exercise training program

The aim of the RET program was to improve muscular strength and endurance, and achieve skeletal muscle growth (muscular hypertrophy). Muscular strength is defined as the maximal force that a muscle or muscle group can generate (Wilmore & Costill, 1999) and this is often assessed using the one repetition maximum test (1-RM). Muscular strength is achieved using resistance that requires maximal or near maximal muscle tension over relatively few repetitions, while muscular endurance is developed using lesser resistance but performing a greater number of repetitions. Current guidelines advise 8-12 repetitions resulting in near failure on the final repetition to elicit improvement in both muscular strength and endurance in healthy individuals (Franklin, 2000). Based on this all subjects trained three times a week (Kraemer *et al.*, 2002), with each session lasting approximately 60 minutes. Training intensity (following four weeks of initiation sessions) was 70% 1-RM (Evans, 1999; Rhea *et al.*, 2003) based on single sets (Carpinelli & Otto, 1998) of 12

repetitions (Kraemer *et al.*, 2002; Pollock *et al.*, 1994) with 2 minutes rest between sets. To ensure equipment familiarity and correct technique was adhered to, the first 4 weeks of each subjects RET was at 40-60% 1-RM. Throughout the RET program a trained gym instructor verbally encouraged the subjects and ensured proper form and technique was performed.

1-RM assessments were performed by each subject every four weeks to ensure that the intensity of their training remained relative to their 1-RM regardless of any strength changes that occurred with blood pressure and resting heart rate also measured at four-weekly intervals.

To ensure uniformity between the amounts of training performed by each subject they were excluded from the study in the event of non-compliance, defined as:

- Non-attendance for >6 consecutive sessions
- Less than 75% attendance (< 36/48 sessions)
- Failure to complete the set exercise regime on >15% attendance (> 7 sessions)

Although the order and number of multiple sets per exercise varied between the three sessions each week to prevent boredom and drop-out, the same total numbers of repetitions were performed each session over a total of 8 exercises as detailed below (Table 1.2). A full training log was provided for each subject (Appendix 5), with weight lifted the only variable between subjects according to their 1-RM.

Table 1.2 Resistance-exercise training program.

Exercise	***Primary muscles trained***	***Workout 1 (sets)***	***Workout 2 (sets)***	***Workout 3 (sets)***
Seated Chest Press	Pectorals, Deltoids, Triceps	2	4	1
Lat Pull Down	Latissimus Dorsi, Trapezius, Biceps	2	4	1
Seated Lever Row	Latissimus Dorsi, Biceps	2		4
Leg Extension	Quadriceps	2	4	1
Seated Leg Curl	Hamstrings	2	4	1
Seated Leg Press	Quadriceps, Hamstrings, Gluteals	2		4
Back Extension	Erector Spinae	2		2
Abdominal Curl	Abdominals	2		2
Number of sets		**16**	**16**	**16**

1.3 Statistical analysis

Statistical analyses were performed using Graph Pad Prism (Version 5.02). All data are reported as means ± SEM with significance set at $P<0.05$, unless otherwise stated.

Two-way ANOVA with Bonferroni post-hoc analysis was used to compare age-group differences before and after RET in basal, fed and fed-plus-exercised conditions for measures of MPS, leg blood flow (LBF), leg vascular conductance (LVC) and leg peripheral resistance (LPR). Protein concentrations/ phosphorylation for measures that were made in all three conditions were also measured using 2-way ANOVA and Bonferroni post-hoc analysis after data had been LOG transformed for normality.

Strength, body composition (BC), blood pressure (BP), resting heart rate (RHR) and diet diary analysis were analysed using ANOVA with Bonferroni or Newman-Kelus post-hoc analysis. Basal protein concentrations were also analysed using ANOVA with Bonferroni post-hoc analysis again after LOG transformation.

Correlations between strength and lean mass, body fat % and BMI or LBF and age were displayed using linear regression and analysed using Pearson's correlation.

Myosin heavy chain distribution, RNA: DNA: Protein ratios, Glucose concentrations, insulin concentrations, HOMA, glucose: insulin ratios, cholesterol profiles and cytokine concentrations were analysed using ANOVA with Bonferroni post-hoc analysis between groups and students t-test between paired samples before and after RET.

Glucose and insulin handling were analysed using ANOVA with Bonferroni post-hoc analysis on area-under-the-curve values generated for each individual subject.

CHAPTER 2- INDICATORS OF FRAILTY

CHAPTER HYPOTHESES:

i. There are age-related declines in muscle strength that are associated with lower dietary protein intake in older individuals.

ii. Resistance-exercise training will improve muscle strength but the gains will be less in older individuals.

iii. Age-related declines in muscle strength are associated with a difference in muscle fibre composition.

iv. Resistance-exercise training will alleviate age-related declines in muscle protein synthesis in the basal condition and in response to feeding.

2.1 Introduction to frailty

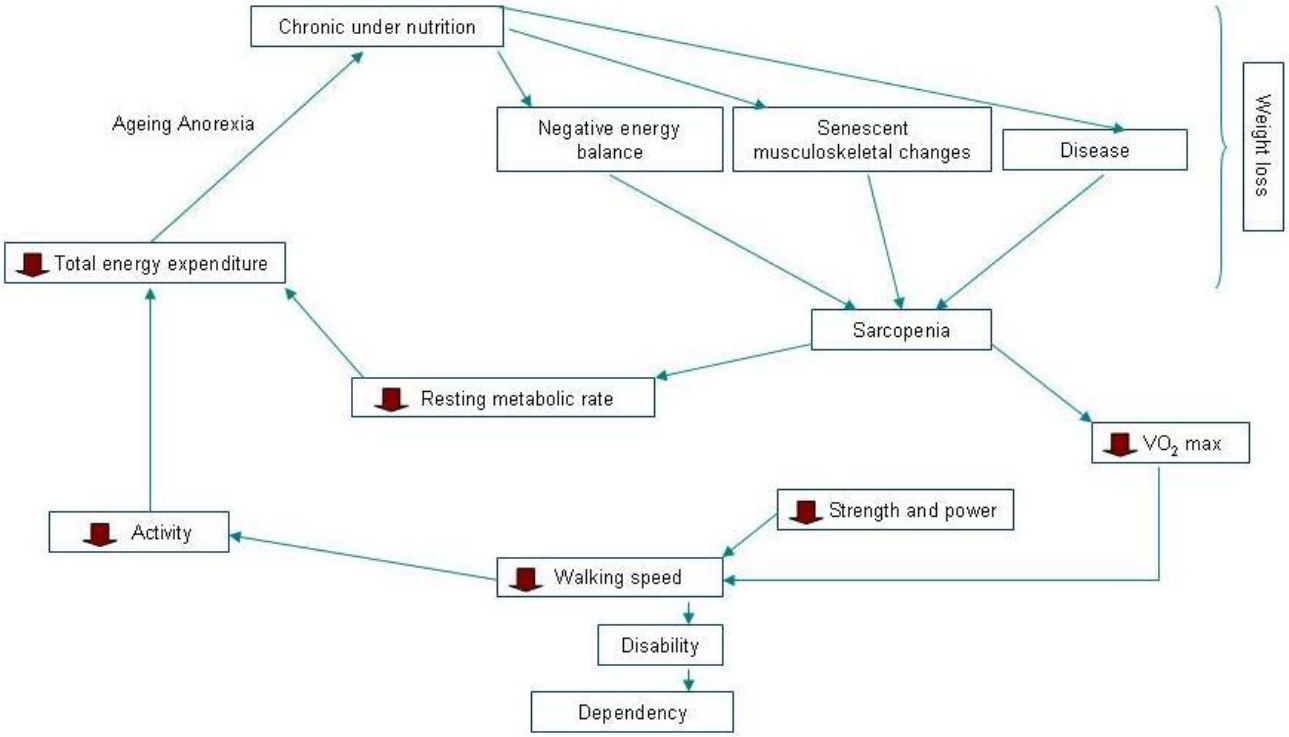
Progressive sarcopenia, the age-related loss of skeletal muscle mass, is ultimately central to frailty (Wolfe, 2006a), a condition that is highly prevalent in old age and is associated with an increased risk of falls, disability, hospitalization and morbidity (Fried *et al.*, 2001). For many years frailty was considered synonymous with disability and co-morbidity but is now being recognized as an independent, distinct clinical syndrome (Fried *et al.*, 2001).

2.1.1 Cycle of frailty

Based on the cycle of frailty (Fried & Watson, 1998) (Figure 2.1) Fried identified the phenotype of frailty as the presence of three or more of the following factors (Fried *et al.*, 2001):

1. Unintentional weight loss of >5% body weight in one prior year.
2. Grip Strength weakness: Grip Strength ability in the lowest 20% of the population, adjusted for gender and BMI.
3. Poor endurance and energy as indicated by self-report of exhaustion.

4. Slowness: Time to walk 15 feet in the slowest 20% of the population, adjusted for age and height.
5. Low Physical Activity: A Physical Activity score in the lowest quintile (based on MET's), adjusted for age and gender.



Adapted from: Fried and Watson, 1998 (Fried & Watson, 1998)

Figure 2.1 Cycle of Frailty

A reduction in physical activity is one component of the cycle of frailty (Figure 2.1) and it is commonly believed that disuse accounts for as much as 50% of the decline in work capacity with ageing. Losses of muscle due to ageing are very similar to losses of muscle due to inactivity (Bortz, 1982). Inactivity has a depressive effect on muscle protein metabolism which may influence muscle protein balance and result in muscle mass loss (Ferrando *et al.*, 1996), although a relatively small volume of RET seems to be enough to alleviate this decrease in MPS (Klitgaard *et al.*, 1990). As well as deleterious effects on muscle protein metabolism disuse is also associated with a decreased capacity to utilize substrates such as fatty acids, glucose and pyruvate.

A combination of decreased levels of physical activity with age and physiological changes (Roth *et al.*, 2000; Voorrips *et al.*, 1993) may go some way to explaining the extent of sarcopenia (which can progressively lead to frailty) in elderly individuals, although it is still unknown if the primary cause is the physiology of ageing or the "physiology of disuse" (Wilmore, 1991).

2.1.2 Muscular changes and frailty

2.1.2.1 Sarcopenia

Skeletal muscle is required for a large range of functions including the preservation of posture, mobility and strength, as well as metabolic functions such as glucoses homeostasis. The term sarcopenia was coined to describe age related losses of skeletal muscle (Rosenberg, 1997), which according to cross-sectional and longitudinal studies has already started in the third decade of life, with a marked acceleration by the fifth (Larsson *et al.*, 1979; Melton, III *et al.*, 2000; Janssen *et al.*, 2004). There are some factors which have been shown to be correlated to adult lean body mass (LBM) that we can do very little about, such as the positive correlation between birth weight and LBM (Gale *et al.*, 2001). We can however mediate a number of lifestyle factors to reduce the risk of sarcopenia, for example both smoking and excessive alcohol consumption have been shown to increase this risk (Szulc *et al.*, 2004; Lang *et al.*, 2003).

Sarcopenia, is associated with muscle weakness, increased fatigue and a loss of independent function; all characteristics of physical frailty (Yarasheski *et al.*, 1999). Current definitions of sarcopenia use a comparison of LBM or skeletal muscle mass of the subject, with that of a height and weight matched young adult. Baumgartner and others take 2 standard deviations (SD) below the mean muscle mass for age as defining significant sarcopenia, (Baumgartner *et al.*, 1998) which results in prevalence estimates of 13-24% for those 65-70 y and greater than 50% in those aged 80 y or older. Others use thresholds in relation to the 'skeletal mass index ratio' (lean body mass (kg)/ height (m^2)) where sarcopenia is denoted as values below 5.75 kg/m^2 for women and 8.5 kg/m^2 for men (Janssen *et al.*, 2004). Another index has come into use recently to characterize the muscle mass of the limbs, the appendicular lean-tissue body mass (ALBM) score, which is also used in relation to height (Kim *et al.*, 2002).

Sarcopenia is a widespread syndrome that can have a devastating effect on quality of life (Wolfe, 2006b) and causes considerable morbidity (Baumgartner *et al.*, 1998). As skeletal muscle is the primary site for glucose and triglyceride oxidation and the most predominant determinant for resting metabolic rate (Zurlo *et al.*, 1994) sarcopenia may contribute to negative conditions of peripheral insulin resistance, dyslipidaemia and increased adiposity (Greiwe *et al.*, 2001), all of which are known to be detrimental to human health.

The average person spends 15% of their lifespan in an unhealthy state requiring hospitalization or care, due to disability, injury or disease occurring in old age (Hunter *et al.*, 2004). Age-associated losses of skeletal muscle mass and strength are widely documented in the current literature and it is estimated that by 80 years of age humans generally lose 30-40% of skeletal muscle fibres (Dirks & Leeuwenburgh, 2005) and voluntary contractile strength is decreased, on average, by up to 40% (Doherty, 2003). The most notable strength reductions are reported in the weight

bearing lower limb muscle groups (Hunter *et al.*, 2004), with decreased strength during both flexion and extension (Evans, 1992). Power output also declines with ageing and may be important in the incidence of falls (Caserotti *et al.*, 2001) and the inability to perform weight bearing tasks (Samson *et al.*, 2000).

Sarcopenia is associated with many important outcomes, both social and clinical, such as:

- Mobility impairments (Melton, III *et al.*, 2000; Sternfeld *et al.*, 2002; Davison *et al.*, 2002)
- Disability (Baumgartner *et al.*, 1998; Visser *et al.*, 1998)
- Falls (Baumgartner *et al.*, 1998)
- Fractures, (Melton, III *et al.*, 2000b)
- Increased body fat; which can in itself cause functional difficulties (Weinsier *et al.*, 2000) and is also associated with increased dyslipidaemia and reduced insulin sensitivity (Hunter *et al.*, 2004)
- Frailty (Puts *et al.*, 2005)
- Loss of independence (Baumgartner *et al.*, 1998; Janssen *et al.*, 2002).

Sarcopenia has been associated with substantial increases in morbidity and premature mortality (Janssen *et al.*, 2004). Severe sarcopenia increases the likelihood of developing a disability by 2-fold in men and by 3-fold in women (Janssen *et al.*, 2002) and doubles the chances of premature death (Cawthon *et al.*, 2007). Sarcopenia also poses a major problem for those admitted to hospital, with post operative hospitalization time and complication rate increased in patients with evidence of muscle wasting (Windsor & Hill, 1988). Diminished hand-grip strength, which is associated with lean tissue losses (Heimburger *et al.*, 2000) is associated with increased mortality due to stroke, pneumonia, heart disease and cancer (Sasaki *et al.*, 2007; Gale *et al.*, 2007).

It is not just the loss of muscle mass that is detrimental to human health and well-being with age. Losses of strength and power are reportedly up to 3-fold greater than losses of mass (Goodpaster *et al.*, 2006), with suggested losses of 1-2% per year in isometric strength (Hughes *et al.*, 2001; Rantanen *et al.*, 1998). These are not however consistent in all individuals as biological variability, lifestyle choices and nutritional status can all have an impact. In fact, in some older individuals strength is maintained over a number of decades (Rantanen *et al.*, 1998), holding back frailty and losses of independence maintenance.

The impaired functional capacity often associated with ageing (Evans & Cyr-Campbell, 1997) and the associated diminished muscle mass and reduced strength (Bassey *et al.*, 1992; Rantanen *et al.*, 1999) can lead to both reduced independence, quality of life (Daley & Spinks, 2000; Winograd *et al.*, 1991) and potentially contribute to falls (Reeves *et al.*, 2004). Whipple and colleagues found that the strength of elderly people who suffered falls was significantly lower than in elderly people who did not fall.

With the detrimental effects of sarcopenia well established, methods by which it may be limited or countered are always welcome and may be sought from the list of established risk factors based on current findings (Whipple *et al.*, 1987), although some are pre-determined and inevitable (Table 2.1).

Table 2.1 Risk factors for sarcopenia

Established risk factors for sarcopenia
Advancing age
Low body weight
Physical inactivity
Female sex
Low birth weight
Impaired lower limb function

Any condition associated with relative anorexia, low physical activity or inflammation increases the risk of muscle wasting and is therefore a significant risk factor for sarcopenia (Poehlman *et al.*, 1995; Kyle *et al.*, 2002).

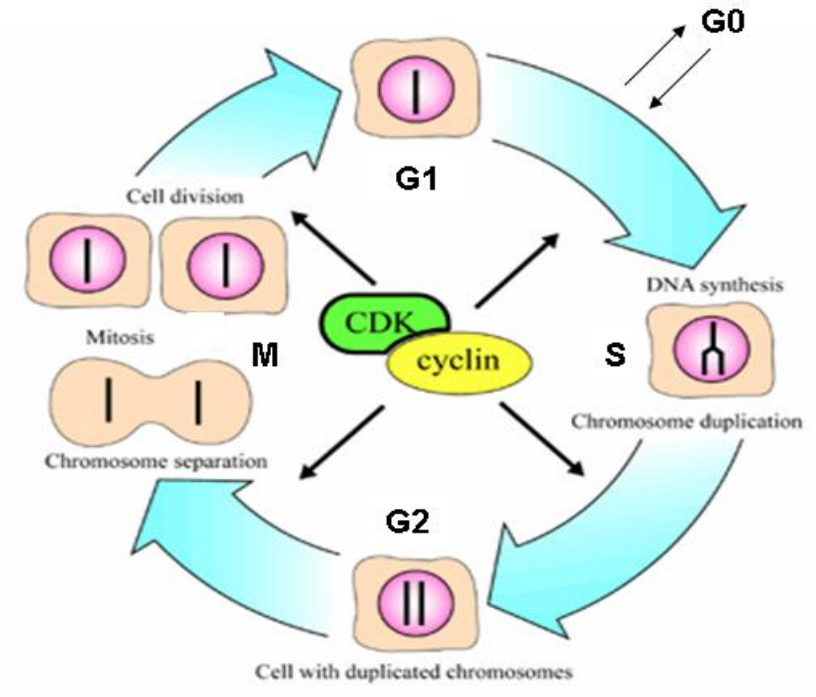
Muscle contains ~35% of all total body protein (Cohn *et al.*, 1980) which is maintained by a continual process of protein synthesis and breakdown. A negative balance in muscle protein metabolism, where MPB exceeds MPS is required for muscle loss. Skeletal muscle mitochondrial and contractile protein synthetic rates decline with age (Rooyackers *et al.*, 1996; Yarasheski *et al.*, 1993) and are associated with reduced muscle mass, decreased muscle strength and decreased endurance capacity (Balagopal *et al.*, 1997; Yarasheski *et al.*, 1993; Welle *et al.*, 1995; Rooyackers *et al.*, 1996; Nair, 1995), although it is not known if the change in net MPS that occurs with ageing is due to change in basal muscle protein metabolism or the result of changing levels of physical activity and/ or nutritional status. Either way, this association suggests that an increase in muscle protein synthesis should result in an improvement in muscle strength and function, and modulate the disability associated with physical frailty and ageing (Yarasheski *et al.*, 1999).

It has been suggested that the basal level of MPS is reduced by ageing (Hasten *et al.*, 2000; Balagopal *et al.*, 1997) therefore leading to a constant decrement in net muscle protein balance and ultimately, may go some way to explain muscle loss with ageing. However, there is great inconsistency in the literature surrounding this concept, with some authors reporting no difference between the FSR of mixed muscle proteins between the young and the elderly (Volpi *et al.*, 2001).

Alternative proposed mechanisms for the cause of sarcopenia in the elderly include a decline in serum anabolic hormones (Morales *et al.*, 1998; Urban *et al.*, 1995), increased myostatin (Thomas *et al.*, 2000; Welle *et al.*, 2002; Corsi *et al.*, 2002), higher levels of TNF-α (Argiles *et al.*, 2000; Langen *et*

al., 2001), an inability to repair fibres, apoptosis and/ or a reduced capacity for satellite cell activation (Bornemann *et al.*, 1999).

Satellite cells are the committed stem cells of adult skeletal muscle, located at the surface of the basal lamina of the myofibre. Their major function is to repair, revitalize and mediate skeletal muscle growth by differentiating into myocytes. Satellite cells are normally non-proliferative, they do however become active when skeletal muscle tissue is injured or heavily used, for example when performing RET. It has been proposed that a reduced capacity for satellite cell activation in the elderly occurs because negative regulators of satellite cell activation, such as myostatin, could possibly down-regulate satellite cell activation by inhibiting the G1 to S-phase transition (McCroskery *et al.*, 2003). For satellite cell division quiescent cells need to enter the S-phase of the cell cycle from either the G1or G0 phase (Figure 2.2).



Adapted from: www.nobelprize.org/medicine/laureates/2001/press.html

Figure 2.2 Satellite cell cycle
"G" represents Gap; "S" represents Synthesis; "M" represents Mitosis

Although the definitive mechanism behind sarcopenia remains unclear, the negative impact it can cause is well established. It is likely a combination of factors interact to contribute to age-related physiological changes which lend themselves to muscle mass losses.

2.1.2.2 Muscle protein metabolism

Although the pathophysiology of sarcopenia is complex and not yet fully understood, it is known that changes in muscle protein turnover are required for alterations in muscle mass to occur. Muscle mass is maintained by a dynamic equilibrium in protein turnover in which net efflux of amino acids during fasting periods is offset by net influx (and incorporation into protein) during fed periods. Both ageing and exercise (topics explored in this project) may modulate the capacity for muscles to incorporate amino acids into protein; the key aspect for the regulation of hypertrophy and atrophy.

There are 20 Amino acids (AA) which are incorporated into protein; 8 are essential (EAA), 10 are non-essential as they are synthesised endogenously and 2 are 'conditionally essential' in certain circumstances. It is the AA sequence which directly determines protein formation, which in turn determines structure and function (Table 2.2).

Table 2.2 Amino Acid Classifications

Essential	***Non-Essential***	***Conditionally Essential***
Isoleucine	Alanine	Arginine
Leucine	Asparagine	Histidine
Lysine	Aspartate	
Methionine	Cysteine	
Phenylalanine	Glutamate	
Threonine	Glutamine	
Tryptophan	Glycine	
Valine	Proline	
	Serine	
	Tyrosine	

The functions of protein include:

1. Maintaining the structure of muscles and connective tissue
2. Acting as messengers for hormones
3. Acting as enzyme catalysts
4. Acting as transporters
5. Genetic regulation roles
6. Fluid and pH balance

Protein regulation accounts for about 20% of daily energy needs and this can increase during times of starvation or trauma when as well as increasing energy demands, muscle provides AA's to sustain protein synthesis in other tissues.

Unlike carbohydrates and fatty acids, protein is not stored in the body, although muscle can provide AA's during fasting. After feeding MPS is increased in response to the increased availability of AA's, but the duration of this increased response is limited to ~2 hours, even if the increased AA availability is maintained (Atherton *et al.*, 2010). During fasting/ starvation MPB is increased to meet the demand, but there is a limit to how much protein can be catabolised to sustain the resultant negative nitrogen balance.

The balance between protein synthesis and protein breakdown is closely regulated. Feeding (especially if EAA's are incorporated) and exercise are the most potent stimulators of protein anabolism and act synergistically to promote muscle mass via an increase in MPS and a decrease in MPB. Starvation and immobilization promote protein catabolism (Phillips *et al.*, 2009) though it appears that synthesis decreases under these conditions before a smaller increase in breakdown, which results in a net loss of muscle protein. Only in advanced stages of disease or sepsis does protein breakdown increase greatly, although the rate of synthesis is also increased to preserve protein mass.

It is understanding the mechanisms of MPS and MPB and the ‘patterns’ for the loss and gain of protein that is essential to design effective therapies to prevent wasting and restore muscle mass.

2.1.2.3 Muscle protein synthesis

Protein synthesis requires the transfer of genetic information from deoxyribonucleic acid (DNA) into protein which involves the transcription of mRNA and subsequent translation on ribosome’s involving tRNA. Three nucleotides (bases) in the mRNA are required to specify particular AA’s for inclusion.

The translation process consists of three stages: Initiation, elongation and termination. The initiation and termination stages can be regulated to affect the rate of protein synthesis. The initiation complex is formed when the ribosome assembles on the mRNA in the presence of various initiation factors. The ribosome moves down the mRNA to the start AUG codon and forms the 70-S initiation complex. Each AA has a specific aminoacyl-tRNA synthetase, which catalyzes the binding of the AA to an acceptor site on the t-RNA forming aminoacyl-tRNA. This carries the AA to the ribosome where it binds and is committed for protein synthesis.

Elongation involves the binding of subsequent aminoacyl-tRNA complexes. The ribosome moves one complex down the mRNA and the initiation process is repeated. The protein is released when a stop codon is reached; this is termination.

Post-translation proteins may be modified or spliced, further increasing the number of proteins. A single protein may also have many interactive domains allowing it to simultaneously have multiple functions. Although the AA constituents of a protein do not necessarily indicate the role of that protein in a cell they can suggest a common function between proteins.

2.1.2.4 Muscle protein breakdown

There are three main systems of protein breakdown: i. Proteolytic systems, ii. Metabolism of the amino-nitrogen and iii. Metabolism of the carbon backbone.

1. There are three proteolytic pathways used. i. The *lysosomal proteolytic pathway* effects surface membrane proteins and extracellular proteins. These proteins are taken up by endocytosis and degraded within the lysosomes by proteases. ii. The *calcium-ion activated calpains pathway* is activated in response to cell injury. The resultant rise in calcium concentration leads to increased proteolytic activity within the damaged tissue and/ or DNA damage leads to the activation of caspases and results in apoptosis within the mitochondrion. iii. The *ubiquitin-proteasine-dependent pathway* degrades most intracellular protein. This system catalyzes the degradation of long-lived proteins and short-lived regulatory proteins. It is this pathway that causes atrophy in times of trauma, fasting or immobilisation, although this pathway does result in amino acid release which can be re-used as incorporation into new proteins.

2. The metabolism of the amino-nitrogen system of degradation is initialised by the removal of the amino group, which may be accomplished by oxidative deamination, transamination or dehydration all of which result in different paths to amino acid degradation.

3. Metabolism of the carbon backbone contributes to protein breakdown as there is continual oxidation of AA's as metabolic fuel.

2.1.2.5 Muscle protein balance

As previously mentioned, skeletal muscle contains ~35% of all total body protein (Cohn *et al.*, 1980), which is maintained by the continual processes

of MPS and MPB outlined above. Skeletal muscle accounts for between 25 and 50% of all protein turnover in the body, dependent upon feeding status (i.e. postabsorptive or postprandial) (Tessari *et al.*, 1996). Amino-acids which are released during protein turnover can be used for cell growth and repair, or branched-chain amino acids (BCAA's) can be used as a fuel for skeletal muscle, although they contribute no more than 15% of total energy during exercise (Rennie *et al.*, 2006). Different stimuli, both metabolic and physical can influence MPS and MPB. In general, MPS is responsible for gradual changes in muscle mass and MPB adapts to preserve tissue (Rennie & Millward, 1983).

In healthy adults, in postabsorptive (PA) conditions MPB exceeds MPS, whereas in the postprandial condition both MPS and MPB increase, but increases in MPS are greater than increases in MPB resulting in a net gain. Typical in the PA condition varies between 0.04 and 0.08%.h^{-1}, although this is dependent upon the stable isotope tracer and analysis/interpretation techniques used (Smith *et al.*, 2007b). For example, measurements taken with leucine are approximately 20% greater than those taken using phenylalanine.

2.1.2.5.1 Regulation of muscle protein synthesis by nutrients

The anabolic effects of feeding are driven through two principal mechanisms: first, FSR of muscle proteins increase ~300% (Atherton *et al.*, 2010) and second, MPB rates are depressed ~50% (Wilkes *et al.*, 2009). As the magnitude of change in MPS is greater than those of MPB, increases in MPS are the main driver of anabolic responses to feeding.

Amino acids (Rennie *et al.*, 1982), specifically EAA (Smith *et al.*, 1992;Volpi *et al.*, 2003;Tipton *et al.*, 1999) are the most significant anabolic stimulants of muscle protein turnover, with EAA required for sustained muscle growth. Of course, teleologically, the anabolic effects of EAA must be short-lived otherwise one could achieve hypertrophy through overfeeding.

The anabolic effect of EAA on FSR correlates with extracellular availability (Bohe *et al.*, 2003), although there appears to be a delay of approximately 30 min before MPS is increased (Bohe *et al.*, 2001). A 2-3 fold increase in MPS has been observed following a 50% rise in EAA availability (Smith & Rennie, 1996), but MPS returns to baseline levels after ~150 min, even in the presence of continued EAA availability (both muscle and plasma AA) (Bohe *et al.*, 2001;Atherton *et al.*, 2010) suggesting an innate 'muscle-full' response, confirming the premise that anabolic effects of EAA must be short-lived and discounting the possibility of MPS being driven by plasma/intracellular amino acid bioavailability *per se*. This 'window' of ~150 min is similar to the response shown after a mixed meal, where physiological increases in AA typically return to baseline within 180 min (Bergstrom *et al.*, 1990). The time-lapse required before a repeated increase in EAA availability can again increase MPS is not yet known.

We are now beginning to understand the mechanisms of increased MPS in response to EAA. Confirmation of a role for mammalian target of rapamycin complex 1 (mTORc1) signalling was recently provided in a study in which administration of rapamycin (a specific inhibitor of mTORc1) blocked increases in mTORc1 signalling and MPS after oral EAA ingestion in humans (Dickinson *et al.*, 2011). Work from our own group is also in agreement with this as after a feed of 48g whey protein rising MPS rates were matched closely with increases in mTORc1 substrate phosphorylation (Atherton *et al.*, 2010). There is however a caveat to this seemingly straight forward association, in the latter study by our group MPS returned to baseline 90 min after feeding despite continued up regulation of mTORc1 signalling, thus revealing some level of dissociation between MPS and mTORc1 .

Another possible mechanism involves amino acid transporters which may serve as more sophisticated import mechanisms than first thought. For instance, it was recently shown that the anabolic effects of leucine require glutamine efflux via sodium-coupled neutral amino-acid transporter

member 2 (SNAT2) so the system-L amino-acid transporter 1 (LAT1) heteroexchange system can import leucine (Nicklin *et al.*, 2009). Importantly, these transporters have been demonstrated to be acutely regulated by oral EAA intake in humans, with reported increases in mRNA and protein for both LAT1 and SNAT2 (Drummond *et al.*, 2010).

Insulin is another (postulated) feeding-based anabolic stimulus when accompanying other nutrients or after resistance exercise. Insulin promotes muscle gains primarily by inhibition of MPB (Louard *et al.*, 1992), although it has been suggested that insulin also has a role to play in increasing MPS (Newman *et al.*, 1994), a concept that some disagree with after demonstrating no effect of insulin-alone on MPS (Chow *et al.*, 2006) or no additional effect of insulin/ carbohydrate on EAA promoted MPS (Greenhaff *et al.*, 2008; Staples *et al.*, 2011).

2.1.2.5.2 Regulation of muscle protein synthesis by exercise

It is now well established that nutrient sufficiency represents a necessary component of muscle remodeling and hypertrophy (Moore *et al.*, 2009a) and that EAA potentiate acute anabolic responses to exercise. Recent work has however, provided new information surrounding the independent and synergistic anabolic effects of exercise and nutrients.

It is not only bodybuilders and recreational weight-lifters who aim to increase muscle mass through RET, it also represents the most popular and accepted method in offsetting declines in muscle mass in both ageing and other muscle wasting conditions. An emerging theme in trying to understand the mechanisms of muscle hypertrophy is that of an intrinsic capacity of muscle to respond to RET. This concept is supported by a study from West and colleagues (West *et al.*, 2009) whereby ‘high’ and ‘low’ hormone environments were created through varying muscle recruitment volume. Systemic concentrations of ‘anabolic’ hormones (growth hormone (GH) and testosterone) did not impact on acute MPS responses to exercise or adaptive responses to RET. This concept is also supported by a study in which 14 days recombinant GH administration did not affect MPS despite

increases in serum GH, insulin-like growth factor (IGF-1) and IGF-1 mRNA expression (Doessing *et al.*, 2010). Support for this notion leads to the suggestion that anabolic hormones do not drive MPS or loading-induced adaptations in humans and that these must instead be controlled by intrinsic autocrine/ paracrine factors and mechanotransduction processes.

Not only do muscle cells appear to have an intrinsic signal to increase MPS but also selectivity over which proteins are to be synthesized; explaining how RET increases myofibre size, whereas endurance training increases fatigue resistance. This diversity in response to different training modes can be seen when MPS is measured in distinct muscle fractions. Wilkinson and colleagues reported that RET upregulated myofibrillar protein synthesis whereas endurance training specifically upregulated mitochondrial protein synthesis (Wilkinson *et al.*, 2008). Additional work also shows that RET induced sustained increases in myofibrillar but not sarcoplasmic MPS (Moore *et al.*, 2009b). Taken together, these results support the notion that myofibrillar protein accretion is the most important muscle fraction for muscle hypertrophy.

Contractile exercise, like EAA intake is an important anabolic stimulus for MPS in skeletal muscle. High force contractions increase MPS by 50-100% compared to basal rates, although this is accompanied by a 50% increase in MPB (Biolo *et al.*, 1995). The response to acute exercise is seemingly determined by the duration and intensity of the exercise and whether the muscle is trained in that mode of exercise. i.e., heavily resistance-trained muscle does not appear to increase FSR in response to an acute bout of that type of exercise (Tipton *et al.*, 1996);(Roy *et al.*, 1997). Following resistance exercise, the increased FSR has been reported to last ~48 h (Phillips *et al.*, 1997).

The combination of exercise and EAA feeding may have an important role in MPS. Fujita and colleagues suggested that a combination of AA and CHO feeding 60 min before a resistance exercise bout attenuates the FSR reduction that occurs during exercise compared to unfed controls (Fujita *et*

al., 2009) and when looking at possible mechanisms for the heightened synergistic response to these stimuli Moore et al, reported that phosphorylation of mTORc1 and mitogen-activated protein kinase (MAPK) were shown to be greater following the combination of exercise-plus-feeding compared to exercise alone which may explain additive effects on MPS (Moore *et al.*, 2011a).

The question of optimal timing for nutrient intake in combination with exercise has long been a hotly debated topic and recent findings from Burd et al. demonstrate good reason to question the importance of timing. Burd et al. demonstrated that even 24 h after a single bout of resistance exercise, provision of EAA caused a much greater increase in MPS in the exercised leg compared to the rest leg (Burd *et al.*, 2011a), suggesting that the additive effects of exercise to EAA ingestion are long-lived, and lead to the speculation that consuming adequate dietary EAA is more important than timing *per se*.

As with feeding, there appears to be a latency period after an acute resistance exercise bout; of which the time is still not yet confirmed. Some authors report 2 (Kumar *et al.*, 2009) to 4 h (Tang *et al.*, 2008);(Biolo *et al.*, 1997) as the time before maximum increases in MPS are reached and others have described no MPS changes at 3 h but maximal responses 6 and 24 h after exercise (Cuthbertson *et al.*, 2005). This inconsistency suggests that the latency period prior to maximal responses may be due to differences in exercise intensity. This inconsistency may also explain the lack of change in FSR in resistance-trained individuals mentioned earlier in this section; it may not be that they do not have the ability to respond, but rather they may have a much longer latency that was not accounted for when final measurements were taken at 5 (Tipton *et al.*, 1996) and 10 h (Roy *et al.*, 1997) post-exercise.

Taken together, AA's and acute exercise increase MPS by 2-3 fold, compared to baseline values (Cuthbertson *et al.*, 2006), with no increase is MPB (Biolo *et al.*, 1997), possibly due to the increased blood insulin

concentrations, and therefore result in a positive net balance and allow for the accretion of muscle. Data about the optimum timing of AA intake post-exercise to elicit the best increases in MPS has been inconsistent, and this may be due to the number of variables in these studies, including AA source, exercise intensity and duration and the training status of the subjects involved. There are however suggestions that early feeding post-exercise increases the maximal rate of MPS after exercise (Levenhagen *et al.*, 2001) and recent data from Moore and colleagues (Moore *et al.*, 2009a) shows a dose-response relationship between MPS and post-exercise protein, up to a maximum of 20g of protein. This synthesis date is supported by data from a recent training study, showing that protein supplementation immediately after exercise results in greater gains in LBM, than in those who received delayed feeding (Hartman *et al.*, 2007).

In summary, high-force contractions can increase MPS rates increase by ~100% (Chesley *et al.*, 1992; Biolo *et al.*, 1995) but MPB rates increase by ~50% (Biolo *et al.*, 1995) and as MPB is greater that MPS in the PA condition these changes only result in neutral net balance. Therefore, to form a positive net balance a combination of these two anabolic stimuli; exercise and EAA feeding are essential.

2.1.2.5.3 Regulation of muscle protein synthesis in catabolic conditions

Causes of muscle atrophy may be broadly separated into wasting-associated diseases, disuse and sarcopenia. The latter two of these categories are often entwined with levels of physical activity decreasing with advancing age. Although it has long been known that declines in PA MPS and/or increases in MPB are a catalyst for muscle atrophy, recent work has uncovered a new layer of dysregulation termed “anabolic resistance” that seems to transcend the causes of atrophy. In brief, anabolic resistance is a deficit in the capacity to mount anabolic responses to activity and nutrients (Rennie & Wilkes, 2005; Fry & Rasmussen, 2011); the key influences of muscle mass maintenance. Anabolic resistance may represent a premature “muscle-full” state that underlies and/or exacerbates

atrophy and perhaps contributes to maladaption to exercise as seen in ageing (Figure 2.3).

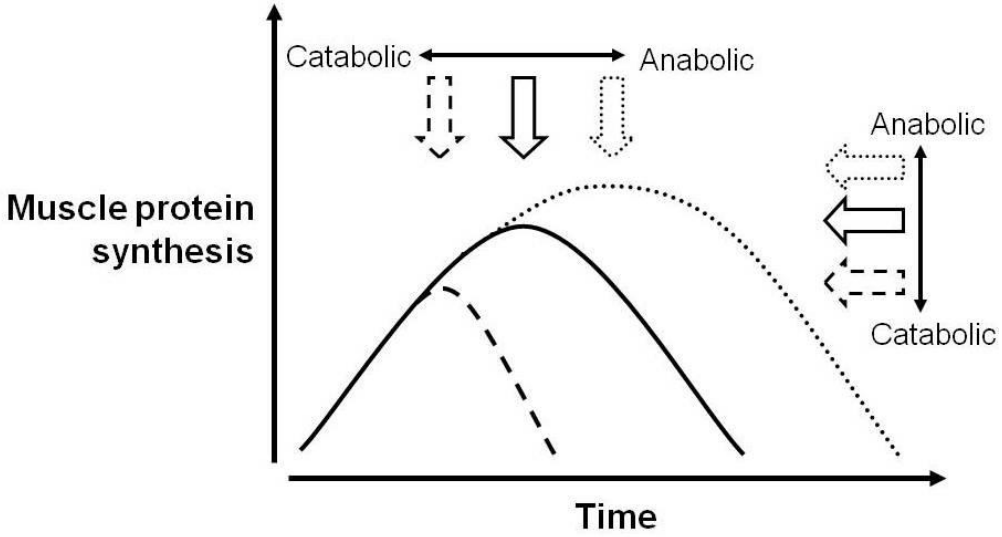


Figure 2.3 Schematic showing muscle protein synthesis responses in normal (—), catabolic (- -) and anabolic states (···).
Arrows indicate the 'muscle-full' set point, which can be modulated in terms of amplitude and/ or duration of MPS.

2.1.2.5.4 Anabolic resistance to feeding in ageing muscles

Although sarcopenia must involve an imbalance between MPS and MPB, rates of MPS and MPB during PA periods are unchanged with age (Volpi *et al.*, 2000; Cuthbertson *et al.*, 2005; Yarasheski *et al.*, 1999; Katsanos *et al.*, 2006) and this has been reported consistently for all fractions of muscle with only a few groups reporting diminished synthesis rates in the sarcoplasmic fractions of older muscle (Hasten *et al.*, 2000; Balagopal *et al.*, 1997).

With this in mind, other mechanisms for sarcopenia have been sought; one of which is the concept of anabolic resistance. In support of this concept Cuthbertson et al. compared responses in MPS to oral EAA over a wide availability (2.5-40 g) and found that above 5 g EAA, older men exhibited smaller increases in MPS to those seen in young people, work which is also supported by others (Cuthbertson *et al.*, 2005; Paddon-Jones *et al.*, 2004). In these studies demonstrating anabolic-blunting with advancing

age it appears to be related to reduced activation of the AA induced signalling pathways that promote MPS (Cuthbertson *et al.*, 2005) and suggests that in response to AA older individuals are less likely to accumulate muscle protein. This blunted signalling response has been cited as having a role in the progression of sarcopenia (Paddon-Jones *et al.*, 2008).

In contrast to this work, others have reported anabolic resistance but only at lower doses of EAA (Volpi *et al.*, 2000) and Symons et al. found that administration of 113 g of lean beef (~30 g protein) raised MPS by approximately 50% in both young and old healthy subjects (Symons *et al.*, 2009). Similarly, Chevalier et al. (Chevalier *et al.*, 2011) found no blunting in the anabolic response under hyperglycaemic, hyperinsulinaemic, hyperaminoacidaemic conditions in which blood concentrations of insulin, total amino acids and glucose were maintained at ~300-400 $pmol.l^{-1}$, ~3300 $\mu mol.l^{-1}$ and ~8 $mmol.l^{-1}$, respectively. Taken together these findings support the notion that overcoming anabolic resistance is simply a matter of increasing total amino acid load; although remain at odds to reports of anabolic resistance at 20-40 g EAA (Cuthbertson *et al.*, 2005) and the concept of a premature "muscle-full" signal Atherton (Atherton *et al.*, 2010).

Consequently, perhaps it is the quality rather than the quantity of AA that is important for overcoming anabolic resistance. Pennings et al. (Pennings *et al.*, 2011) compared acute anabolic responses of old men to casein, casein hydrolysate and whey protein. MPS rates were significantly higher following whey ingestion ($0.15\%.h^{-1}$) than casein ($0.08\%.h^{-1}$) or casein hydrolysate ($0.10\%.h^{-1}$); a result which the authors explained as being due to the faster absorption rates and a higher peak plasma concentration of leucine. These findings in combination with data from the Phillips' lab, in which MPS in young males was more effectively stimulated by a large single bolus of protein that by the sum of quantitatively equivalent smaller boluses (West *et al.*, 2011) support the concept that rapid exposure of

muscle to amino acids and/ or peak leucine concentration may be important in determining anabolic sensitivity.

The addition of novel dietary interventions have also proved efficacious and suggest that overcoming anabolic resistance may not be simply due to AA quantity/ quality. For example, 8 weeks supplementation of omega-3 fish oils ameliorated anabolic resistance in elderly men smith (Smith *et al.*, 2011). Although consensus still needs to be reached on optimal feeding strategies to overcome anabolic blunting with age, this initial work is promising.

2.1.2.5.5 Anabolic resistance to exercise in ageing muscles

It is not only the anabolic responses to feeding that appear blunted in the elderly but also their responses to exercise. Young individuals have been shown to double their FSR after an acute bout of resistance exercise, while this does not appear to be so in older individuals, with a deficit in MPS responses of approximately 30% reported over a wide-span of exercise intensities (20-90% 1-RM) (Kumar *et al.*, 2009). These findings were further corroborated by Fry et al. (Fry *et al.*, 2011) who showed that ageing impairs contraction-induced human skeletal muscle mTORc1 signalling and MPS when sampling up to 24 h after exercise. Collectively these data may explain age-related reductions in trainability (i.e. muscle hypertrophy) with RET (Bickel *et al.*, 2011). These findings of anabolic resistance with age are not restricted to RET, Durham et al. (Durham *et al.*, 2010) reported age-related decline in MPS responses to walking in the fed state. Therefore, it may be that anabolic insensitivity even to mild, habitual activity may exacerbate the catabolic effects of sedentarism associated with ageing.

As with feeding there is conflicting data that casts doubt on the existence and/ or severity of anabolic resistance to exercise with age. For example, Symons et al. (Symons *et al.*, 2011) found that when a bout of resistance exercise was combined with a high protein meal there was no difference in MPS responses between young and older individuals. Drummond et al.

(Drummond *et al.*, 2008) reported that the 'cumulative' anabolic response to resistance exercise and EAA is similar between young and old but that the response is simply delayed with ageing. Based on this proposition shorter-duration post exercise studies must be read with caution as they may not be able to capture the complete long-term anabolic effects of exercise (Burd *et al.*, 2011). A study design which encompasses a longer post-exercise measurement period (Fry *et al.*, 2011) is more likely to identify potentially important differences that could be masked under short-study formats (Phillips *et al.*, 2012).

For those supporting the notion of anabolic blunting with age suggestions have been made that these blunted responses in the elderly can be improved after a relatively short period of RET (of as little as 2 wk) (Hasten *et al.*, 2000), while others report that even after 3 months of high intensity RET anabolic blunting was still apparent in older individuals (Welle *et al.*, 1995). The data from Hasten was measured 3 h after acute exercise, compared to 24 h after by Welle and colleagues and this discrepancy renders these results difficult to compare as explained above.

It is clear to see the benefits of exercise and/ or activity on anabolic status when you examine the situation of disuse, something which becomes much more likely with age. Consider the following:

- Normal turnover is $0.05\%.h^{-1}$ (or $1.2\%.day^{-1}$) in which MPS and MPB are equal and opposite
- MPS increases three-fold for 1.5 h with feeding
- Approximately 5 h per day is spent in fed periods (Atherton *et al.*, 2010)

Based on conservative assumptions from previous findings MPS is suppressed ~50% in both PA and fed periods with disuse which would equate to:

Protein accretion = (0.025 x 19 (PA hours)) + (0.025 x 1.5 x 5 (fed)) = 0.66%.day^{-1}

Thus, if MPB remained constant:

Muscle loss = 1.2-0.66 = 0.54%.day^{-1}

a figure entirely consistent with that measured (0.6%.day^{-1}) over the first 30 days of immobilization (Phillips *et al.*, 2009).

It is not only exercise training but also changes to the format of an acute exercise bout that may improve anabolic responses in the elderly. Following on from their study in which anabolic blunting with age to resistance exercise over a range of intensities (20-90% 10RM) was demonstrated (Kumar *et al.*, 2009), Kumar and colleagues doubled work by increasing the number repetitions performed which significantly increased FSR in the older men, this time regardless of the intensity (40 or 75% 1-RM) and bought the FSR of the older men to a level not significantly different to that of the younger men who performed the original 'single' workload at the same intensity (Kumar *et al*, Gerontology, in press), effectively restoring their anabolic response.

2.1.2.3 Muscle fibre type

A muscle fibre is linked to a single motor neuron and expresses characteristic molecules to determine its contractile function, namely different myosin heavy chain (MHC) isoforms and metabolic enzymes. The mechanisms of how different contractile functions are determined are still not fully understood although both the motor neuron and muscle fibre type have been implicated to play a role in this process. Diversity within human muscle is based on genetic predisposition and future differential expression and gene regulation is regulated by the two mechanisms outlined below (Bottinelli & Reggiani, 2000):

1. A qualitative mechanism whereby many muscle proteins exist in forms that are similar but not identical, these are known as isoforms. Isoforms can derive from the same gene through alternative splicing or from different genes of the same family (isogenes). Isoform generation is the first mechanism in generating diversity within muscle fibres.

2. Differential expression of the same gene is a quantitative mechanism whereby the proportion of up and down-regulating products such as neural discharge, mechanical load and hormones differ between the same gene modifying and/or promoting new functional or structural features.

The number of possible combinations and therefore variations as a result of the combination of the above two mechanisms is limited by constraints set either by structural requirements or by rules of expression which set preferential associations between isoforms (Bottinelli & Reggiani, 2000). It is for this reason that some combinations diminish and more frequent phenotypes of muscle fibres appear.

Although the above mechanisms were not then understood, the diversity of skeletal muscle has been recognized since as early as the 1870's (Spangenburg & Booth, 2003). In 1873, Ranvier categorized muscles as red with slow muscle contraction and white with fast contraction, based upon appearance and stimulation of the muscles with electrical current. In 1970, Brooke and Kaiser recognized more than one type of fast/ white fibre (Type II), labeling the fibre types: Type I, Type IIa and Type IIb (Brooke & Kaiser, 1970). This terminology was altered in 1972 by Peter and colleagues who devised a dual-functioning naming system based on both contractile properties and oxidative capacity. Type I became slow-twitch oxidative red (SO), Type IIa became fast-twitch oxidative white (FOG) and Type IIb became fast-twitch glycotic white (FG) (Peter *et al.*, 1972). By the mid-1980's groups had begun to identify the correlations between myosin heavy chain isoform expression (via electrophoresis),

contraction speeds and myosin ATPase activity (Reiser *et al.*, 1985; Staron & Pette, 1986). In 1989, Schiaffino et al, described a third possible fibre type in fast twitch muscle when they discovered IIx MHC protein (Schiaffino *et al.*, 1989).

Current classifications of human muscle fibre types commonly refer to three types based on histochemical staining of MHC, these being: type I 'slow-red', type IIa 'fast-red' and type IIx 'fast-white'(Smerdu *et al.*, 1994), with type IIb identified mainly in non-human species such as mice, rats, rabbits and sheep, although some type IIb fibres can be found in the facial and eye muscles of humans. Type I, IIa and IIx fibres all differ in contractile and energetic properties that are known to be associated with their MHC isoform content (Bottinelli & Reggiani, 2000).

A summary of the characteristics of these fibre types is given below (Table 2.3):

Table 2.3 Summary of skeletal muscle fibre type characteristics

Classification Scheme	***Fibre Type***		
Contractile speed	Slow-twitch	Fast-twitch	Fast-twitch
Myosin heavy chain	I	IIa	IIx
Metabolic function	Oxidative	Oxidative	Glycotic
Average % in human vastus lateralis (Andersen & Aagaard, 2000)	40	30	30

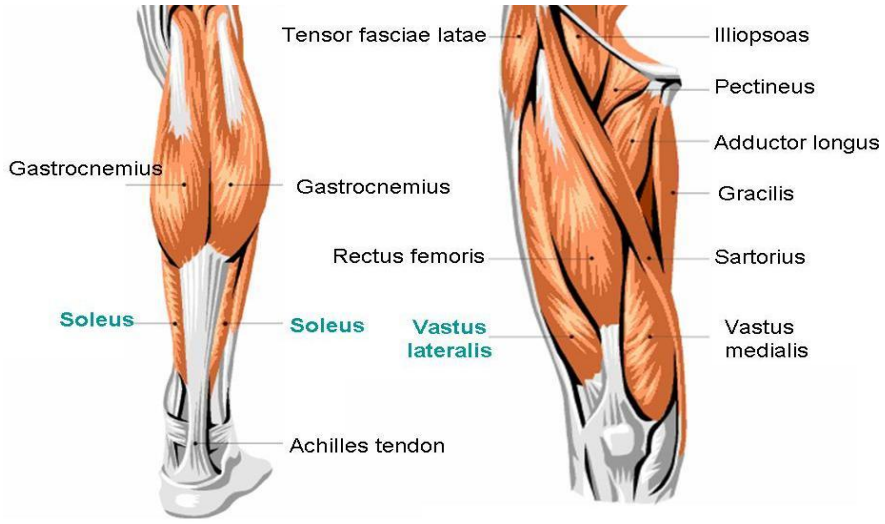
Adapted from: (Spangenburg & Booth, 2003)

It must be acknowledged that the majority of these characteristics are based on the idea of a continuum with much individual variation. Type I fibres contain the MHC 1 isoform and have lower maximum shortening velocity, maximum power and ATPase activity and slower kinetics of stretch than type IIx fibres. Type IIx fibres contain MHC 2x and have a higher specific force than type I. Type IIa fibres are an intermediate classification containing MHC 2a isoforms, they have shortening velocity, maximum power, ATPase activity and kinetic stretch speed that is in between that of type I and type IIx fibres, although specific force which is the same as that seen in type IIx fibres (Hilber *et al.*, 1999).

Within a single human, fibre type distribution and ratio will vary greatly, even among the large limb and trunk muscles. Not all of an individual's skeletal muscles will have the same fibre type distribution. Johnson and colleagues (Johnson *et al.*, 1973) demonstrated this when he found that in 6 male autopsied cadavers the *soleus* muscle contained 75-98% type I fibres while the *vastus lateralis* contained only 20-46% type I fibres. These significant differences may be explained by the different functionality of these muscles and therefore the related differences in the firing patterns of the innervating motor neurons.

The *soleus* muscle is situated inferior to the *gastrocnemius* in the calf (see Figure 2.4). It originates from the posterior surface of the head of the fibula and the internal border of the tibia. At the other end it forms a tendon with the *gastrocnemius*; commonly known as the Achilles tendon, this inserts onto the posterior surface of the calcaneus. The *soleus* is for the majority of the time in steady tension. This is required for posture, without the constant pull of the *soleus* the body would fall forward. When activated the *soleus* also aids in plantar flexion of the foot.

In comparison, the *vastus lateralis*, the largest muscle in the *quadriceps femoris*, situated in an anterior position in the upper leg (see Figure 2.4) is more active without postural need for steady tension. Attached to the anterior and inferior borders of the greater trochanter, to the lateral aspect of the gluteal tuberosity and to the lateral aspect of the linea aspera, three quarters of this muscle is attached. Inserting at the patella and the tibial tuberosity via the patellar ligament, the *vastus lateralis* is involved in the extension and stabilization of the knee joint- an action required for running, jumping, dancing and walking, for example, and therefore plays a very different role to that of the fore mentioned *soleus*.



Adapted from: www.orgs.jmu.edu/strength/images/male_muscles

Figure 2.4 Anatomical positions of *soleus* and *vastus lateralis*

Findings of heterogeneous expression of MHC's within a single fibre (Pette & Staron, 2000) in combination with new screening methods such as 2D-gels and micro-arrays have made evident the fact that there are many more than three types of protein expressed in human skeletal muscle. In 2001 it was suggested that the co-expression of MHC isoforms with the same muscle fibre may form a sub-population of hybrid fibres with high adaptive potential; able to switch phenotype to meet new functional demands (Baldwin & Haddad, 2001).

The cadaver samples used by Johnson (Johnson *et al.*, 1973) were from healthy but relatively sedentary individuals. Among sportspeople the range of muscle fibre type distribution variation within individuals' muscles is greater still. Exercise training (both resistance and endurance type) is a major factor in determining muscle phenotype. RET is well known to elicit both muscle hypertrophy and a shift of fibre type distribution (Bottinelli & Reggiani, 2000; Schiaffino & Reggiani, 1996; Fluck & Hoppeler, 2003). With regard to fibre type distribution and training, longitudinal studies have often shown a small and surprisingly similar training-induced shift for endurance and RET (Adams *et al.*, 1993; Liu *et al.*, 2003; Andersen *et al.*, 1994). Despite the differences in muscle mass and metabolism induced by

the two forms of training a type IIx → IIa shift is most commonly observed (Fluck & Hoppeler, 2003; Jansson & Kaijser, 1977) with little evidence to suggest that type I fibres may be increased through training.

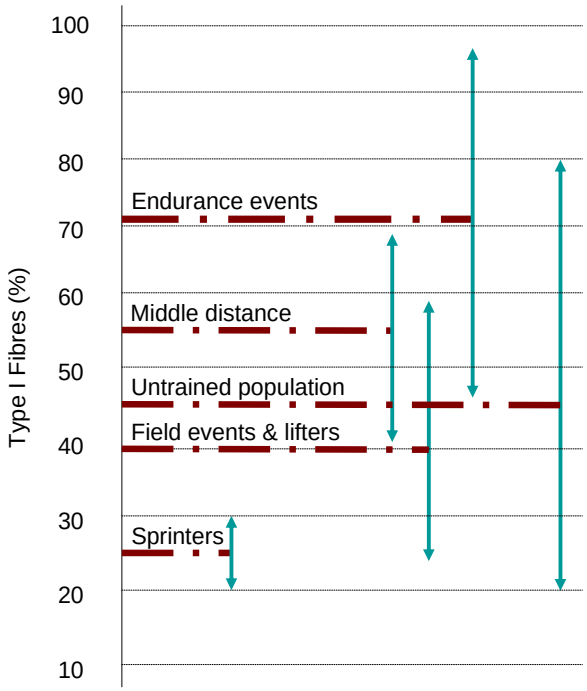
An increase in the number of type I fibres has been observed in animal studies, although 24 hour a day muscle stimulation was required, something that is near impossible to achieve in humans (Trumble *et al.*, 2001). In 2000 Thayer and colleagues (Thayer *et al.*, 2000) reported an increase in type I fibre number after 10 years of endurance training, however the cross-sectional design of this study raises the question of whether this was an age or training effect. This question also followed a study by Trappe five years earlier where after 20 years the same increase in type I fibres was seen in both endurance trained and untrained males (Trappe *et al.*, 1995).

There is some disagreement in the literature as to what happens to fibre type distribution with ageing. In 1983, Larsson and colleagues reported a slight increase in relative proportions of Type I fibres with age (Larsson, 1983). This increase however, was not confirmed by other later cross-sectional studies (Porter *et al.*, 1995). Indeed when Frontera and colleagues studies the muscle of the same subjects at aged 65 y and then 12 y later, they observed a decrease of Type I fibres from ~60% to 40% (Frontera *et al.*, 2000).

With regard to RET, Booth and Pette both found that sprint and power training caused some shift of type IIx to type I and type IIa fibres which can be attributable to increased activity levels but the most noticeable difference was the increased size (cross-sectional area) of type IIx fibres (Booth *et al.*, 1998; Pette, 1998). Howald and more recently Putman, both reported a significant increase in the size of type IIx fibres following RET (Howald, 1982; Putman *et al.*, 2004). One potential mechanism proposed for this increase in size is stretch as a stimulus to growth (Goldspink *et al.*, 1992). It has also been proposed that the increase in type I and IIa fibres following training, including that of a resistance/ power variety may be due

to the elevated levels of human growth hormone (HGH) and testosterone following RET which in turn has the capacity to increase oxidative metabolism as favored by muscle fibre types I and IIa (Kraemer, 1992).

In contrast to longitudinal studies, cross-sectional studies have shown a strong bias in fibre type distribution towards fast-twitch fibres in elite sprinters (~70% type IIa and IIx fibres) and slow-twitch fibres in elite marathon runners (60-90% type I fibres) (Sjostrom *et al.*, 1988; Andersen *et al.*, 2000) (see Figure 2.5). The inconsistency between these two types of study may be explained by either the longer or more intense training of elite athletes, or by a genetically determined bias of fibre type distribution (D'Antona *et al.*, 2006), or both.



Adapted from: Spurway, 2006 (Spurway, 2006)

Figure 2.5 Relative percentages of type I fibres in different athletes and untrained people
Red horizontal lines = means; Green vertical lines = ranges. This figure is representative of fibre number and not size.

2.1.3 Nutrition and frailty

As people age a loss of muscle mass, strength and declines in metabolic function begin to occur. This must be considered when developing programs to change diet and lifestyle.

When trying to maintain or improve muscle mass and/ or strength at any stage of life it must be acknowledged that muscle protein is directly affected by protein in the diet. A high dietary protein intake increases MPS by increasing systemic amino acid availability (Motil *et al.*, 1981) and the amino acids absorbed as a result of protein digestion stimulates MPS in a dose-dependent way. Several studies indicate that healthy older adults may need more protein than the dietary recommended intake (DRI) to increase muscle mass and strength (Campbell *et al.*, 1994; Campbell *et al.*, 2001), although studies involving commercial nutritional protein supplements or high protein diets in the elderly have mainly reported negative results (Campbell *et al.*, 1995; Welle & Thornton, 1998; Fiatarone *et al.*, 1994). These negative results may be in part due to a calorie-for-calorie reduction in other food intake if a supplement is involved (Fiatarone *et al.*, 1994), or the absence of exercise training in these studies.

Rates of MPS are responsive to RET and also to feeding. RET in the absence of nutrients will not result in optimal gains. The anabolic effects of exercise are amplified by protein intake. Protein intake above the EAR of $0.66 g.kg.day^{-1}$ stimulates the fractional synthetic rate (FSR) of muscle protein which has been shown to be positively correlated with muscle strength (Balagopal *et al.*, 1997). FSR can be defined as a parameter which can estimate protein turnover from kinetic tracer data using validated formulae. Stimulation of muscle via RET should interact with nutrients in any meals consumed in the 24 hours following and result in muscle growth. Interaction of nutrient intake and RET has been demonstrated to increase MPS without a concomitant increase in MPB, thus resulting in a positive net muscle protein balance (Biolo *et al.*, 1997; Tipton *et al.*, 1999; Rasmussen *et al.*, 2000). The ingestion of amino acids post exercise has been shown to further stimulate muscle protein synthesis (Tipton *et al.*,

1999; Borsheim *et al.*, 2002), ultimately resulting in greater muscle fibre hypertrophy (Kim *et al.*, 2005).

The need for exercising individuals to consume greater dietary protein than sedentary people is because those exercising will need to off-set the oxidation of amino acids during exercise with protein intake. This oxidation can account for ~ 1-5% of the total energy cost of exercise, plus there will be a need for protein to repair any exercise induced muscle damage and to provide substrate for the synthesis of lean tissue (Tarnopolsky, 2004). Research has shown that this increased need for protein in exercising individuals is true regardless of training mode (i.e. resistance or endurance based) or indeed training status (Friedman & Lemon, 1989). Risks in consuming insufficient amounts of protein, especially for exercising individuals include the creation of a negative nitrogen balance leading to muscle catabolism and impaired recovery (Campbell *et al.*, 2007). This extra protein requirement is thought to be even more so for those undertaking RET, especially during the initial stages of training or if sharp increases in volume occur (Campbell *et al.*, 2007). There is however evidence that training over time can lead to biological adaptations that may improve net protein retention (Rennie & Tipton, 2000).

The additional protein requirements of exercising individuals may be consumed in dietary foods or by the use of supplementation, which in some circumstances can prove to be convenient (Tipton & Wolfe, 2004). Common sources of protein include meat, milk, whey, casein and soy-based products, all of which provide different protein bioavailability; explained as the amount and variety of amino acids that are digested and absorbed into the blood stream after protein ingestion (Campbell *et al.*, 2007). The quality of a protein source is often determined by the protein digestibility corrected amino acid score (PDCAAS), established by the Food and Agriculture Organization. Milk-derived whey protein isolate presents the highest PDCAAS of all the common protein sources, thought to be due to its high content of essential and BCAA's (Campbell *et al.*,

2007). BCAA's (leucine, isoleucine and valine) make up ~30% of skeletal muscle protein and current literature suggests that of the three BCAA's, leucine is most significant in stimulating MPS (Rennie *et al.*, 2006). Research has shown that whey protein elicits a sharp, rapid rise of plasma amino acids following ingestion (Boirie *et al.*, 1997) and induces a greater protein gain than the common alternative supplement, casein (Dangin *et al.*, 2003).

The timing of protein intake can prove integral in preserving muscle mass, eliciting hypertrophy and/ or ensuring successful recovery from exercise. There is much research advocating the positive role of protein intake post RET on increases in muscle mass (Willoughby *et al.*, 2007; Esmarck *et al.*, 2001; Tipton *et al.*, 2003; Cribb *et al.*, 2007) and high levels of blood amino acids following RET have found to be significant in promoting MPS (Biolo *et al.*, 1997). Protein intake when combined with exercise may also have a role in sustaining optimal immune function (Campbell *et al.*, 2007). A study by Flakoll and colleagues showed that the ingestion of a mixed (carbohydrate, fat and protein) nutritional supplement, rich in protein, immediately after exercise resulted in 33% less medical visits, 28% less infection-related visits, and 37 % less orthopedic-related visits. Self-reported post-exercise muscle soreness was also significantly reduced, compared to those who had ingested a placebo supplement of no nutritional value (Flakoll *et al.*, 2004).

Overall, the muscular growth, recovery aid and health benefits that can be achieved by adequate protein intake all lead to the conclusion that protein requirements equal to or above the DRI's should reached by all through healthy whole-food intake. It is however safe and may prove very convenient for exercising individuals with a higher protein requirement to ingest some high quality protein in the form of supplementation. Claims that high protein diets can be detrimental to health due to metabolic strain on the kidneys and increased calcium excretion are unfounded with no substantive evidence to support these reports in healthy individuals (Campbell *et al.*, 2007). On the contrary preliminary clinical studies have

suggested benefits of high-protein diets on major risk factors for chronic diseases, such as kidney disease, hypertension, diabetes, obesity and the metabolic syndrome (Martin *et al.*, 2005) and data is now showing that those most susceptible to osteoporosis should consume dietary protein above current recommended levels to optimize bone mass by increasing circulating levels if IGF-1 (Dawson-Hughes *et al.*, 2004).

2.1.4 Implications of frailty

Frailty is a common and important syndrome that is increasingly prevalent with advancing age. Associated with adverse health outcomes such as disability and admission to hospital or long-term care (Clegg *et al.*, 2011), frailty is often responsible for reduced quality of life, reduced independence maintenance and increased health-care costs for an ageing population. Frailty is characterised by cumulative physiological decline, which results in a vulnerability to sudden changes in health status that can be triggered by relatively minor stressor events (Walston *et al.*, 2006). The frailty phenotype includes weight loss, reduced energy expenditure, slower gait speed, losses of strength and power and sarcopenia (as outlined in Figure 2.1). A recent study reported a frailty prevalence rate of 8.5% for women, and 4.1% for men in 638 people aged 64-74 years (Syddall *et al.*, 2010). Frailty is cyclic; its development results in a situation that leads to worsening frailty and increased risk of adverse health consequences.

Because of the associated adverse health consequences, frailty impacts directly on quality of life, well-being and independence maintenance and has important economic implications. Any intervention therefore, that has the potential to attenuate prevalence or severity of frailty is likely to have benefits for not only the individual in question but also for their families and society as a whole (Clegg *et al.*, 2011).

2.1.5 Frailty and resistance-exercise training

As one of the key components of frailty, sarcopenia is characterised by loss of muscle mass and strength reflective of a progressive withdrawal of anabolism and increased catabolism. Sarcopenia is mediated by multiple

factors including alpha-motor neuron death, altered hormone concentrations, inflammation and inactivity (Jones *et al.*, 2009). Sarcopenia is a potential target for frailty prevention initiatives as interventions to increase muscle mass and strength through exercise have been shown to improve basic mobility skills such as getting up from a chair, climbing stairs and walking to the toilet. These mobility skills, which most people would take for granted are critical for maintenance of independence in older age and loss of these skills can lead to increased care requirements (Clegg *et al.*, 2011). RET has been shown in numerous studies to improve the strength and muscle mass of divergent training groups (Benton & Schlairet, 2012; Macdonald *et al.*, 2012; Geirsdottir *et al.*, 2012), including very old geriatric patients already classed as frail (Binder *et al.*, 2005), although strength increments are typically much larger than hypertrophic responses (Strasser *et al.*, 2009).

Age-related declines in skeletal muscle mass may also contribute to other age-associated changes such as reductions in BMD, insulin insensitivity and decreased aerobic capacity. This association suggests that elderly people gaining both muscle mass and strength with adequate RET may not only improve functional independence but may also decrease the prevalence of many age-associated chronic diseases that have links with declines in skeletal muscle mass (Evans, 1992).

2.2 Methodology

2.2.1 Muscle protein synthesis

In order to determine AA kinetics a primed constant infusion of trace quantities of stable isotope amino acids; "the tracer", were administered through a forearm vein to minimise changes in plasma AA profiles. The tracer infusion contained [1, 2-$^{13}C_2$]- Leucine, (Cambridge Isotopes, Cambridge, MA, USA) which was quality assessed for purity and sterility prior to tracer manufacture, and which were then aseptically dissolved in 250 ml of 0.9% at the Clinical Trials Sterile Production Unit, Pharmacy, Queens Medical Centre, Nottingham. The desired tracer infusion rate for

each subject was calculated by subject body weight to obtain a constant level of pre-cursor labeling at ~6% (Table 2.4) and was administered via an infusion pump (Volumed µVP5005) at a prime dose of 0.7 $mg \cdot kg^{-1}$, followed by a constant infusion rate of 1 $mg \cdot kg^{-1}$. During the fed phase of the study the tracer infusion rate was increased to ~1.6 $mg \cdot kg \cdot hr^{-1}$ to ensure a constant level of labeling, accounting for the 6% increase in leucine from the feed.

Table 2.4 Calculations of tracer infusion rate

Amino acid	Prime	Fasted Infusion	Fed Infusion
[1,2-$^{13}C_2$]- Leucine	0.66 $mg \cdot kg^{-1}$	1 $mg \cdot kg \cdot hr^{-1}$	1.6 $mg \cdot kg \cdot hr^{-1}$

The tracer infusion of [1,2-$^{13}C_2$]- Leucine coupled with the sampling of venous blood and the muscle biopsies allowed us to measure muscle specific protein synthesis by measuring directly and indirectly the uptake of tracer by the muscle (Figure 2.6).

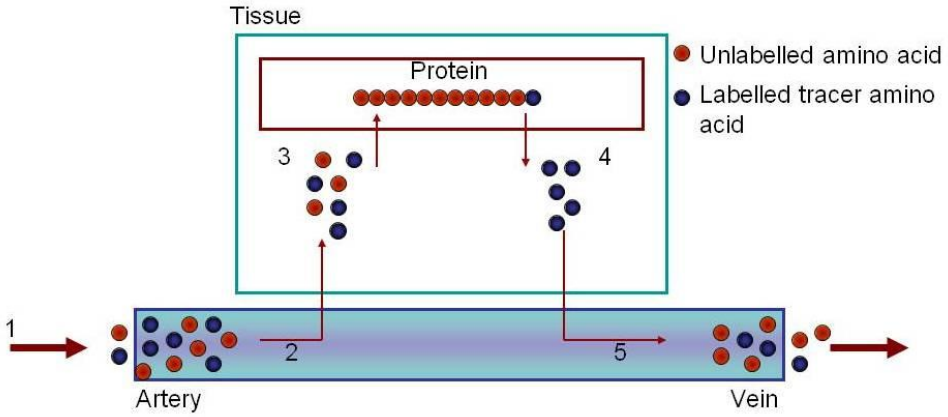


Figure 2.6 Principle of tracer uptake by skeletal muscle
1. Injection of labelled and unlabelled tracer amino acids
2. Uptake of amino acids into tissue
3. Incorporation into tissue protein
4. Unlabelled amino acid release
5. Venous concentration

The primed constant infusion method that we used relies on the assumption that steady state aminoacyl-tRNA (the immediate pre-cursor for protein synthesis) is attained, however as this is extremely difficult to measure, labelling in the α-keto acid of leucine was used as a surrogate measure as it

is metabolized intracellularly and closely reflects the labelling of the true leucyl-tRNA (Watt *et al.*, 1991).

To determine the labelling of α-ketoisocaproate (α-KIC), plasma was deproteinised with 100% ethanol and dried. Lipids were removed by ethyl acetate extraction and then samples were converted to their *tert*-butyldimethylsilyl (t-BDMS) derivatives. Enrichments were determined by gas-chromatography-mass spectrometry using a Trace DSQ GC-MS (Thermo Fisher Scientific, Hemel Hempstead, UK) and the use of appropriate internal standards.

The rate of muscle protein synthesis between the biopsies was calculated using standard equations:

$$\mathbf{FSR\ (\%\cdot h^{-1}) = (\Delta E_m/E_p\ x\ 1/t)\ x\ 100}$$

Where ΔE_m is the change in labelling of muscle protein leucine between the two biopsy samples, E_p is the mean enrichment over time of the precursor for protein synthesis (venous α-KIC $^{13}C_2$ labelling), and t is time between the biopsies with venous α-KIC representing the immediate precursor for protein synthesis, i.e. leucyl-*t*RNA as discussed above (Nair *et al.*, 1988).

To determine the change in labelling of muscle protein leucine between the two biopsy samples it was required that we isolated the myofibrillar protein from our muscle biopsy samples. Myofibrillar protein isolation was achieved using the following method:

Muscle sample was snipped using fine point scissors in cold extraction buffer (0.02 M Tris, 0.15 M NaCl, 0.1 M EDTA, 0.1 M Triton-X) using 10µl per mg of tissue. The homogenate was centrifuged at 1’600 x *g* for 20 min, the supernatant removed and the myofibrillar/ collagen pellet re-suspended in 0.3 M NaOH. The supernatant was centrifuged further (7’000 g for 15 min) and the sarcoplasmic fraction was precipitated by bringing

the supernatant to 70% with ethanol and centrifuging again (1'600 g). The pellet containing the myofibrillar and collagen protein fractions was twice washed with 0.7 M KCl and the soluble myofibrillar protein and the insoluble collagen were separated by centrifugation at 1'600 g for 20 min.

The myofibrillar fraction was precipitated using 1 M perchloric acid and the pellet washed twice with 70% ethanol. Myofibrillar protein was hydrolysed in 0.05 M HCl/Dowex 50W-X8-200 at 110°C overnight (Balagopal *et al.*, 1994), and the liberated amino acids purified then eluted in 2 M NH_4OH. The AA's were then derivatized as their N-acetyl-n-propyl (NAP) ester (Meier-Augenstein, 1999).

The incorporation of [1,2-$^{13}C_2$] leucine into protein was then analysed by gas chromatography combustion-isotope ratio mass spectrometry (GC-C-IRMS; Delta-plus XL, Thermo Fisher Scientific, Hemel Hempstead, UK). Separation was achieved on a 25m x 0.25 mm x 1.0μ film DB17-1 capillary column (Agilent Technologies, West Lothian, UK) (Bohe *et al.*, 2001; Moore *et al.*, 2005).

2.2.2 Muscle protein concentrations by immunoblotting

Muscle biopsies (~10-20 mg) were homogenized with scissors in ice-cold extraction buffer (10 μl/mg^{-1}) containing 50 mM Tris-HCl (pH 7.4), 0.1% Triton X-100, 1 mM EDTA, 1 mM EGTA, 50 mM NaF, 0.5 mM activated sodium orthovanadate (all Sigma Aldrich, Poole, UK) and a complete protease inhibitor cocktail tablet (Roche, West Sussex, UK). Homogenates were rotated on a Vibramax for 10 min at 4°C and centrifuged at 10'000 g for 10 min at 4°C before recovery of supernatants representing sarcoplasmic fractions. Bradford assays were used to determine sarcoplasmic protein concentrations after which samples were standardized to 1 mg/ml^{-1} by dilution with Laemmli loading buffer in order to measure relative protein concentrations of our proteins of interest. Samples were mixed and heated at 95°C for 5 min before fifteen μg of protein/lane was loaded on to Criterion XT Bis-Tris 12% SDS-PAGE gels (Bio-Rad, Hemel

Hempstead, UK) for electrophoresis at 200 V for ~60 min. Gels were equilibrated in transfer buffer (25 mM Tris, 192 mM glycine, 10% methanol) for 30 min before proteins were electroblotted on to 0.2 μm PVDF membranes (Bio-Rad) at 100 V for 30 min. After blocking with 5% low-fat milk in TBS-T (Tris Buffered Saline and 0.1% Tween-20; both Sigma-Aldrich, Poole, UK) for 1 h, membranes were rotated overnight with primary antibody (all AbCam, Cambridge, UK) against our target proteins at a concentration of 1:2000 at 4°C. Membranes were washed (3×5 min) with TBS-T and incubated for 1 h at room temperature with HRP-conjugated anti-rabbit secondary antibody (New England Biolabs, UK), before further washing (3×5 min) with TBS-T and incubation for 5 min with ECL reagents (enhanced chemiluminescence kit, Immunstar; Bio-Rad). Blots were imaged and quantified by assessing peak density after ensuring bands were within the linear range of detection using the Chemidoc XRS system (Bio-Rad, Hemel Hempstead, UK). Where required, phosphorylation of signalling proteins was corrected for loading anomalies to an appropriate loading control.

2.2.3 Whole body strength

Whole-body strength was measured as the sum of 6 1-RM assessments performed at the beginning of the RET and every four weeks throughout. The 1-RM assessments were on 6 resistance training machines (Cybex International UK Ltd, Derbyshire, UK) with the assessments carried out by a fully qualified exercise professional following a standard operating procedure that included a pre-determined warm up set of 10 repetitions, 3 min rest between warm-up and assessment beginning and 2 min rest in-between each 1-RM attempt. The final 1-RM was to be determined within 5 attempts, or the assessment was declared void and was performed again ~48h later. The exercises assessed were three using the upper-body; latissimus pull-down, seated chest press and seated lever row and three for the lower body; leg extension, seated leg curl and leg press.

2.2.4 Diet diary analysis

Analysis of the subjects three-day diet diaries from prior to and during the exercise training program were analyzed using Microdiet diet analysis software, version 5 (Downlee Systems Ltd) to assess the subjects dietary habits, macro and micro-nutrient composition. This data was then assessed to determine if there were differences in the habitual diets of our three age groups or if they altered in response to RET.

2.2.5 Muscle fibre typing

Taking 25 mg of basal muscle sample from before and after RET, myofibrillar protein was extracted as described earlier in this chapter. The pellet containing myofibrillar and collagen fractions was then snipped using fine point scissors in 250 µl of lysing buffer (10% Glycerol, 5% 2-Mercaptoethanol, 2.3% Sodium dodecyl sulphate (SDS), dissolved in 62.5 mM Tris-HCl-solution (pH 6.8)). Samples were mixed and heated at 90°C for 3 min and then diluted 1:20 with lysing buffer (as above) before 1 µl of sample was loaded on to self-cast 6% SDS-PAGE gels (separation gel: 13.4 ml Acrylamide/ Bis-acrylamide buffer (30 ml 2% bis-solution, 150 ml 40% Acrylamide solution bought to 200 ml with ddH_2O), 6.7 ml Lower-Tris solution (36.34 g Tris-base, 8 ml 10% SDS bought to 200 ml with ddH_2O (pH 8.8)), 15.0 ml Glycerol, 2.0 ml 10% SDS, 5.0 ml 1M Glycine, 7.5 ml ddH_2O, 750 µl 10% Ammonium persulfate, 60 µl TEMED; stacking gel: 3.72 ml 30% Acrylamide solution, 4.71 ml 2M Tris-HCl-solution (pH 6.8), 10 ml Glycerol, 250 µl 10% SDS, 6.24 ml ddH_2O, 125 µl 10% Ammonium persulfate, 20 µl TEMED), alongside a protein ladder to confirm isoform size at analysis.

The gels were placed into a mini-system electrophoresis tank (Bio-Rad, Hemel Hempstead, UK) filled with 1 litre running buffer (3.02 g Tris-Base, 14.41 g Glycine, 1.01 g SDS bought to 1 litre with ddH_2O) and 400 µl of mercaptoethanol in the upper chamber. An electrical current was passed through the tank at 50 V for 30 hours. Gels were then silver stained according to the manufacturer's instructions (Proteosilver Silver Stain Kit,

Sigma Life Sciences, UK). Gels were imaged and the proportion of identified type I, IIa and IIx bands quantified by assessing peak density after ensuring bands were within the linear range of detection using the Chemidoc XRS system (Bio-Rad, Hemel Hempstead, UK) (Figure 2.7).

MHC-IIx - top, MHC-IIa – middle, MHC-I - bottom.

Figure 2.7 Image of a bis-acrylamide gel after electrophoresis to identify MHC isoforms.

2.2.6 RNA: DNA: Protein ratios

Taking 15 mg of basal muscle sample from before and after RET; the sample was snipped using fine point scissors in 1ml 0.2M percholric acid (PCA; BDH Laboratories) before vortexing and centrifugation at 4°C at 11’000 rpm for 8 minutes to form a pellet. The pellet was then washed twice in 1ml 0.2M PCA before 800µl of 0.3M sodium hydroxide (NaOH; Sigma Aldrich, Poole, UK) was added to the pellet before they were placed in a hot block at 37°C for 30 min to allow the pellet to dissolve.

Once dissolved 20µl of sample was removed to measure total protein using the Bradford assay. In brief, 967µl of Bradford reagent (Sigma Aldrich, Poole, UK) was mixed with 26.4µl 0.3M NaOH and 6.6µl of sample before reading on a spectrophotometer (Bichrom WPA Lightwave, UK) at 595nm after 10 minutes at room temperature.

400µl of 1M PCA was added to the remaining dissolved samples and they were cooled at 4°C for 10 min before vortexing and centrifugation at 4°C at 5’000 rpm for 5 minutes to form a pellet. The supernatant was then removed and stored in new vials before 300µl 0.2M PCA was added to the pellet before vortexing and centrifugation at 4°C at 5’000 rpm for 5 min.

The supernatant from this was then added to the new vials giving a volume of 1.5ml which was used to measure RNA.

In brief, RNA measurements were made on a spectrophotometer at 260 and 270nm with 0.2M PCA as the blank. The equation used to calculate RNA taking account of peptide material was:

$$\textbf{RNA } \mu g/ml^{-1} = (abs260nm \times 11.87) - (abs275nm \times 10.4) \times (100/9.7)$$

1ml 2M PCA was then added to the pellet and the samples were placed in a heated block at 70°C for 60 min. Samples were then vortexed and centrifuged at 4°C at 5'000 rpm for 5 min to form a pellet. The supernatant was then removed and stored in new vials. 300µl 2M PCA was added to the pellet before vortexing and centrifugation at 4°C at 5'000 rpm for 5 min. The supernatant from this was then added to the new vials giving a volume of 1.3ml which was used to measure DNA.

In brief, DNA measurements were made on a spectrophotometer at 268 and 274nm with 2M PCA as the blank. The equation used to calculate RNA taking account of peptide material was:

$$\textbf{DNA } \mu g/ml^{-1} = (abs268nm - abs284nm) \times (100/9.7)$$

2.3 Results

2.3.1 Effect of resistance-exercise training on muscle protein synthesis

There was no effect of age on basal MPS either before or after RET and RET had no effect on basal MPS in any of the age-groups.

All groups demonstrated increased MPS in response to feeding before RET (Y: 0.044±0.004 vs. 0.082±0.010, $P<0.001$; M: 0.041±0.004 vs. 0.064±0.005, $P<0.01$; O: 0.042±0.003 vs. 0.065±0.006%·h^{-1}, $P<0.01$) but there was no difference in fed MPS between the age-groups before RET, although there was a trend for it to be higher in the young than in the

middle-aged group (P=0.08). After RET, the young and the middle-aged groups still demonstrated increased MPS in response to feeding compared to basal values (Y: 0.053±0.006 vs. 0.085±0.011, P<0.01; M: 0.047±0.003 vs. 0.061±0.004 %·h^{-1}, P<0.05). Surprisingly, the old did not demonstrate a significant increase in MPS in response to feeding alone after RET (0.045±0.006 vs. 0.061±0.006%·h^{-1}, P>0.05) and this may be due to the slightly increased (but not significant) basal value observed after RET (0.042±0.003 vs. 0.045±0.006%·h^{-1}, P>0.05). This unusual finding may also be due to the statistical analysis used as when comparing the old groups basal MPS to their fed MPS after RET with a paired t-test there was a significant difference (P=0.02).

Both before (Y: 0.044±0.004 vs. 0.081±0.008; M: 0.041±0.004 vs. 0.081±0.005; O: 0.042±0.003 vs. 0.083±0.006%·h^{-1}, all P<0.001) and after RET (Y: 0.053±0.006 vs. 0.105±0.011; M: 0.047±0.003 vs. 0.085; O: 0.045±0.006 vs. 0.090±0.008%·h^{-1}, all P<0.001) all three groups responded to the dual stimuli of exercise-plus-feeding when compared to basal values. In the young, MPS in response to exercise-plus-feeding was significantly higher after RET compared to before (0.081±0.008 vs. 0.105±0.011%·h^{-1}, P<0.05). The old group demonstrated an additional response of exercise-plus-feeding compared to feeding alone only after RET (0.61±0.006 vs. 0.90±0.008, P<0.001).

The only difference between the age-groups either before or after RET was that the MPS in response to exercise-plus-feeding was significantly lower in the middle-aged group compared to the young after RET (0.105±0.011 vs. 0.085±0.007, P<0.05) (Figure 2.8).

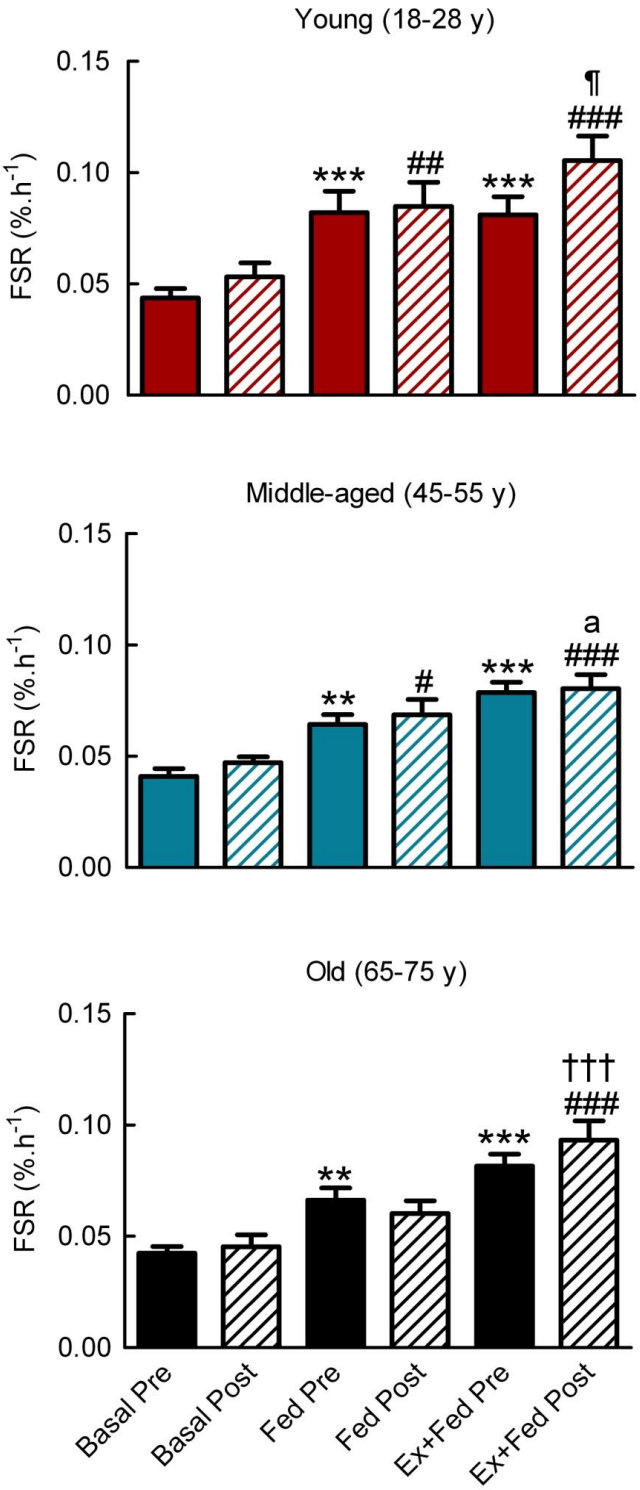


Figure 2.8 Myofibrillar fractional synthetic rates in young, middle-aged and older subjects before and after RET. Values are means±SEM. Statistical analysis via 2-way ANOVA with Bonferroni post-analysis. **= P<0.01 vs. basal pre-training; ***= P<0.001 vs. basal pre-training; #= P<0.05 vs. basal post-training; ##= P<0.01 vs. basal post-training; ###= P<0.001 vs. basal post-training; †††= P<0.001 vs. feeding post-training; ¶= P<0.05 vs. exercise-plus-feeding pre-training; a= P<0.05 vs. young in the same condition.

2.3.1.1 Molecular markers of muscle protein synthesis

Before RET the young did not increase p70S6K (p70) phosphorylation in response to any of the anabolic stimuli. After RET, the young had increased phosphorylation in response to exercise-plus-feeding (0.91±0.05 vs. 1.25±0.05, $P<0.001$). The middle-aged group demonstrated increases phosphorylation in response to exercise-plus-feeding both before (1.00±0.00 vs. 1.19±0.03, $P<0.001$) and after (0.96±0.03 vs. 1.17±0.05, $P<0.001$) RET with phosphorylation after exercise-plus-feeding higher that after exercise alone both before (1.19±0.03 vs. 1.04±0.04, $P<0.01$) and after RET (1.17±0.05 vs. 1.04±0.04, $P<0.05$) and also higher than after feeding alone before RET (1.19±0.03 vs. 1.07±0.04, $P<0.05$). The old group displayed increased p70 phosphorylation after exercise-plus-feeding both before (1.00±0.00 vs. 1.19±0.04, $P<0.01$) and after RET (0.97±0.04 vs. 1.25±0.04, $P<0.001$) with phosphorylation in response to exercise-plus-feeding higher than after exercise alone before RET (1.19±0.04 vs. 1.00±0.04, $P<0.05$). The old group also demonstrated increased phosphorylation in response to feeding alone after RET (0.97±0.04 vs. 1.21±0.04, $P<0.001$). At no time point was there a significant difference between the age groups (Figure 2.9).

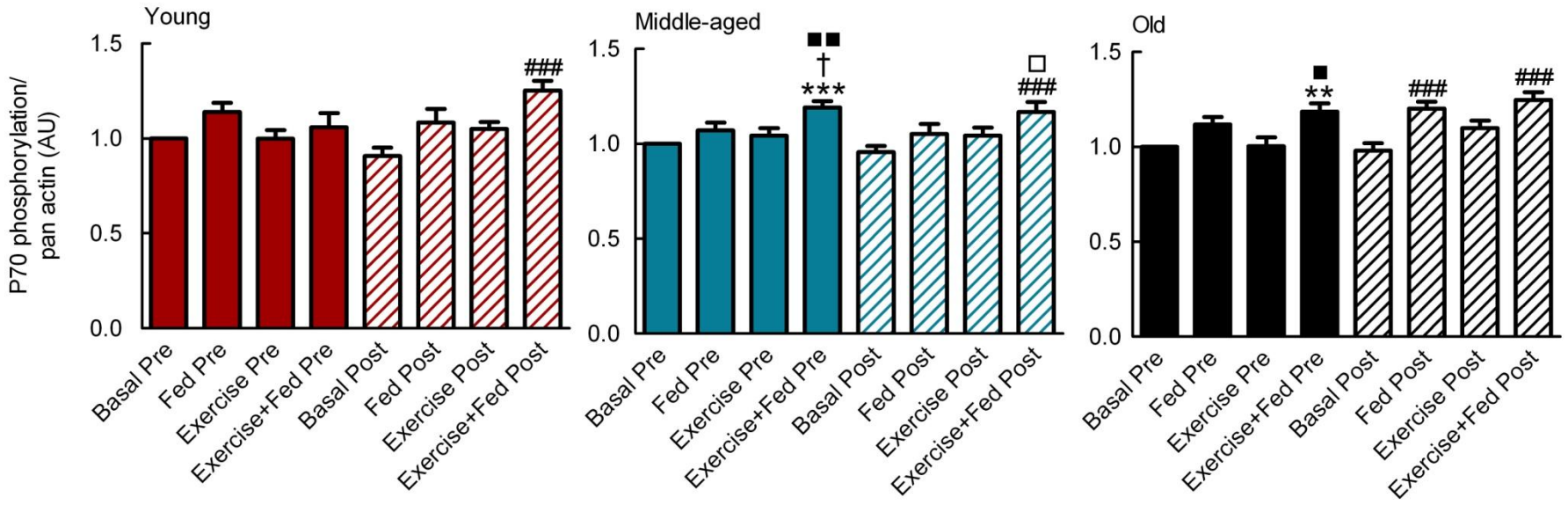


Figure 2.9 P70 phosphorylation in young, middle-aged and old subjects, in basal, fed, exercised and fed-plus-exercised conditions, before and after RET. Analysis by ANOVA with Tukey post-hoc analysis. **= P<0.01 vs. basal pre;***= P<0.001 vs. basal pre; ###= P<0.001 vs. basal post; †= P<0.05 vs. fed pre; ■= P=<0.05 vs. exercise pre; ■■= P= <0.01 vs. exercise pre; □= P<0.05 vs. exercise post.

The young did not increase 4EBP1 in response to any of the anabolic stimuli either before or after RET, although both before and after RET the phosphorylation in response to feeding alone (before: 1.27±0.05 vs. 0.82±0.06, $P<0.001$, after: 1.19±0.06 vs. 0.83±0.05, $P<0.01$) and exercise-plus-feeding (before: 1.15±0.09 vs. 0.82±0.06, after: 1.21±0.05 vs. 0.83±0.05, both $P<0.01$) was significantly higher than after exercise alone. The middle-aged group also showed that exercise suppressed phosphorylation compared to feeding alone (1.13±0.05 vs. 0.86±0.04, $P<0.001$) and exercise-plus-feeding (1.15±0.05 vs. 0.86±0.04, $P<0.001$) before RET but this was not apparent after RET. The old group showed increases in 4EBP1 phosphorylation before and after RET in response to both feeding alone (before: 1.00±0.00 vs. 1.20±0.03, after: 1.04±0.04 vs. 1.23±0.05, both $P<0.01$) and exercise-plus-feeding (before: 1.00±0.00 vs. 1.24±0.04, P<0.001, after: 1.04±0.04 vs. 1.22±0.06, $P<0.01$). The old group also showed significantly higher phosphorylation after feeding alone and exercise-plus-feeding compared to after exercise alone both before (1.20±0.03 and 1.24±0.04 vs. 0.87±0.04 respectively, $P<0.001$) and after (1.23±0.05 and 1.22±0.06 vs. 0.94±0.05 respectively, $P<0.001$) RET. At no time point was there a significant difference between the age groups (Figure 2.10).

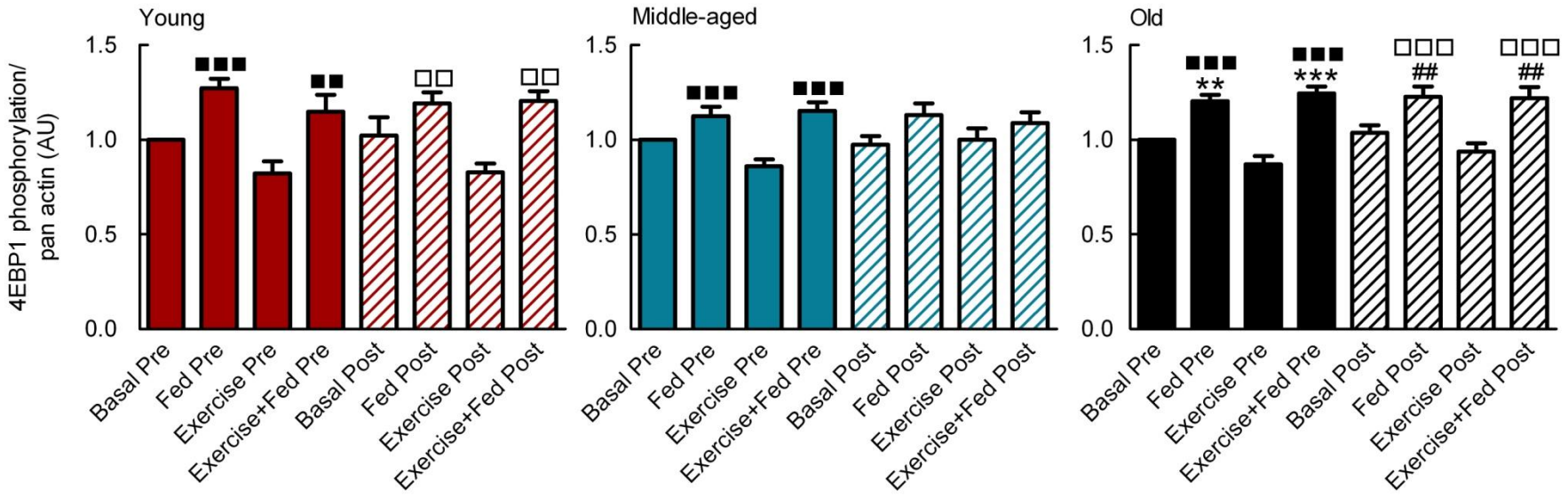


Figure 2.10 4EBP1 phosphorylation in young, middle-aged and old subjects, in basal, fed, exercised and fed-plus-exercised conditions, before and after RET. Analysis by ANOVA with Tukey post-hoc analysis. **= P<0.05 vs. basal pre; ***= P<0.01 vs. basal pre; ##= P<0.01 vs. basal post; ■■= P<0.01 vs. exercise pre; ■■■= P<0.001 vs. exercise pre; □□= P<0.01 vs. exercise post; □□□= P<0.001 vs. exercise post.

Neither before or after RET did the young did demonstrate increased mTOR phosphorylation in response to any of the anabolic stimuli; although they did demonstrate significantly higher phosphorylation in response to exercise-plus-feeding after RET than before (1.26±0.06 vs. 0.92±0.15, $P<0.05$). The middle-aged group had increased mTOR phosphorlyation in response to feeding and exercise-plus-feeding after RET (0.94±0.04 vs. 1.08±0.05, $P<0.05$ and 1.19±0.05, $P<0.001$ respectively) when phosphorylation after exercise-plus-feeding was significantly higher than after exercise alone (1.19±0.05 vs. 1.02±0.05, $P<0.01$). The old did not respond with increased mTOR phosphorylation to any of the stimuli before RET but did display increased phosphorylation in response to feeding alone and exercise-plus-feeding after RET (0.98±0.05 vs. 1.22±0.05 and 1.22±0.06 respectively, $P<0.01$). At no time point was there a significant difference between the age groups (Figure 2.11).

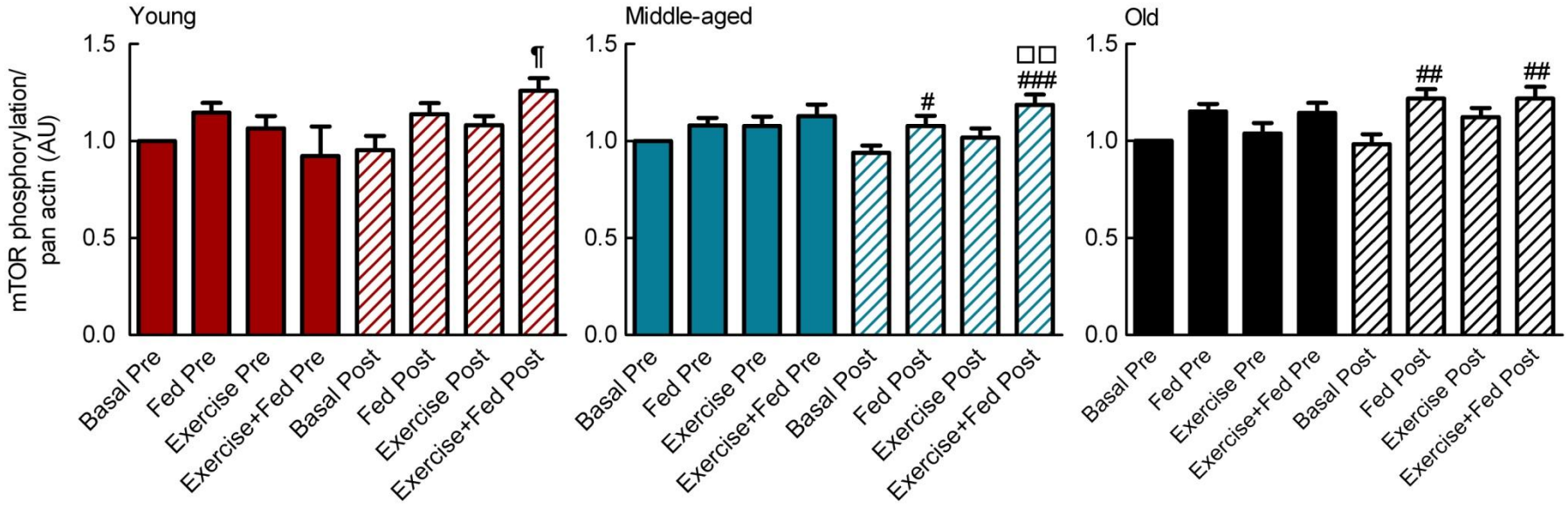


Figure 2.11 mTOR phosphorylation in young, middle-aged and old subjects, in basal, fed, exercised and fed-plus-exercised conditions, before and after RET. Analysis by ANOVA with Tukey post-hoc analysis. #= P<0.05 vs. basal post;##= P<0.01 vs. basal post; ###= P<0.001 vs. basal post;□□= P<0.01 vs. exercise post; ¶= P<0.05 vs. ex+fed pre.

The young group showed a significant depression in EEF2 phosphorylation before RET in response to exercise-plus-feeding that was significantly lower than phosphorylation at basal and after feeding alone (0.73±0.08 vs. 1.00±0.00, $P<0.01$ and 0.94±0.03, $P<0.05$ respectively). This depression also resulted in the phosphorylation of EEF2 after exercise-plus-feeding being significantly lower than in the middle-aged and older groups under the same stimulated conditions before RET ($P<0.001$). This depression was not apparent after RET resulting in higher phosphorylation in response to exercise-plus-feeding after RET when compared to before (0.96±0.06 vs. 0.73±0.08, $P<0.05$). Neither the middle-aged nor the older group showed increases in EEF2 phosphorylation in response to any of the anabolic stimuli either before or after RET. At no time-point was there a significant difference between the age groups (Figure 2.12).

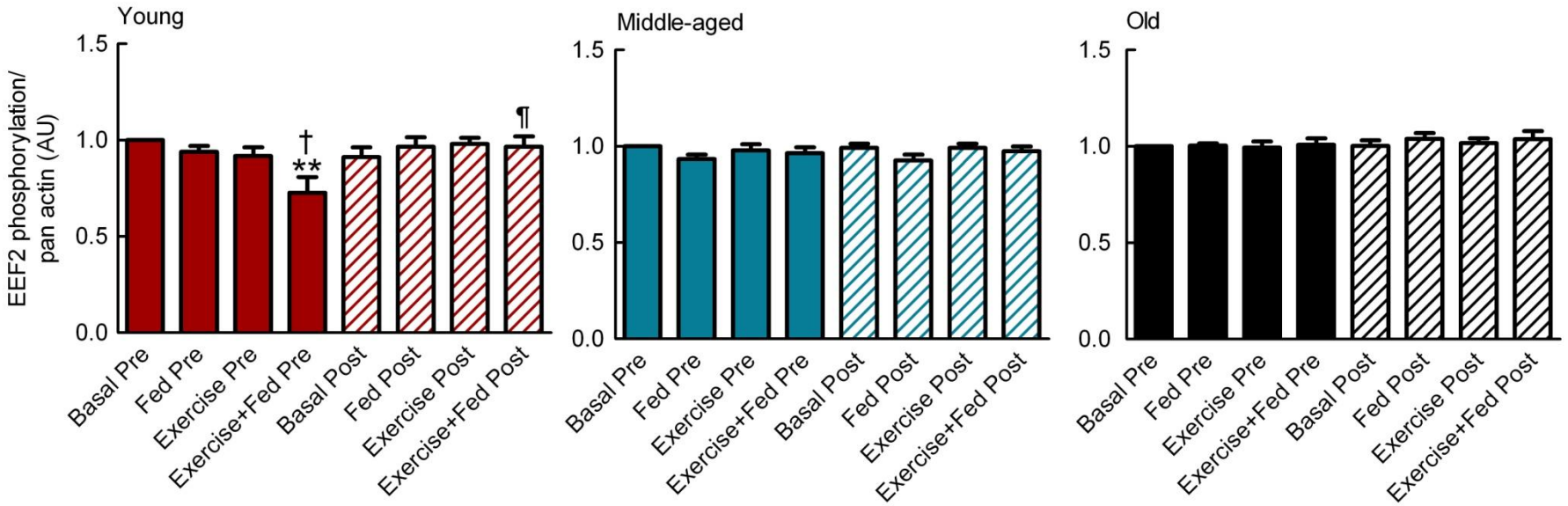


Figure 2.12 EEF2 phosphorylation in young, middle-aged and old subjects, in basal, fed, exercised and fed-plus-exercised conditions, before and after RET. Analysis by ANOVA with Tukey post-hoc analysis.**= P<0.01 vs. basal pre; †= P<0.05 vs. fed pre; ¶= P<0.05 vs. ex+fed pre.

2.3.2 Effect of resistance-exercise training on strength

There were no significant age-related differences in the lean mass of our subjects before RET (see chapter 3), but there were substantial age-related reductions in strength, assessed as the sum of 6 1RM assessments both before (Figure 2.13) and after RET.

Before RET the muscle strength of the young was 5729±405 N which was not significantly different to that of the middle-aged group (4921±387 N) but was significantly higher than that of the older group whose strength was 4082±202 N (P<0.05).

There were no significant differences inrelative strength gains between the groups, with the young increasing from 5729±405 N to 7609±377 N (+~36%), the middle-aged from 4921±387 N to 6535±460 N (+~35%) and the older group from 4082±202 N to 5630±374 N (+~39%). It is interesting to note that after RET, following their 39% increase, the strength of the old group was not significantly different to that of the young group before RET (5630 vs. 5729 N) (Figures 2.13 and 2.14).

In each group males were stronger than females (before training: Y, men 6472 N vs. females 4392 N; M, males 6309 vs. females 3534 N; O, males 4680 vs. females 3227 N) but in each group the strength gains made between men and women were not significantly different.

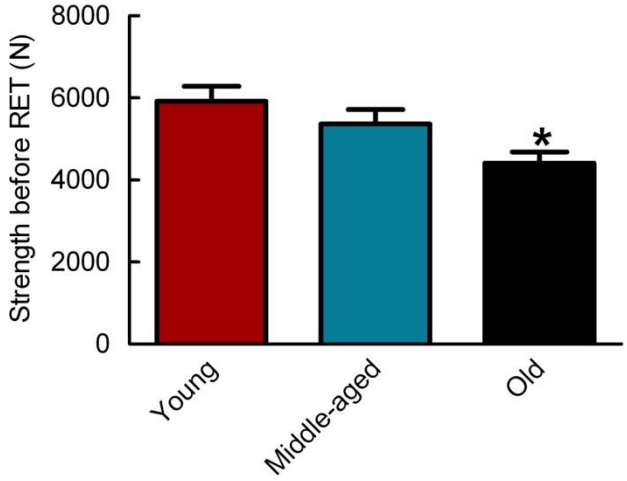


Figure 2.13 Strength in young, middle-aged and older subjects before RET. Values are means±SEM. Statistical analysis via ANOVA with Bonferroni post analysis. *=*P*<0.05 vs. young.

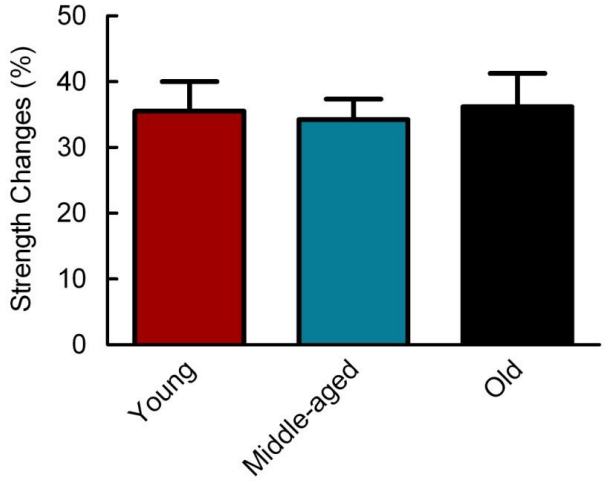


Figure 2.14 Strength gains in young, middle-aged and older individuals following RET. Values are means±SEM. Statistical analysis via ANOVA with Bonferroni post analysis.

The temporal aspect of strength gains was not significantly different between the age-groups with all groups displaying the fastest rate of strength gains between weeks 8 and 12 (discounting weeks 1-8 where the rapid acceleration was likely based on learning and familiarisation as well as physiological changes). The rate of increase in the young between weeks 8 and 12 was significantly higher than at any other time (*P*<0.01) and greater than the middle-aged and old group (9.8% vs. 6.7% and 6.2% respectively) both of whom sustained a greater rate of increase than the young in the subsequent four weeks (4.0% vs. 4.9% and 6.0%). Rate of

increase in the final four weeks of training was greatest in the old group with the middle-aged group demonstrating the least improvement (O, 4.3%; M, 2.5%; Y, 3.0%) (Figure 2.15), which may suggest that the old would have continued to improve had the RET been of a longer duration.

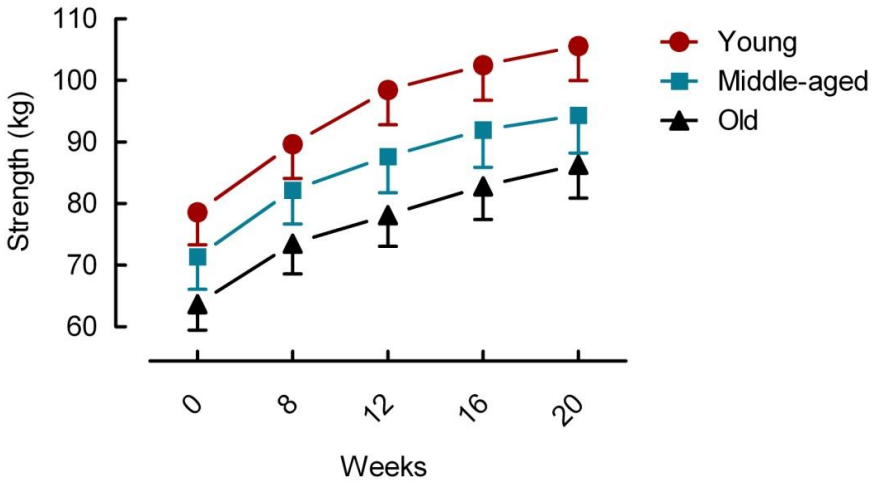


Figure 2.15 Strength in young, middle-aged and older individuals during the RET. Values are given as the average of 6 1-RM assessments and are means±SEM. Statistical analysis via ANOVA with Bonferroni post analysis.

Only in the young group was there a correlation between strength gains and increases in lean mass ($P<0.05$), although there was a trend for this in the middle-aged also ($P<0.06$) (Figure 2.16). This suggests that perhaps in the middle-aged, and certainly in the older subjects the mechanism for improved strength cannot be explained by increases in lean mass and must exist due to changes in alternative mechanisms such as improved neural firing or muscle/ tendon architectural properties.

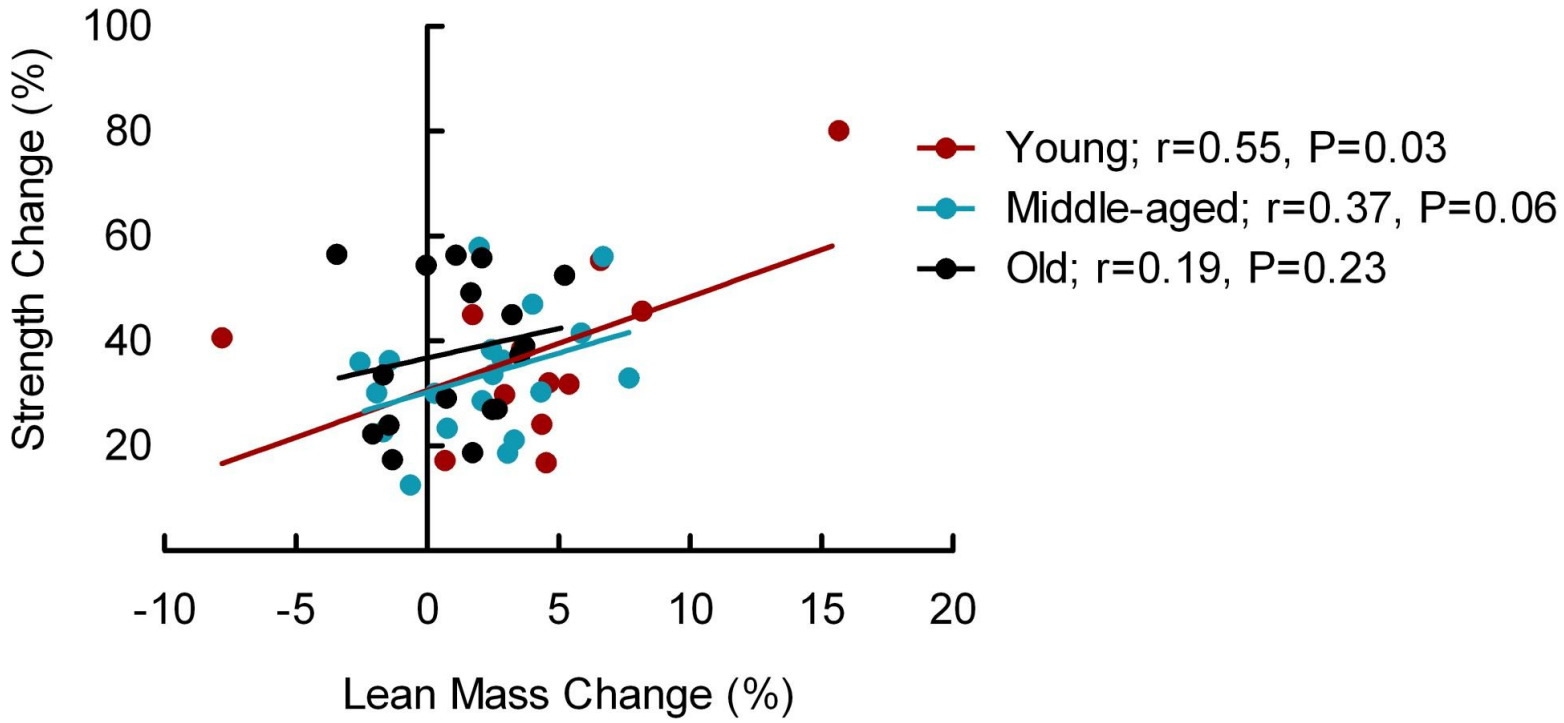


Figure 2.16 Relationship between changes in strength and changes in lean body mass in young, middle-aged and older individuals following RET. Statistical analysis by Pearson's correlation.

2.3.3 Dietary intake with ageing and resistance-exercise training

Before RET the young consumed significantly more kcal per day (2127±156.0 kcal) than both the old (1706.93±84.2 kcal, $P<0.01$) and the middle-aged (1547.52±70.66 kcal, $P<0.001$) groups. There was no difference between the energy intake of the middle-aged and old. During RET there were no significant differences between the calorie intakes of the age-groups. None of the groups significantly changed their energy intake during RET compared to before, although there was a trend for it to be lower in the young (2127.71±156.00 vs. 1784.99±109.396 kcal, $P=0.07$) (Figures 2.17 and 2.18).

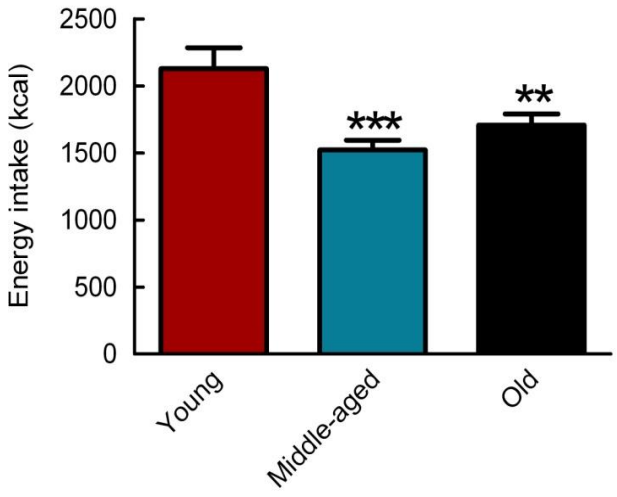


Figure 2.17 Total energy intakes in young, middle-aged and older subjects before RET. Values are mean±SEM. Statistical analysis via ANOVA with Newman-Kelus post analysis. **=P<0.01 vs. young, ***=P<0.001 vs. young.

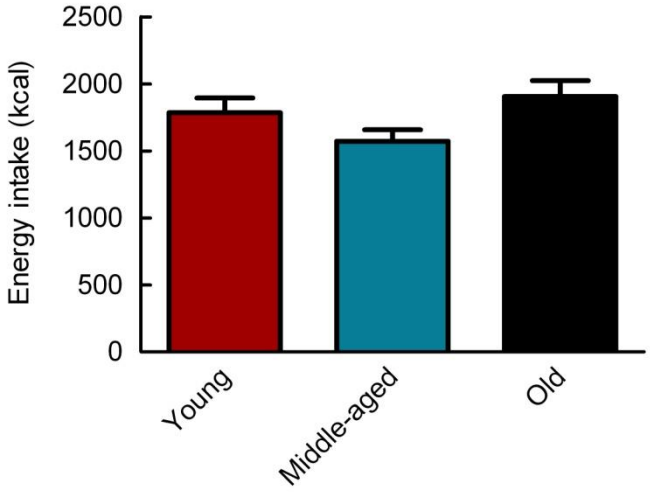


Figure 2.18 Total energy intakes in young, middle-aged and older subjects during RET. Values are mean±SEM. Statistical analysis via ANOVA with Newman-Kelus post analysis.

Neither before or during RET was the percentage of dietary intake composed of protein significantly different between the three age groups. Before RET the percentage of intake composed from fats was significantly lower in the middle-aged group than in the young (28.65±1.42 vs. 34.49±1.76%, $P<0.05$) but was not different to that of the old (32.73±1.20%). During RET there were no differences in the percentage intake from fat between the age groups. The percentage of intake composed from carbohydrates was significantly higher in the middle-aged group than in the young or the old (51.17±1.89 vs. 43.26±1.63 and 44.25±1.44%, respectively, $P<0.01$) before RET with no significant difference between the groups during RET. The middle-aged group had a significantly lower percentage intake from carbohydrates during RET (51.17±1.89 vs. 46.77±1.65%, $P<0.05$) when compared to before and this may be related to the observed trend for an increase in the percentage of energy obtained from fat during RET (28.65±1.42 vs. 31.89±1.14, $P=0.06$). The young did not change their relative percentage intakes from protein, fat and carbohydrates during RET although there was a trend towards a reduction in percentage intake from fat (34.49±1.76 vs. 29.56±2.06%, $P=0.06$). There were no differences in the percentage intake

from fat, carbohydrate and protein in the old group during RET when compared to before RET (Figures 2.19 and 2.20).

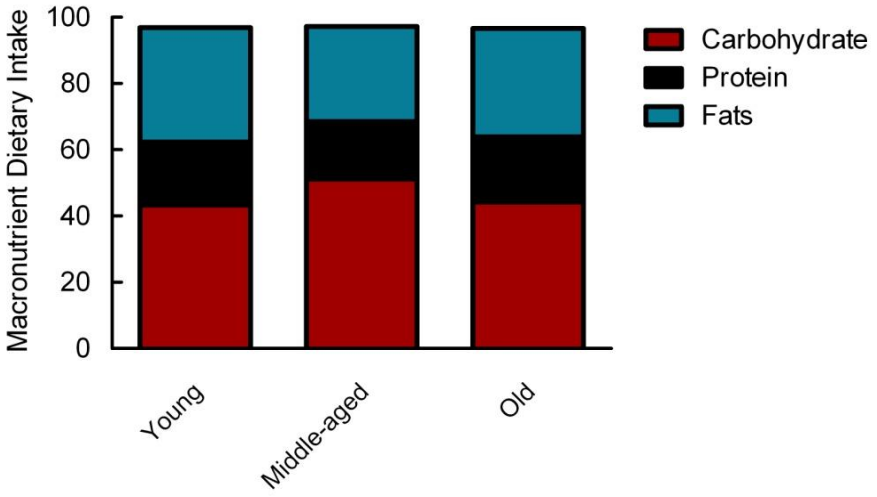


Figure 2.19 Percentage of fat, protein and carbohydrate in the diets of young, middle-aged and older subjects before RET. Values are means. Statistical analysis via ANOVA with Newman-Kelus post analysis.

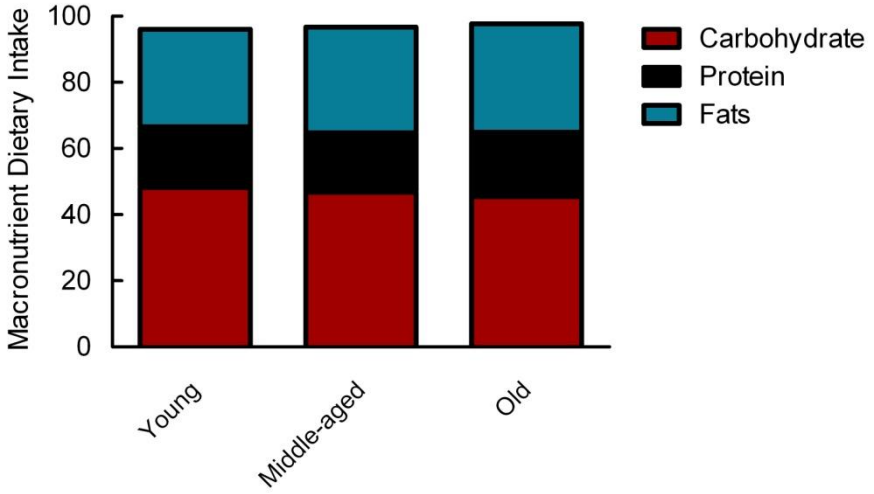


Figure 2.20 Percentage of fat, protein and carbohydrate in the diets of young, middle-aged and older subjects during RET. Values are means. Statistical analysis via ANOVA with Newman-Kelus post analysis.

Absolute protein intake before RET was significantly higher in the young than in the middle-aged (100.04±8.98 vs. 69.96±9.42 g, $P<0.05$), with a trend for it to also be higher than the old (100.04±8.98 vs. 82.03±4.07 g, $P=0.08$). During RET there were no significant differences in absolute protein intake between the age groups. There were no differences in the

middle-aged and old groups absolute protein intakes during RET when compared to before. There was a trend for absolute protein intake to be lower in the young during RET (100.04±8.98 vs. 81.46±7.47 g, *P*=0.07) (Figures 2.21 and 2.22).

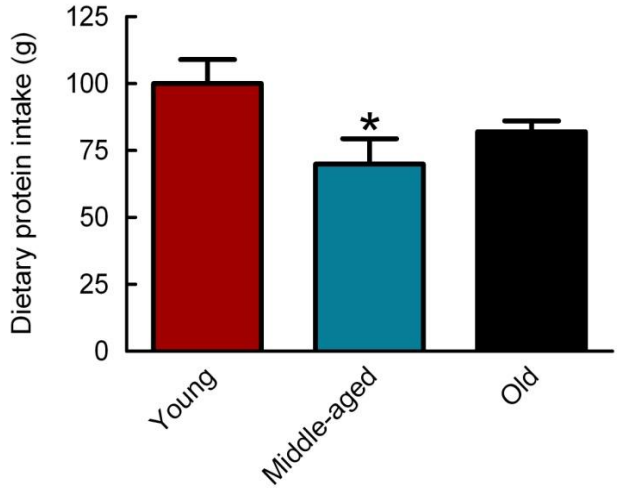


Figure 2.21 Protein intakes in young, middle-aged and older individuals before RET. Values are mean±SEM. Statistical analysis via ANOVA with Newman-Kelus post analysis. *=P<0.05 vs. young.

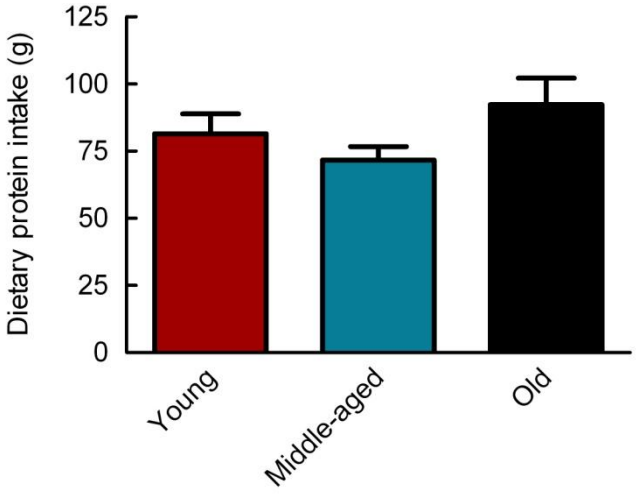


Figure 2.22 Protein intakes in young, middle-aged and older individuals during RET. Values are mean±SEM. Statistical analysis via ANOVA with Newman-Kelus post analysis.

Before RET leucine intake was significantly higher in the young than in the middle-aged and the old (3226.69±624.53 vs. 1495.07±206.12 and 1851.14±187.54 mg, $P<0.01$, respectively). There was no significant difference between the middle-aged and the old. During RET there were no significant differences between the three age groups. None of the groups showed any differences in leucine intake during RET compared to before RET (Figures 2.23 and 2.24).

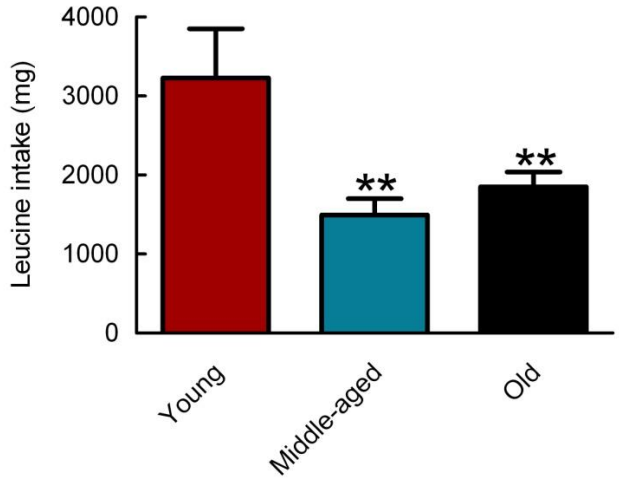


Figure 2.23 Leucine intakes in young, middle-aged and older individuals before RET. Values are mean±SEM. Statistical analysis via ANOVA with Newman-Kelus post analysis. **=P<0.01 vs. young.

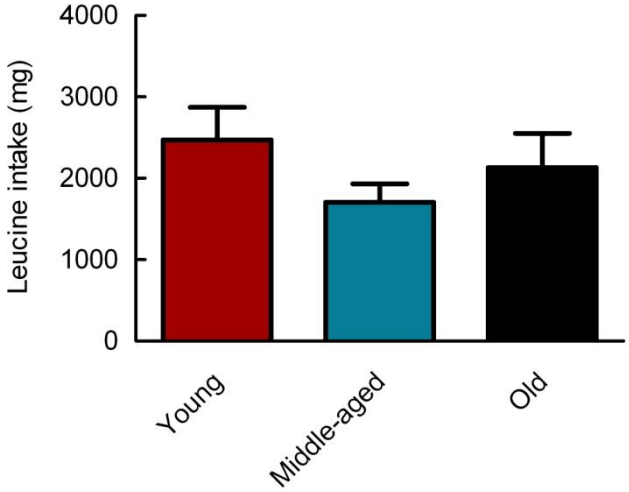


Figure 2.24 Leucine intakes in young, middle-aged and older individuals during RET. Values are mean±SEM. Statistical analysis via ANOVA with Newman-Kelus post analysis.

Before RET saturated fat intake was significantly higher in the young than in the middle-aged and old (31.07±2.93 vs. 16.99±1.55 g, $P<0.001$ and 23.53±1.83 g, $P<0.05$, respectively). The saturated fat intake of the old was significantly higher than the middle-aged ($P<0.01$). During RET there were no significant differences between the age-groups. The young group consumed significantly lower saturated fats during RET compared to before (31.07±2.93 vs. 21.67±2.92 g, $P<0.05$). There was a trend for saturated fat intake to be increased in the middle-aged during RET (16.67±1.44 vs. 19.88±1.97 g, $P=0.08$) with no difference in the old group (Figures 2.25 and 2.26).

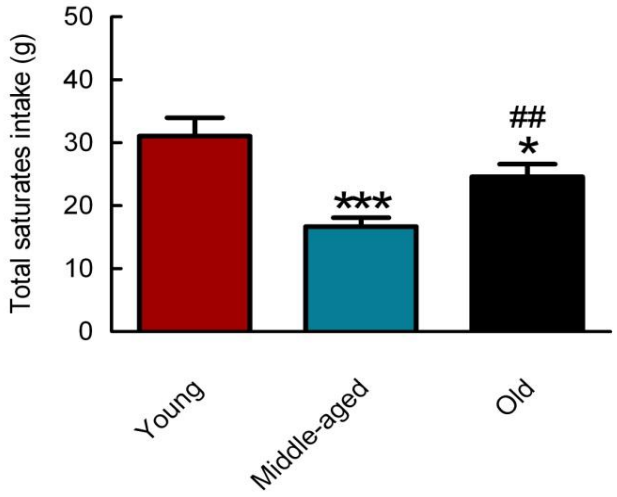


Figure 2.25 Saturated fat intakes in young, middle-aged and older subjects before RET. Values are mean±SEM. Statistical analysis via ANOVA with Newman-Kelus post analysis. *=P<0.05 vs. young, ***=P<0.001 vs. young, ##=P<0.01 vs. middle-aged.

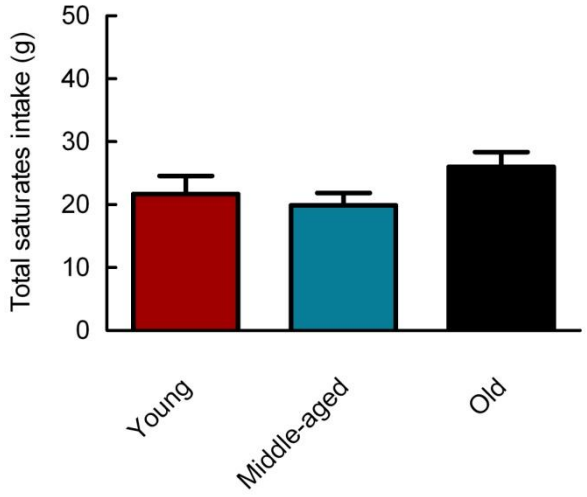


Figure 2.26 Saturated fat intakes in young, middle-aged and older subjects during RET. Values are mean±SEM. Statistical analysis via ANOVA with Newman-Kelus post analysis.

Before RET the young consumed significantly more monounsaturates than the middle-aged and the old (28.64±3.03 vs. 17.31±1.24 g, $P<0.001$ and 21.83±1.13 g, $P<0.05$, respectively) but there were no significant differences between the age groups during RET. In keeping with the reduction in saturated fat intake, there was a trend in the young for lower monosaturated fat intake during RET when compared to before (28.64±3.03 vs. 21.11±2.40 g, $P=0.06$). Neither the middle-aged nor the old group changed their monosaturated fat intake during RET (Figures 2.27 and 2.28).

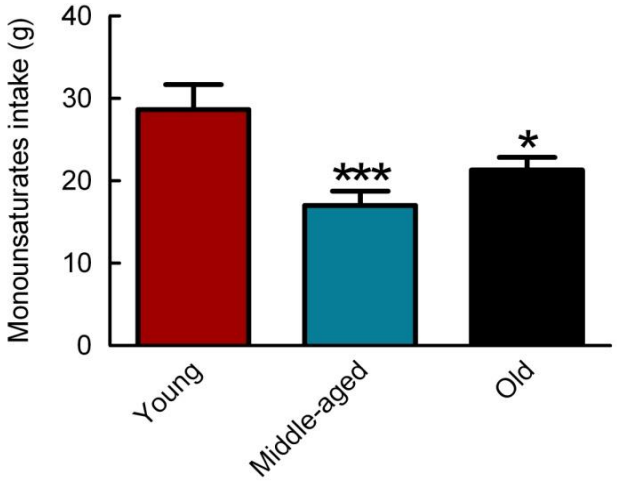


Figure 2.27 Monounsaturated fat intakes in young, middle-aged and older subjects before RET. Values are mean±SEM. Statistical analysis via ANOVA with Newman-Kelus post analysis. *=P<0.05 vs. young, ***=P<0.001 vs. young.

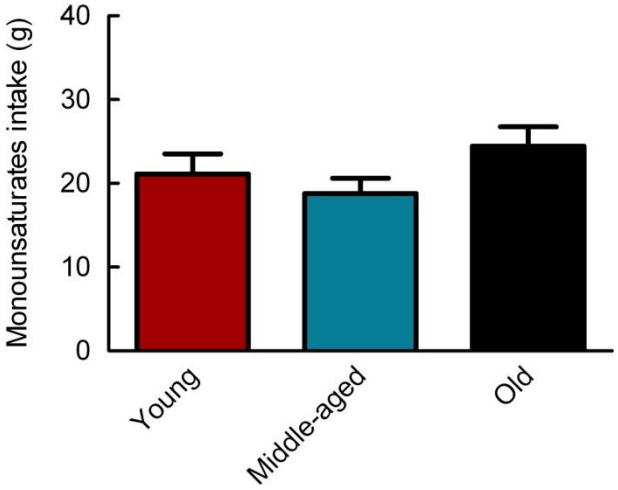


Figure 2.28 Monounsaturated fat intakes in young, middle-aged and older subjects during RET. Values are mean±SEM. Statistical analysis via ANOVA with Newman-Kelus post analysis.

Neither before or during RET was intake of polyunsaturates significantly different between the three age groups and it was unchanged in all three groups when comparing before RET to during RET (Figures 2.29 and 2.30).

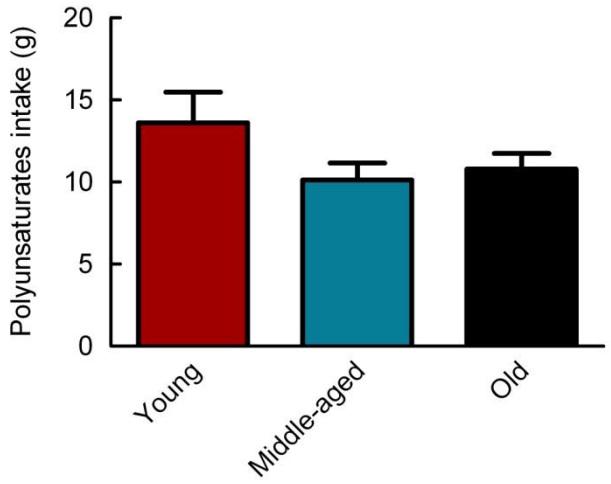


Figure 2.29 Polyunsaturated fat intakes in young, middle-aged and older subjects before RET. Values are mean±SEM. Statistical analysis via ANOVA with Newman-Kelus post analysis.

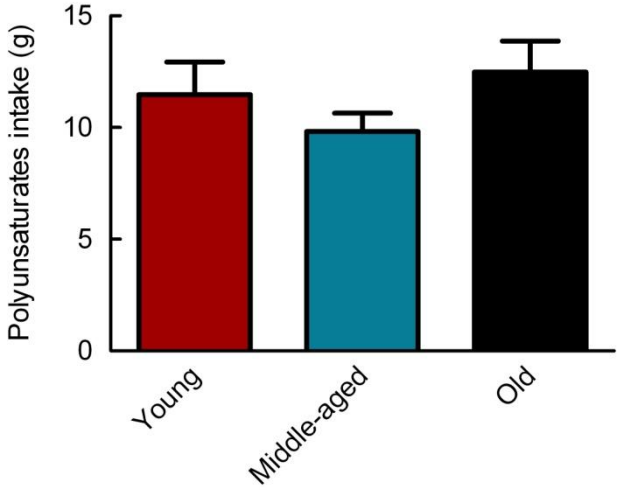


Figure 2.30 Polyunsaturated fat intakes in young, middle-aged and older subjects during RET. Values are mean±SEM. Statistical analysis via ANOVA with Newman-Kelus post analysis.

Cholesterol intake was significantly higher in the young than in the middle-aged group before RET (324.85±47.37 vs. 178.93±35.32 mg,

P<0.05), although neither group had significantly different cholesterol intake to the old (273.58±28.69 mg). During RET there were no significant differences in the cholesterol intakes of the three age groups. None of the groups displayed a significant reduction in cholesterol intake during RET compared to before RET but there was a trend for it to be lower in the young (324.85±47.37 vs. 215.66±36.37 mg, *P*=0.08) (Figures 2.31 and 2.32).

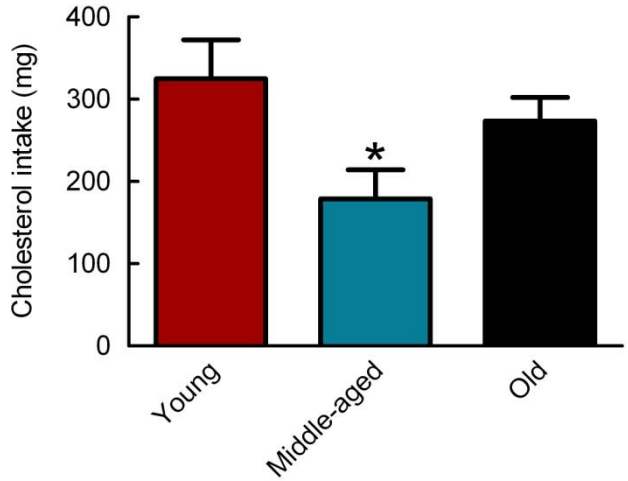


Figure 2.31 Cholesterol intakes in young, middle-aged and older individuals before RET. Values are mean±SEM. Statistical analysis via ANOVA with Newman-Kelus post analysis. *=P<0.05 vs. young.

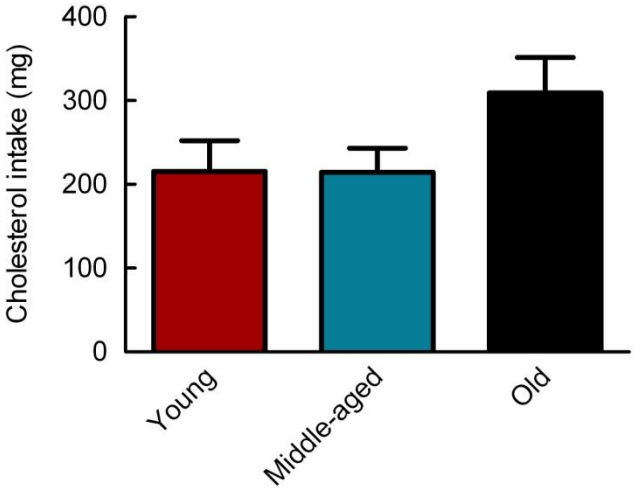


Figure 2.32 Cholesterol intakes in young, middle-aged and older individuals during RET. Values are mean±SEM. Statistical analysis via ANOVA with Newman-Kelus post analysis.

Sodium intake was not significantly different between the three age groups before RET, although during RET the sodium intake of the old was significantly higher than both the young and middle-aged groups (3148.75±265.14 vs. 2156.29±243.99 and 2341.75±2112.33 mg respectively, $P<0.05$).

The young reduced their sodium intake during RET compared to before RET (3129.53±435.301 vs. 2156.29±243.99 mg, $P<0.05$) while the old increased theirs (2511.07±238.731 vs. 3148.75±265.136 mg, $P<0.05$). There was no significant change in the middle-aged group (Figures 2.33 and 2.34).

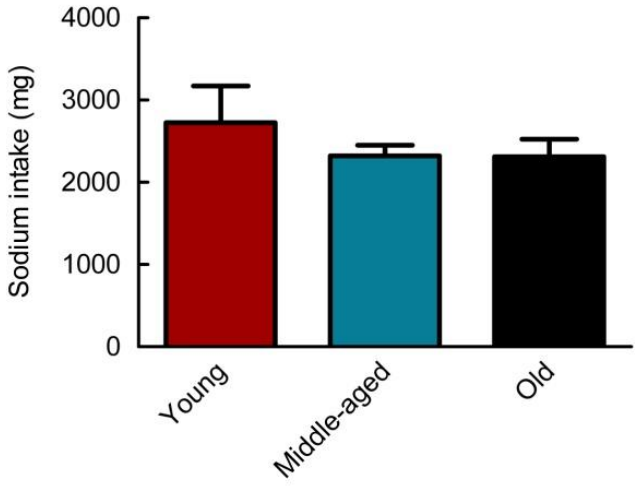


Figure 2.33 Sodium intakes in young, middle-aged and older individuals before RET. Values are mean±SEM. Statistical analysis via ANOVA with Newman-Kelus post analysis.

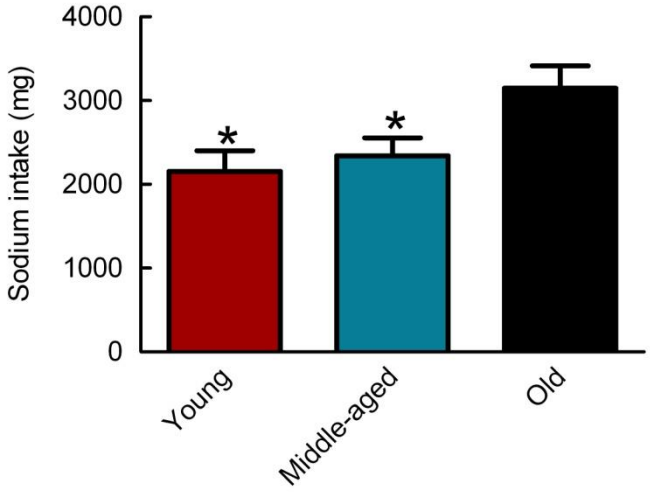


Figure 2.34 Sodium intakes in young, middle-aged and older individuals during RET. Values are mean±SEM. Statistical analysis via ANOVA with Newman-Kelus post analysis *=P<0.05 vs. old.

There were no significant differences in calcium, potassium or vitamin D intake between the three age groups before RET or during RET, although the middle-aged group did have significantly higher vitamin D intake during RET compared to before (2.29±0.42 vs. 3.57±0.62 µg, P<0.05). Iron intake was significantly higher in the young than in the middle aged (13.63±1.45 vs. 10.03±0.70 mg, P<0.05), with a trend for it to also be higher than the old (11.25±0.79 mg, P=0.08) before RET but it was not different between the three age groups during RET due to a significant reduction in iron intake in the young during RET compared to before RET (13.63±1.45 vs. 10.43±0.68 mg, P<0.05) (Figures 2.35 and 2.36).

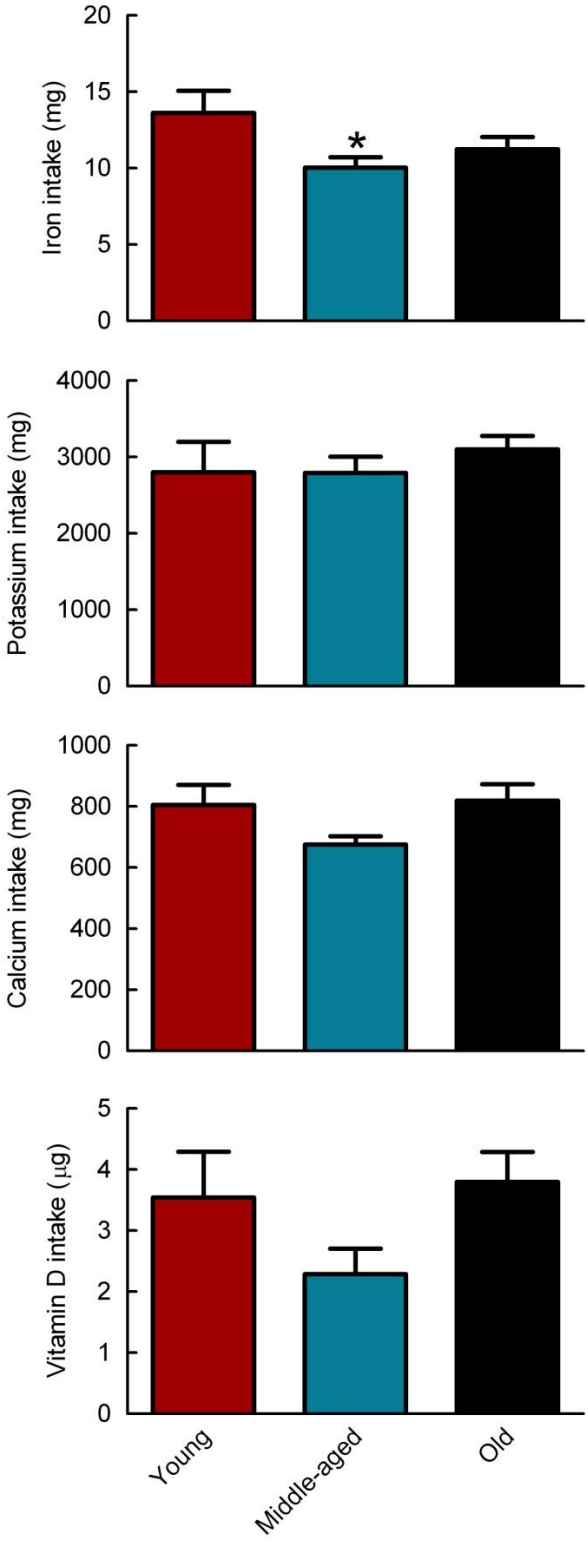


Figure 2.35 Micronutrient intake in young, middle-aged and older individuals before RET. Values are mean±SEM. Statistical analysis via ANOVA with Newman-Kelus post analysis. *=P<0.05 vs. young.

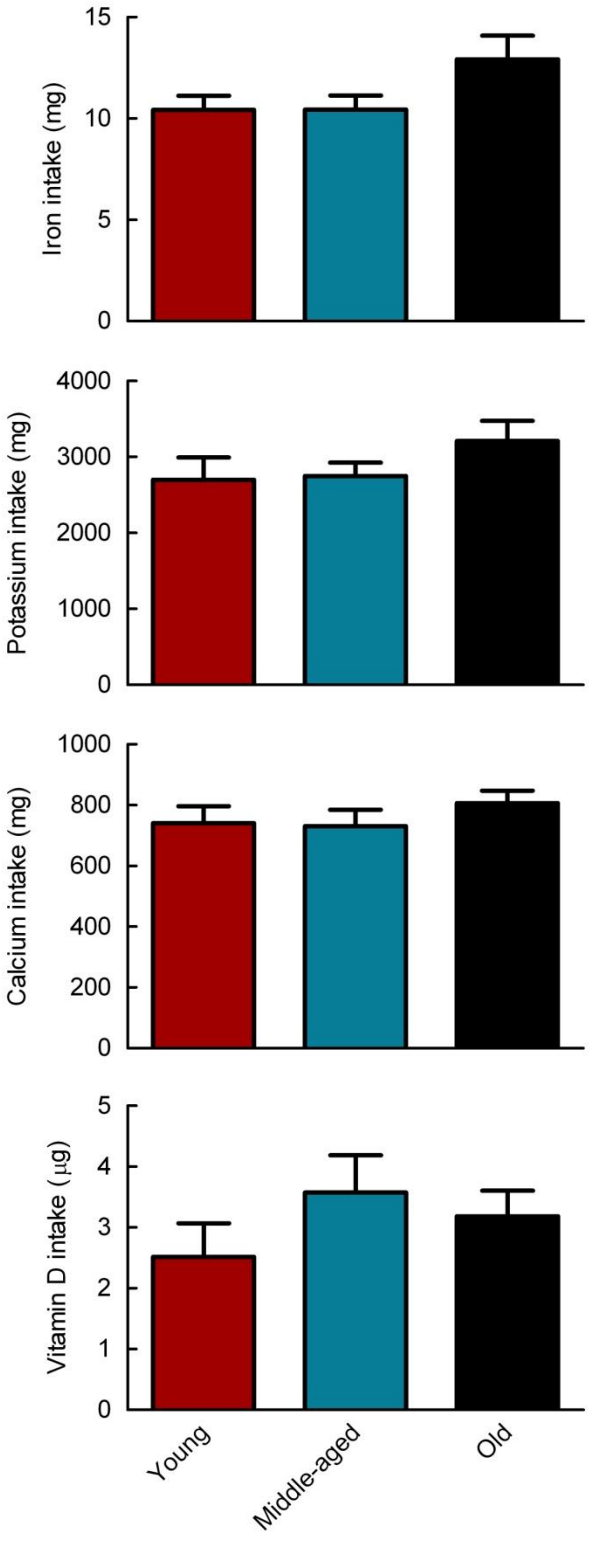


Figure 2.36 Micronutrient intake in young, middle-aged and older individuals during RET. Values are mean±SEM. Statistical analysis via ANOVA with Newman-Kelus post analysis.

2.3.4 Effect of resistance-exercise training on muscle fibre type

Before RET the fibre type distribution between the three age groups was not significantly different. The young and middle-aged groups had even distribution of the three isoforms before RET (Y: I=32.6±0.9, IIa=33.3±0.5, IIx=34.2±0.7; M: I=32.6±0.7, IIa=33.1±0.6, IIx=34.4±0.5%), while the old had a greater percentage of Type IIx than Type I fibres (I=32.0±0.9 vs. IIx=34.9±0.6 P=<0.01 (IIa=33.1±0.6%)).

After RET, the young had a significant reduction in the proportion of Type IIa fibres (33.3±0.5 vs. 31.7±0.5%, P<0.05) and a significant increase in the proportion of Type IIx fibres (34.2±0.7 vs. 35.3±0.9%, P<0.05). The middle-aged group showed a significant increase in the percentage of Type I fibres after RET (32.57±0.7 vs. 33.48±0.5%, P<0.05) as did the older group (32.0±0.9 vs. 33.2±0.9%, P<0.01) who also demonstrated a significant decrease in their percentage of Type IIa fibres (33.1±0.6 vs. 31.8±1.1%, P<0.05). The changes demonstrated by the old group were mirrored by the results for all when the data was grouped together. The grouped data showed a significant increase in the proportion of Type I fibres from 32.3±0.5 to 33.3±0.4%, P<0.001 and a significant reduction in the proportion of Type IIa fibres from 33.1±0.4 to 32.1±0.4%, P<0.01 (Figure 2.37).

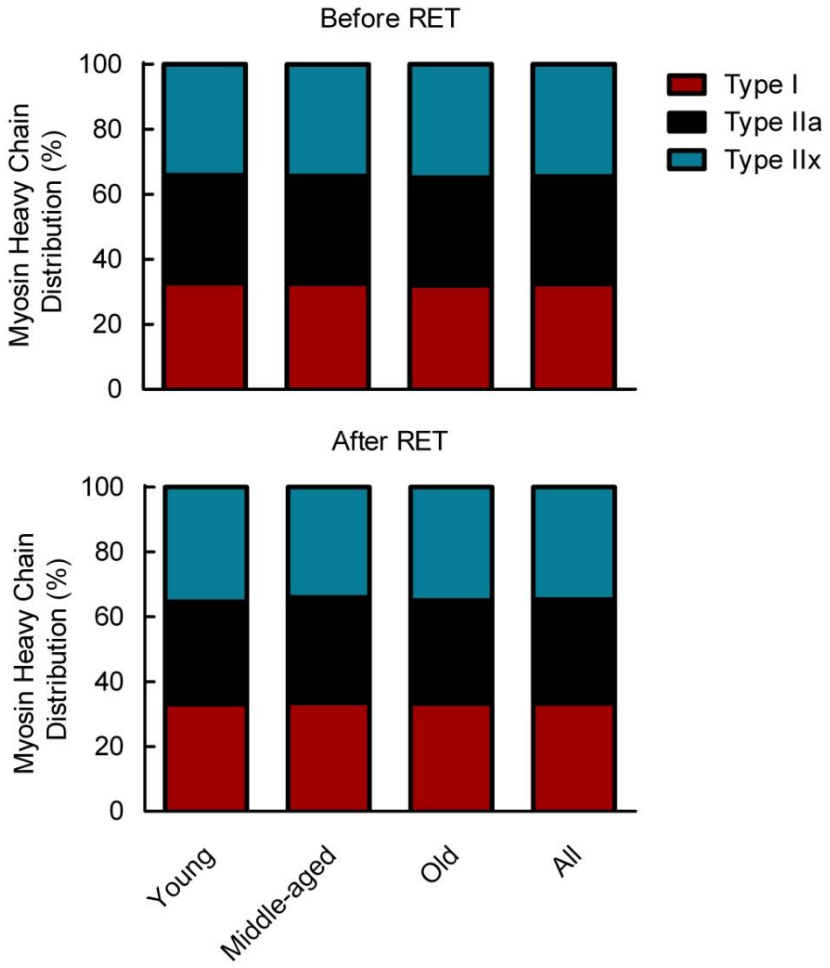


Figure 2.37 MHC isoform distribution in young, middle-aged and older subjects before and after RET. Values are mean±SEM. Statistical analysis via ANOVA with Bonferroni post analysis.

In the young group, after RET the distribution between the three isoforms was no longer even with a significantly higher proportion of Type IIx fibres compared to Type IIa (35.3±0.9 vs. 31.7±0.5%, $P<0.05$). The isoform distribution in the middle-aged group remained even after RET. In the older group the distribution was still not even after RET but the distribution pattern had changed from before RET with the old group displaying more Type IIx fibres than Type II a fibres after RET (35.0±0.6 vs. 31.8±1.1%, $P<0.01$).

When the data from the three age groups was grouped the distribution patterns were the same as that shown by the older group with a significantly higher proportion of Type IIx fibres compared to Type I before RET (34.5±0.4 vs. 32.3±0.5%, $P<0.01$) and a significantly higher proportion of Type IIx fibres compared to Type IIa (34.6±0.4 vs. 32.1±0.4%, $P<0.001$) after RET (Figure 2.38).

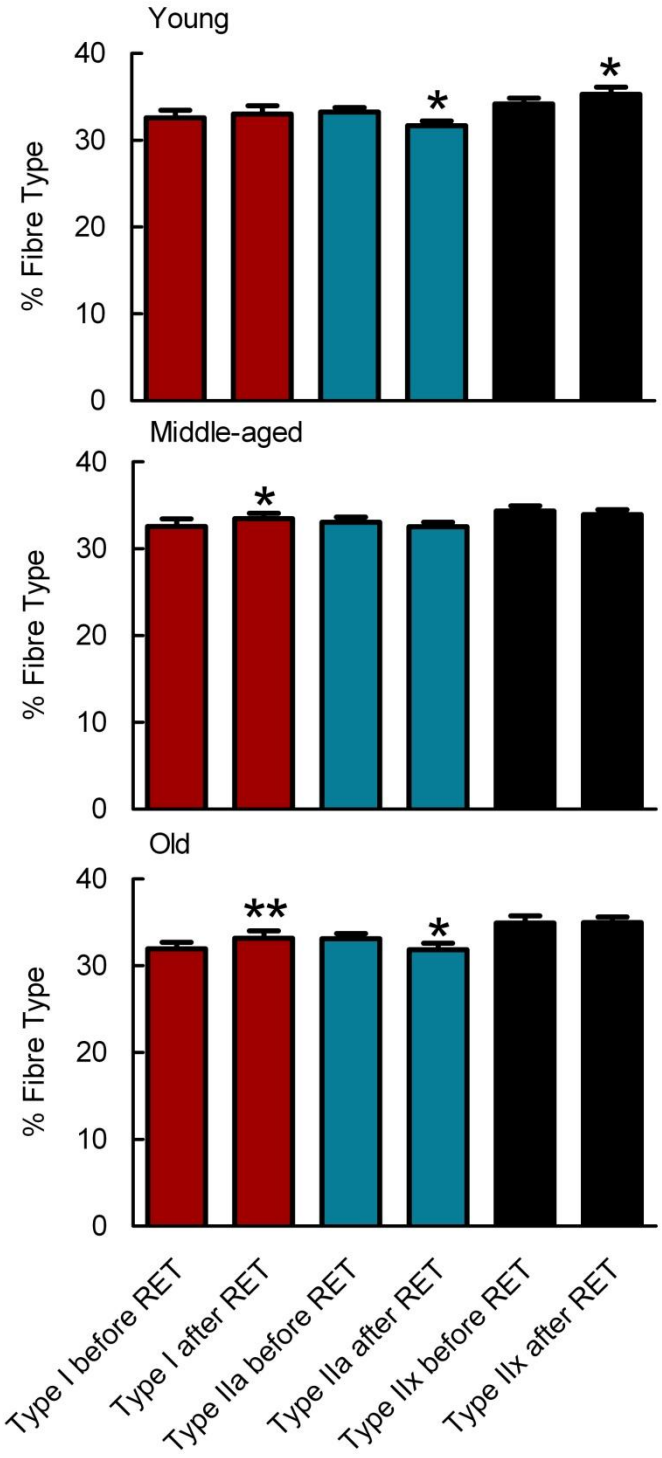


Figure 2.38 Changes in MHC isoform distribution after RET in young, middle-aged and older subjects. Values are mean±SEM. Statistical analysis via ANOVA with Bonferroni post analysis.*=P<0.05 vs. before RET, **=P<0.01 vs. before RET.

2.3.5 Effect of resistance-exercise training on RNA: DNA: Protein ratios

Before RET the protein: tissue ratio was significantly higher in the middle-aged group than in the young (201.40±9.43 vs. 149.45±7.46, $P<0.01$). None of the groups demonstrated a significant change in their protein: tissue ratio after RET, although the ratios of the different age-groups were not significantly different after RET (Y, 159.73±11.90; M, 200.86±8.02; O, 181.29±10.45) (Figure 2.39).

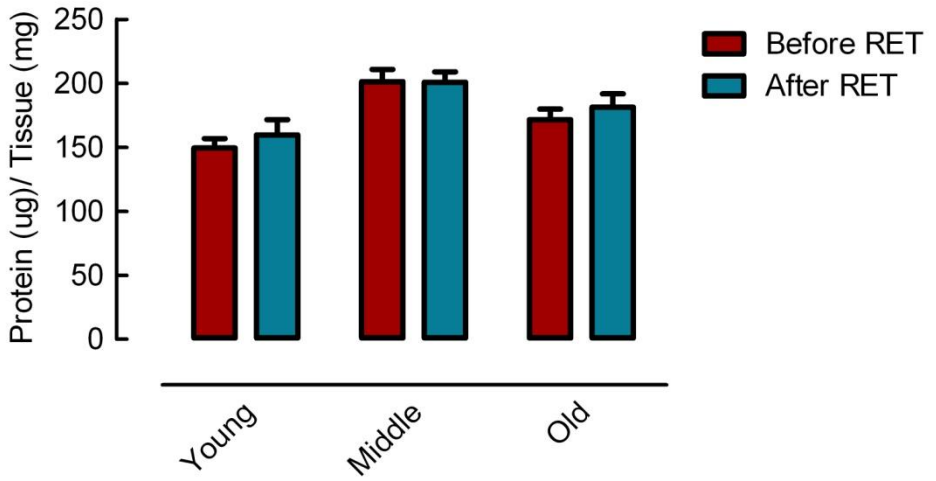


Figure 2.39 Protein (µg)/ Tissue (mg) in young, middle-aged and older subjects before and after RET. Values are means±SEM. Statistical analysis via ANOVA with Bonferroni post analysis and students t-test.

The RNA: tissue ratios were not different between the age-groups either before or after RET. RET had no effect on the RNA: tissue ratio in any of the age groups (before: Y, 0.66±0.05; M, 0.73±0.04; O, 0.76±0.06, after: Y, 0.73±0.05; M, 0.74±0.06; O, 0.79±0.04) (Figure 2.40).

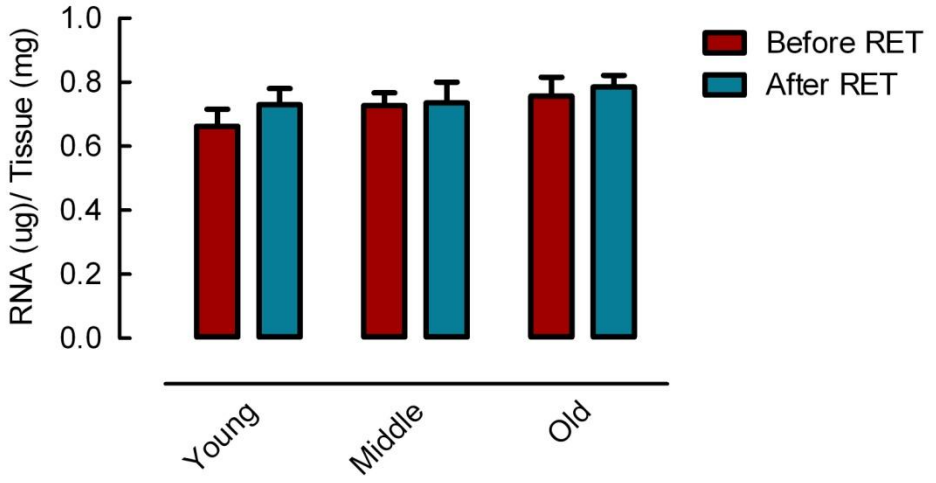


Figure 2.40 RNA (µg)/Tissue (mg) in young, middle-aged and older subjects before and after RET. Values are means±SEM. Statistical analysis via ANOVA with Bonferroni post analysis and students t-test.

Before RET the DNA: tissue ratios were not different between the age-groups (Y, 0.80±0.05; M, 0.77±0.03; O, 0.91±0.04). The young increased their DNA: tissue ratio after RET (0.80±0.05 vs. 0.98±0.06, $P<0.05$) resulting in a ratio that was significantly higher than the middle-aged group after RET (0.98±0.06 vs. 0.79±0.03, $P<0.05$). The middle-aged and old groups DNA: tissue ratios were not significantly different after RET (M, 0.79±0.03; O, 0.87±0.05) (Figure 2.41).

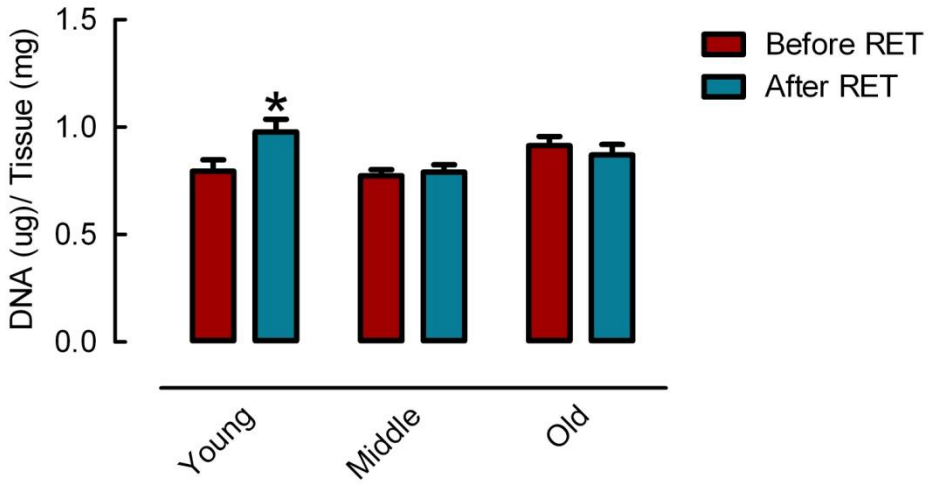


Figure 2.41 DNA (µg)/Tissue (mg) in young, middle-aged and older subjects before and after RET. Values are means±SEM. Statistical analysis via ANOVA with Bonferroni post analysis and students t-test. *=$P<0.05$ vs. value before training in the same age-group.

Before RET the protein: DNA ratio of the middle-aged group was significantly higher than that of both the young and older groups (262.92±12.50 vs. 191.39±8.33 and 189.00±7.58, respectively, $P<0.001$). After RET the young had a significantly lower protein: DNA ratio (191.39±8.33 vs. 164.49±10.88, $P<.05$) and the old had a significantly higher protein: DNA ratio (189.00±7.58 vs. 211.89±12.20, $P<0.05$). After RET the middle-aged still had a higher protein: DNA ratio than both the young (259.28±10.82 vs. 164.49±10.88, $P<0.001$) and old groups (259.28±10.82 vs. 211.89±12.20, $P<0.05$) (Figure 2.42).

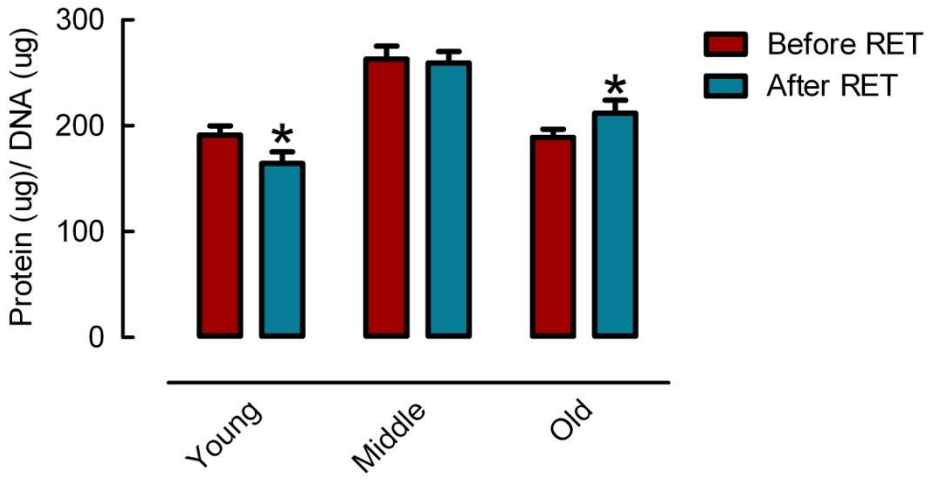


Figure 2.42 Protein (µg)/DNA (µg) in young, middle-aged and older subjects before and after RET. Values are means±SEM. Statistical analysis via ANOVA with Bonferroni post analysis and students t-test. *=$P<0.05$ vs. value before training in the same age-group.

The RNA: protein ratios were not different between the age-groups either before or after RET. RET had no effect on the RNA: protein ratio in any of the age groups (before: Y, 4.41±0.20; M, 3.73±0.23; O, 4.43±0.35, after: Y, 4.70±0.33; M, 3.67±0.26; O, 4.66±0.40) (Figure 2.43).

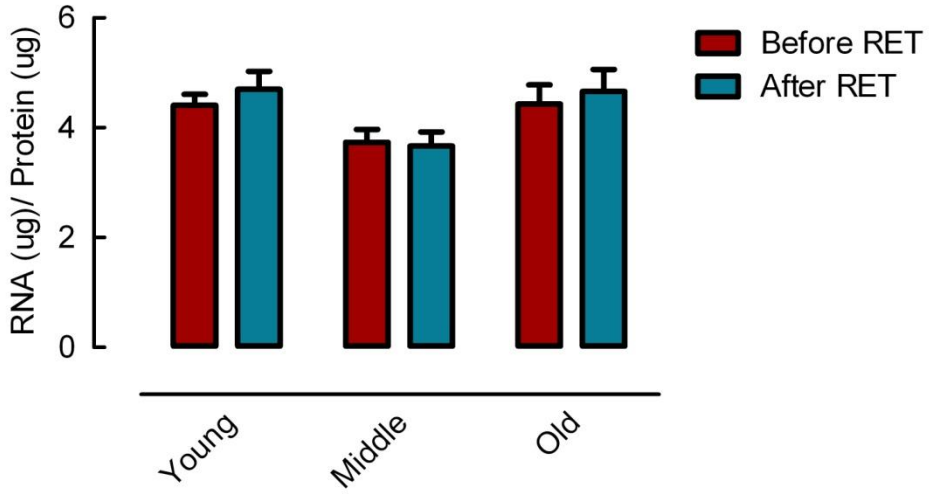


Figure 2.43 RNA (µg)/Protein (µg) in young, middle-aged and older subjects before and after RET. Values are means±SEM. Statistical analysis via ANOVA with Bonferroni post analysis and students t-test.

The RNA: DNA ratios were not different between the age-groups either before or after RET. RET had no effect on the RNA: DNA ratio in any of the age groups (before: Y, 0.84±0.05; M, 0.95±0.04; O, 0.87±0.07, after: Y, 0.76±0.05; M, 0.94±0.07; O, 0.91±0.05) (Figure 2.44).

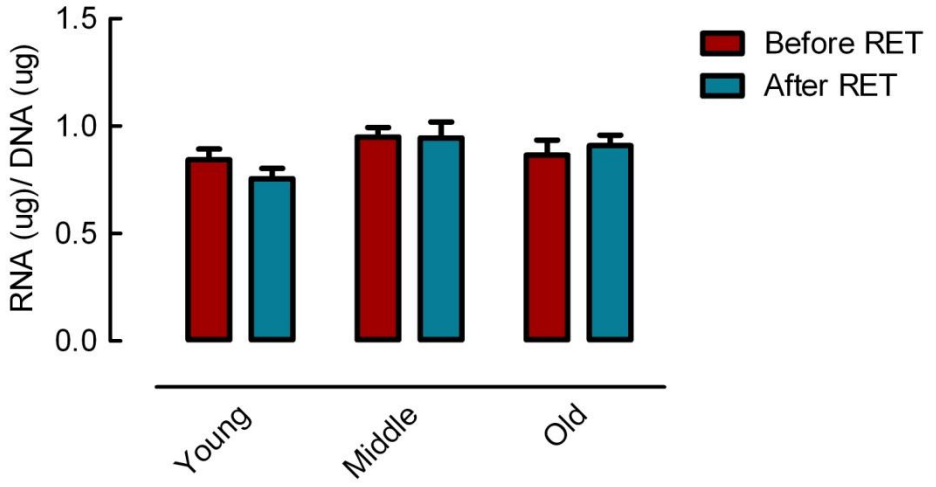


Figure 2.44 RNA (µg)/DNA (µg) in young, middle-aged and older subjects before and after RET. Values are means±SEM. Statistical analysis via ANOVA with Bonferroni post analysis and students t-test.

2.4 Discussion

We hypothesized that RET would rejuvenate age-related declines in muscle protein synthesis in the basal condition and in response to feeding and/ or exercise-plus-feeding. However, all groups demonstrated an increase in MPS in response to feeding and exercise-plus-feeding both before and after RET with no difference in MPS between the age-groups at any time, thus not supporting the hypothesis-based assumption of age-related declines in MPS responses to anabolic stimuli. This data conflicts with work showing anabolic blunting with age across a range of EAA feeds (Cuthbertson *et al.*, 2005; Volpi *et al.*, 2000b). One possible explanation for the lack of differences between the age-groups is that our study design may have missed the optimal time-point for anabolic responses to be measured due to the "muscle full" phenomena recently proposed by Atherton (Atherton *et al.*, 2010). This work demonstrated that

there is approximately a 90 min window in which anabolic responses (increases in MPS and many signalling proteins) are heightened in response to AA feeding and that even with continued AA provision (extracellular and intracellular availability) the MPS and signalling (mostly) responses will return to baseline. Although the feeding strategy for this study did involve repeated feeds with the last feed only ~30 min before the final muscle biopsy, it may be that the final biopsies being 120 min after the initial feeding bolus is not truly representative of maximal anabolic responses and that studies using a single AA dose or protein meal, which do demonstrate anabolic blunting, are more likely to represent practicable feeding strategies employed by most individuals in everyday life.

Although there were no significant differences between the age-groups there was a trend for the fed MPS values before and after training to be lower in both of the older groups compared to the young, illustrating subtle anabolic blunting with age, although this does not appear to extend to the combined stimuli of exercise-plus-feeding.

The issue of timing may also be what prevents us seeing age-related differences or RET effects on FSR in response to exercise-plus-feeding. Adding exercise to feeding has been shown to not only heighten but also prolong the anabolic response (Pennings *et al.*, 2011; Tipton *et al.*, 2001; Dreyer *et al.*, 2008; Witard *et al.*, 2009; Moore *et al.*, 2009a; Moore *et al.*, 2011b), so much so that when measuring FSR for 24 hours after protein ingestion and exercise, synthesis rates are heightened compared to basal even at the end of the study (Burd *et al.*, 2011). RET may therefore have significantly prolonged the duration of increased FSR in response to exercise-plus-feeding but with only subtle increases in the amplitude of response and this would not have been evidenced in our study design.

The increase in the young groups MPS in response to exercise-plus-feeding after RET does suggest positive effects of RET on MPS responses to anabolic stimuli. Also, it is interesting to note that this, the most significant

effect of RET, occurred only in the young group where the most significant increases in muscle hypertrophy occurred (see chapter 3) and where the only correlation between strength and lean mass increases was apparent, despite almost identical strength gains between the groups. This association lends itself to the suggestion that the ability of the young to heighten their MPS responses to exercise-plus-feeding after RET may partially explain their greater improvements in muscle mass. This would be in keeping with the work of some groups who have shown blunted hypertrophic responses to RET with age (Greig *et al.*, 2011; Welle *et al.*, 1996; Dionne *et al.*, 2004; Lemmer *et al.*, 2001), albeit contradicting the work of others (Ivey *et al.*, 2000; Hakkinen *et al.*, 2001) who suggest that older individuals have the same propensity to gain muscle mass through RET as younger subjects.

Another suggestion of the positive benefits of RET comes from the additive effect of the dual stimuli compared to feeding alone in the older group after RET. It may be that older individuals require the dual stimuli of exercise-plus-feeding, which has been shown to be an optimal combination (Moore *et al.*, 2009a; Staples *et al.*, 2011; Tang *et al.*, 2009) to elicit maximal anabolic responses.

Another suggestion for the subtlety of MPS improvements in our older group is that our RET was not truly 'optimal' for this age-group. The optimal patterns of RET to maximize anabolic responses, especially in the elderly still need determining and this represents a major area of study. Work from Kumar and colleagues, has cast new light on the role of exercise intensity in determining MPS responses to exercise. Kumar has shown a sigmoid dose-response to resistance exercise such that, MPS after exercise is greatest at intensities above 60% 1-RM; even when repetition number was increased at lower intensities to match workload, suggesting an anabolic 'ceiling' above 60% 1-RM (Kumar *et al.*, 2009).

Interestingly the intensity of exercise to elicit a robust MPS response can be drastically reduced (20% 1-RM) when combined with vascular

occlusion (Fry *et al.*, 2010), suggesting that high intensity RET is not necessarily a pre-requisite for exercise-induced increases in MPS. In fact, increasing the volume of work at a low intensity (30% 1-RM to failure) was shown to be more effective than high-intensity, low-volume (90% 1-RM to failure) resistance exercise in terms of amplitude and duration of MPS responses after acute exercise (Burd *et al.*, 2010), although more work is required to establish if this would translate into similar training adaptations.

The associated anabolic signalling, as with the FSR does not tell a conclusive story with regard to the effects of either age or RET but does lend itself to some suggestions of improvements with RET. Acute changes in MPS are primarily regulated at the mRNA level via increasing translational efficiency (Kimball *et al.*, 2002). For example, increasing dietary AA alters the phosphorylation state of proteins within the mTOR signalling cascade (Moore *et al.*, 2011a) resulting in increases in the activity of these proteins, including of P70, 4EBP1 and EEF2 (Cuthbertson *et al.*, 2005; Fujita *et al.*, 2007; Glover *et al.*, 2008). It is not only AA feeding, but also acute resistance exercise that is known to promote these activity increases, with the combination of the two stimuli appearing optimal (Karlsson *et al.*, 2004; Witard *et al.*, 2009; Apro & Blomstrand, 2010). RET also appears to act upon a different pathway, independent of mTOR; the MAPK pathway (Williamson *et al.*, 2003). Activation of this pathway seems to affect ERK1/2 which can regulate proteins involved in initiation and elongation of mRNA translation (Roux *et al.*, 2007) and could therefore be involved in post-exercise increases in MPS.

P70 is known for its role in modulating cell-cycle progression, cell size and cell-survival (An *et al.*, 2003) and its activation is required for G1 cell cycle progression (Lane *et al.*, 1993) (see Figure 2.2). P70 phosphorylation only increased in the young in response to exercise-plus-feeding after RET, while in the middle-aged group this increase was apparent both before and after RET. The old group displayed increased phosphorylation in response

to exercise-plus-feeding both before and after RET but increased phosphorylation in response to feeding alone only after RET.

4EBP1 mediates the regulation of protein translation by hormones, growth factors and other stimuli such as contraction and feeding which signal through the P13 kinase pathway (Gingras *et al.*, 1999). 4EBP1 was depressed immediately post-exercise in both the young and the older groups before and after RET and in the middle-aged group before RET. Only in the old group did 4EBP1 phosphorylation increase in response to the anabolic stimuli of feeding and exercise-plus-feeding and this was true both before and after RET.

mTOR is a serine/threonine protein kinase that regulates cell growth, proliferation, motility, survival, protein synthesis and transcription, targeting P70, EEF2 and 4EBP1. mTOR phosphorylation was the signalling target seemingly most affected by RET. At no time-point were there any significant differences between the age-groups before or after RET, however mTOR phosphorylation was increased from basal after RET in all three age-groups in response to exercise-plus-feeding, a response that was not apparent in any of the groups before RET. After RET the middle-aged and old groups also responded to feeding alone with increased mTOR phosphorylation.

EEF2 is essential for protein synthesis by promoting translocation and is activated by inhibition of EEF2K by mTOR and p70S6K. There were no significant changes in EEF2 phosphorylation with any of the anabolic stimuli in any of the age-groups either before or after RET. The surprising depression in EEF2 phosphorylation in response to exercise-plus-feeding in the young before RET is likely an artifact of outliners within the data (although all data is LOG transformed) that may have been caused by possible technique limitations such as mixed tissue (i.e. connective tissue infiltration) biopsies and subsequent non-uniform protein loads.

Although the lack of demonstratable changes in FSR with RET may seem surprising even the most optimal of feeding and/or exercise strategies may not elicit substantial effects in all individuals. In a recent study by Davidsen et al. (Davidsen *et al.*, 2011), a fully supervised RET program in younger individuals elicited strikingly heterogeneous mass and strength gains. Based on a continuum, in which the authors categorized the top 20% 'high' responders and the bottom 20% 'low' responders, four miRNA species were associated with training responsiveness; suggesting that these miRNA's may have a role to play in determining adaptive heterogeneity.
On a similar theme, a study by Mayhew et al. (Mayhew *et al.*, 2011), determined that increased concentrations of eukaryotic initiation factor 2B epsilon (eIF2Bε) protein expression after a single exercise bout was directly associated with the degree of hypertrophy after RET and that in-vitro over expression of eIF2Bε lead to muscle hypertrophy; suggesting that up regulation of eIF2Bε may partly underlie adaptive capacity. Taken together these 2 studies suggest that using biological variability represents a powerful approach in terms of both bio-prediction and gaining mechanistic insight into human studies and although much more work is needed to link measures such as non-coding RNA, mRNA, intracellular proteins and MPS in humans, it could be speculated that heterogeneity in the 'muscle-full' set-point may underlie adaptive capacity.

The remaining hypotheses for this chapter all center around strength losses with ageing and the effects that nutritional intake and RET may have upon these. We hypothesized that RET would improve muscle strength but the gains would be less in older individuals. This hypothesis proved incorrect. RET did result in significant increases in strength, but these improvements were almost identical between the age-groups. The strength improvements resulted in the strength of the old group after RET being not different to that of the young before; effectively the strength of the old group was restored.

There is little argument that older people are able to gain strength with RET (Lambert & Evans, 2005) but agreement on whether older people

have the ability to achieve the same strength increases as younger people has not yet been reached. Some of the literature suggests blunted strength gains with ageing (Lemmer *et al.*, 2001; Greig *et al.*, 2011) while other groups complement our data showing that older individuals retain their ability to gain strength in response to a RET program (Moritani & deVries, 1980; Hakkinen *et al.*, 2001) and in what seems like a possible explanation for the discrepancy in these results, some groups have shown differences in response to certain exercises between young and older individuals (Jozsi *et al.*, 1999; Welle *et al.*, 1995).

We also hypothesised that there would be age-related declines in muscle strength associated with 1. lower dietary protein intake in older individuals or 2. a difference in muscle fibre composition. Age-related declines in strength are consistently shown within the literature and have also been observed in this study. However, these declines in strength are often coupled to declines in lean mass, something which we do not see in this study and will be discussed in more detail in the next chapter.

Muscle wasting alone cannot therefore account for all decreases in muscle strength. This observation means that there must be other factors at play leading to the loss of force per unit area seen in ageing individuals. The reduction in strength with age, when no apparent reduction in lean mass is observed, is commonly explained by differences in tendon properties and a decreased efficiency of excitation-contraction coupling with age (Narici & Maganaris, 2006). Narici and colleagues suggested that 4 factors, other than sarcopenia may contribute to the decreases in strength observed with ageing: 1. Muscle architecture, 2. Tendon mechanical properties, 3. Reduced agonist and increased antagonist muscle activity and 4. Decreased single fibre specific tension.

Numerous groups have demonstrated evidence to support the theories of reduced agonist and increased antagonist muscle activity (Harridge *et al.*, 1999; Scaglioni *et al.*, 2002) and decreased single fibre specific tension (D'Antona *et al.*, 2003). Support for changes in muscle architecture and

tendon mechanical properties has also been shown with a 25% reduction in muscle volume of the plantar flexors associated with a 10% reduction in fibre fascicle length, a 13% reduction in pennation angle, and a 10% reduction in tendon stiffness (Morse *et al.*, 2004).

In relation the strength changes that we have observed following our RET it is interesting that most of the observed changes in muscle architecture and tendon mechanical properties are reversible by 14 weeks of RET; fibre fascicle length was shown to be increased by 10% and tendon stiffness by 64% (Reeves *et al.*, 2004). It may therefore be that our RET program improved muscle architecture and tendon mechanical properties and these contributed to improved strength, especially in the old.

The increases in strength that we observed following our RET may be partially due to changes in muscle architecture and tendon mechanical properties as discussed above, however as no measures of fascicle length, pennation angle or tendon stiffness were made during this study we are unable to comment on if these changes occurred. These changes are however highly likely given that they were observed following a 14 week RET, also 3 times per week and at a similar intensity (80% 5-RM). Changes in these properties may offer great functional benefits to an ageing population as a RET induced increase in tendon stiffness has been associated with a 25% faster development of joint torque, which may be critical in recovering body position from a potential fall, with the risks and outcomes of falls in the elderly discussed earlier in this thesis (see chapter 1).

Another possible explanation for the disconnection between muscle size and strength that is seen with ageing is that of reduced neural efficiency. The ability of muscles to produce force begins with initiation in the nervous system with force production governed by 1. motor unit recruitment and 2. the rate of motor unit firing/ central activation (Lambert & Evans, 2005). Although there appears to be impairment of muscle activation with age for some muscle groups (Jakobi & Rice, 2002; Yue *et*

al., 1999) but not others (Connelly *et al.*, 1999; Kent-Braun & Ng, 1999; Roos *et al.*, 1999; Phillips *et al.*, 1992) there is other evidence of impaired nervous system muscle activation with ageing, such as increased co-activation of antagonist muscles (Hakkinen *et al.*, 2001; Izquierdo *et al.*, 1999; Klein *et al.*, 2001) and reduced foot tapping speed (Kent-Braun & Ng, 1999). Using a curvilinear equation Stackhouse and colleagues calculated a central activation deficit of 11% in old subjects compared to young controls and suggested that this may explain the strength-mass loss discrepancy (Stackhouse *et al.*, 2003); with strength declines of 2.0-2.5% per year after the age of 65 (Frontera *et al.*, 2000) but mass losses of 0.5-1.0% per year after the age of 40 (Janssen *et al.*, 2000).

With regard to our hypothesis of reductions in strength with ageing being related to lower dietary protein intake in our older group there were very few differences in dietary composition between the age groups or with RET. The percentage of dietary intake composed of protein was not different between the age groups either before or after RET and did not change significantly with RET. As hypothesized absolute protein intake was significantly higher in the young than in the middle-aged group before RET with a trend for it to also be higher than in the old group. However, during RET there was a trend for the absolute protein intake of the young to be reduced; (corresponding with a reduction in their energy intake) which resulted in no significant differences in the absolute protein intakes between the age groups during RET. Leucine has been shown to be the primary EAA required for increases in MPS (Breen & Phillips, 2011) and following the pattern of absolute protein, leucine intake was significantly higher in the young than in the middle-aged and old before RET but was no different during owing to a small (but not significant) reduction in leucine intake in the young and increases of a similar magnitude in the middle-aged and old groups. In addition to protein-based intake the young group reduced their total kcal intake during RET and this corresponded with decreased saturated fat intake, monounsaturated fat intake and sodium intake as well as a trend for reduced cholesterol intake, something also observed by Bales and colleagues who found that after 8 months RET

reduced reported energy and fat intake was observed (Bales *et al.*, 2012). These changes resulted in no differences between the age-groups during RET as the kcal, saturated fat and monounsaturated fat intakes had all been significantly higher in the young group than the middle-aged and old groups before RET. The only other significant dietary change was an increased sodium intake in the old during RET.

Diet-diary results must however be interpreted with caution as under-reporting of energy intake (EI) when using diet diaries is common (Biltoft-Jensen *et al.*, 2009). This is likely to be the case in our results as the maximum reported EI is only ~2130 kcal and this was for our young group only, with the middle-aged and older groups significantly lower (before RET values), averaging an EI of just 1790 kcal if all age-groups were taken together. When EI reported by diet diaries were quantified against a validated position and motion instrument, reported EI was underestimated by 12% on average (Biltoft-Jensen *et al.*, 2009). It is not only EI that is misreported in these diet diaries, the quality of the diet is often under-reported by virtue of underreporting EI and this is seemingly true for all 3 common methods of dietary recording; diaries, recall and history (Livingstone & Black, 2003).

Diet manipulation for the duration of the recording is another problem associated with diet diaries. Rebro and colleagues found that people tended to reduced their snack intake, number of foods consumed and the complexity of their diet during times of monitoring, even if the reporting was over non-consecutive days and in subjects deemed as highly motivated (Rebro *et al.*, 1998).

The duration and chosen days for the diet diary also seems to affect the extent of underreporting, with the highest degrees of underreporting on the 'weekend' days of Friday and Saturday (Whybrow *et al.*, 2008), not Saturday and Sunday which are traditionally classified as 'the weekend' when people are requested to include one weekend day in a 3 day diet diary. These daily discrepancies support the use of 7-day diaries as

superior to 3-day, in that they will catch a complete weekly cycle of human feeding behavior and is also allow more days of accurate captures as the lowest reported intake occurs on day-1 of a diet diary (Whybrow *et al.*, 2008). The observation of a trend for reduced reported EI between days 2-7 of a diet diary may lead some to speculate that a shorter diary would be favorable, but this trend was only for a reduction of 49 kJ per day (Whybrow *et al.*, 2008) while shorter diaries induce greater between-subject variability as they may choose different days of the week. Overall, the correlation between a 3-day and 7-day diet diary was found to be 0.860, $P<0.001$ with the 3-day less effortful for both investigators and subjects.

Of course not all people will under-record to the same extent, it has been shown that 1. there is a correlation between BMI and degree of underreporting, 2. Under reporting is more common in females and 3. sugars and fats are the most under-reported (Rasmussen *et al.*, 2007). Based on these observations our results should still allow us to assess with confidence differences between the age-groups and with RET as there were no significant differences between the BMI of our groups and the female: male ratio was almost equal in all the groups. Smoking status and educational level appear to have little effect on the degree of underreporting.

In general it is agreed that dietary intake cannot be estimated without some degree of problem (Beaton, 1994). However, stringent guidelines on how and when to record may reduce some elements of inaccuracies which is key if dietary intake represents a primary endpoint, shorter diaries do however seem suitable for recording group trends and changes with an intervention.

In relation to our final hypothesis for this chapter we did observe differences in myosin heavy chain isoform distribution (representative of muscle fibre-type composition) between the age-groups, these do not however lend themselves to explaining the loss of strength observed with

age. Before RET the old displayed a unique fibre type distribution compared to the other age groups, with a higher percentage of Type IIx fibres compared to Type I while both the young and middle-aged groups showed an even distribution of the three fibre types. This is at odds with the work of Lexell and colleagues who reported a greater loss in the number and size of Type II fibres with age when compared to Type I fibres (Lexell, 1995). This is also at odds with knowledge that the number and size of Type II muscle fibres are a primary determinant of muscle force production as they produce more force than Type I fibres (Bottinelli *et al.*, 1996), with a positive relationship between muscle maximal torque production and the percentage of Type II fibres in that muscle (Thorstensson *et al.*, 1976).

Despite all subjects undertaking the same exercise regime there were differences in the fibre type shifts seen with RET. The young group had a reduced percentage of Type IIa fibres after RET with increased Type IIx, resulting in more Type IIx than Type IIa fibres after RET. The old group also showed this profile after RET but in this group was due to a reduction in Type IIa fibres after RET without an increase in Type IIx. These shifts, in both the young and old group are contrary to the literature where across all ages RET has been shown to decrease the percentage of Type IIx fibres and increase the percentage of Type IIa (Hikida *et al.*, 2000; Staron & Johnson, 1993). The old group did however increase their Type I fibres after RET, something which has been shown as the results of RET in numerous studies (Hakkinen *et al.*, 2001; Frontera *et al.*, 1988; Trappe *et al.*, 2000; Hikida *et al.*, 2000). The middle-aged group also increased their proportion of Type I fibres, although the distribution of the three fibre types remained even in the middle-aged group after RET, rendering their fibre type profile unchanged by the RET.

Overall it seems unlikely that our hypothesis of age-related declines in muscle strength would be associated with a difference in muscle fibre composition is correct. Both the difference between the groups and the changes with RET are very small (yet significant) and it may be that the

use of ratios has given greater power to the statistical analysis of this data set. If a conclusion was to be drawn about the age-related differences and RET-induced changes observed it would be that the young appear to get 'faster' while the old appear to get 'slower'. Type II fibres have been shown to hypertrophy more due to greater recruitment and this may be another partial explanation to the greater gains in lean mass shown by our young group with RET.

We also measured RNA, DNA and protein content in postabsorptive, pre and post RET muscle samples from all of our subjects. Protein: DNA ratio gives a sensitive index of muscle protein mass (Crossland *et al.*, 2010) and is an indication of the muscle DNA unit size (Cuthbertson *et al.*, 2005); a measure of the amount of cytoplasm managed by each nucleus in the muscle. Of all the calculated ratios this was most effected by RET with the young group showing a decrease in their Protein: DNA ratio after RET while the old group had an increased ratio.

Changes in Protein: DNA may be explained by alterations in the DNA: Tissue ratio, where the young showed a significant increase after RET and the old showed a small (non-significant) decrease. The DNA: Tissue ratio is representative of nucleus to fibre ratio and increases may represent increased satellite cell proliferation and migration into the cell which in turn would support muscle growth and result in a reduced Protein: DNA ratio as observed in our young group. Conversely the old groups Protein: DNA ratio would be increased as they may not have the ability to increase their satellite cell pool within the muscle.

The middle-aged group did not change either Protein: DNA or DNA: Tissue with RET but Protein: DNA ratio was higher than both of the other groups before and after RET. The spurious results from the middle-age group for this data set and others in this work may be due to the large "ageing" variation in our middle aged group; i.e. we have biologically 'young' and 'old' middle-aged subjects forming this group; something

which could be evidences by using a combination of RNA profiling with single-gene DNA marker association (Timmons *et al.*, 2010).

The RNA: Protein and RNA: DNA ratios represent the total capacity of the muscle for protein synthesis and in work by Cuthbertson et al were found to be lower in elderly compared to young subjects (Cuthbertson *et al.*, 2005). However, we found no differences in either of these ratios either between our age groups or with RET. This discrepancy between our work and the work of Cuthbertson is similar to the one observed for the FSR data and it may be that subject characteristics explain some of the variation observed. This study was promoted as "Nottingham and Derby Active Ageing" and this in combination with the enticement of 20 weeks free personal trainer sessions may have led to a degree of self-selecting bias in those who participated in this study. Although our exclusion criteria restricted this study to healthy adults, our older age group had a lean muscle mass that was not different to that of our younger groups and therefore was not representative of the age-related declines in muscle mass that can be seen in larger population cohorts. This discrepancy may offer another explanation (alongside the concept of an anabolic window and the feeding strategy) as to why we are unable to observe (with statistical power) the commonly reported concept of anabolic blunting with age both with regard to FSR and the associated anabolic signalling.

CHAPTER 3- BODY COMPOSITION

CHAPTER HYPOTHESES:

i. There are age-related decreases in total body and lean leg mass and increases in fat mass.

ii. Age-related changes in body composition are associated with fat distribution linked to increased risk of cardiovascular health risks.

iii. Resistance-exercise training increases lean mass and improved measures of bone quality.

3.1 Introduction to body composition

Maintaining a healthy body weight and level of body fatness is fundamental to a healthier and longer life. Overweight or underweight individuals with levels of body fat at, or near to the extremes of the body fat continuum are likely to have serious health problems that reduce life expectancy and threaten quality of life.

Individuals who are overweight or obese have a higher risk of developing various cardiovascular, pulmonary and metabolic diseases, as well as osteoarthritis and certain types of cancer (US Department of Health and Human Services, 2000).

Underweight individuals with extremely low levels of body fat are often malnourished and have a higher risk of fluid-electrolyte imbalances, renal and reproductive disorders, osteoporosis, osteopenia and muscle wasting (Fohlin, 1977; Mazess *et al.*, 1990).

At present a common tool to assess someone's weight is the body mass index (BMI), which is calculated using the following formula:

$$\frac{\text{Weight (kg)}}{\text{Height (m)}^2}$$

With current guidelines classifying:

- < 18.5 as underweight
- 18.5 – 24.9 as healthy
- 25- 29.9 as overweight
- >30 as obese, further categories then split those with a BMI over 30 into levels of obesity (http://www.euro.who.int/nutiriton).

The BMI method does however have limitations. With no accounting for the ratio of fat mass (FM): FFM, the BMI can misclassify people with extreme musculature into an overweight or obese category based on their weight: height ratio when they are in fact carrying very little body fat.

For any given BMI there is considerable variation in body composition, often related to age and/ or gender as discussed later in this chapter. Some individuals with low BMI's may have as much relative body fat as those with high BMI's because composition of an individual's body weight cannot be assessed using BMI.

The best model for the assessment of body composition, including measurement of muscle mass is magnetic-resonance imaging (MRI). MRI is validated against anatomical analysis of cadavers and has been reported to display errors of as little as 2% (Engstrom *et al.*, 1991; Mitsiopoulos *et al.*, 1998), application of this method is however limited by expense and availability.

A slightly less expensive option is computerized tomography (CT), with reported errors of ~6% (Janssen & Ross, 2005), this method is limited by radiation exposure and also by the lack of readily available composition quantification software, meaning that data manipulation using this method is often subject to operator variability.

One commonly used method, more accurate than BMI but less so than the gold-standard of MRI, to estimate an individual's body composition is

DEXA. DEXA is a body composition measurement method used to measure total body bone mineral density, bone mineral content, fat and lean soft tissue mass. Based on the principle of using the attenuation of X-rays to identify body tissues, DEXA provides an accurate measure of total body bone mineral (TBBM) and BMD, and can provide *estimates* of bone free lean tissue mass (LTM), fat mass (FM), soft-tissue mass (STM) (STM = LTM + FM), FFM and % body fat (BF).

The principle underlying DEXA is that the attenuation of X-rays with high and low photon energies is measurable and dependent upon the thickness, density and chemical composition of the underlying tissue. The attenuation of X-ray energies through fat, lean tissue and bone varies due to the differences in densities and chemical composition of these tissues. These attenuation ratios at two different X-ray energies are thought to be constant for all individuals (Pietrobelli *et al.*, 1996).

As with all body composition measurement methods, DEXA is based on assumptions, with the main three assumptions for the DEXA method being:

1. The amount of fat over bone is the same as the amount of fat over bone-free tissue. With DEXA the composition of STM is calculated only from pixels that do not contain bone which is approximately 60-65% of pixels in a whole body scan. This means that STM estimates in bony regions may not be as accurate as they are in bone-free regions.

2. Measurements are not affected by the anterior-posterior thickness of the body. In theory the attenuation of any given substance is constant, but these values may change with differences in thickness. Calibration of the DEXA machine with a phantom attempts to correct this limitation as the quantity and density of the phantom is known and can be used to check the accuracy of a DEXA scan.

3. The hydration and electrolyte content of the LTM is constant. Although not exact, research has reported that changes in hydration of up to 1kg are not likely to greatly affect the accuracy of DEXA measurements (Going *et al.*, 1993; Pietrobelli *et al.*, 1998; Pietrobelli *et al.*, 1996) and that a 5% change in the water content of FFM will only affect DEXA body fat estimates by 1-2.5% BF (Lohman *et al.*, 2000).

All DEXA models and makes are based on the same principles and assumptions outlined above. Although often referred to as a three compartment (3-C) model of body composition assessment as it provides estimates of TBBM, LTM and FM, it is in reality providing two separate sets of two compartment assessments (Ellis, 2000), where the first distinguishes bone from STM and the second distinguishes lean tissue from fat mass. It is the accuracy of the second distinction that has been questioned in the past (Milliken *et al.*, 1996; Snead *et al.*, 1993), although it has been reported that improvements in software have corrected the inaccuracy of this distinction enabling DEXA to accurately measure exogenous fat regardless of whether it was centrally or peripherally distributed (Kohrt, 1998). One problem that has not yet been overcome is that fluid retention increases DEXA reporting of LBM but not FM, with an additional limitation of DEXA being that no cross-sectional images can be produced.

As well as the limitations and assumptions already discussed a further difficulty of the measurement of muscle mass is that determination of muscle mass by whichever method does not predict functional muscle mass. Alterations or differences in muscle fibre components (i.e. MHC isoforms), myofibre function or metabolic characteristics (e.g. creatine phosphate content), are not given by any of the body composition measurement methods mentioned.

3.1.1 Body composition and energy balance

With levels of obesity and overweight in many westernized countries reaching epidemic proportions it has become an issue which all of society needs to be aware of as it has high reaching numbers of associated health problems. Obesity is clinically characterized by a disproportionately high fat mass (Wolfe, 2006) and excessive body fat has the ability to cause numerous and serious health problems. Excess body fat not only leads to changes in fat tissue development and growth, it also leads to insulin resistance and endothelial dysfunction through the pro-inflammatory and pro-thrombotic effects of adipokines (McCroskery *et al.*, 2003). Adipose tissue is an active endocrine and paracrine organ that releases a large number of cytokines and bioactive mediators that influence not only body weight homeostasis but also inflammation, coagulation, fibrinolysis, insulin resistance, diabetes, atherosclerosis, risk of coronary heart disease (CHD) and some forms of cancer (Kopelman, 2000; Lau *et al.*, 1996; Van Gaal *et al.*, 2006; Mohamed-Ali *et al.*, 1998). In general, for each unit of BMI increment (above 24.9) the risk of CHD increases by 8% (Li *et al.*, 2006).

The only way in which the homeostasis of body weight can be altered is through an imbalance of net energy. Weight gain is inevitable if the energy taken up by the body exceeds that expended. Obesity classifications are often based on weight: height ratios and although generally thought of and defined as excessive accumulation of fat mass, the increased muscle mass in obesity is often less appreciated (Hibbert *et al.*, 1994). In obese individuals the increased energy expenditure associated with larger muscle mass is insufficient to offset the positive net energy balance but may still be capitalized upon to facilitate weight loss, especially when combined with adequate nutrition. The energy to provide adenosine tri-phosphate (ATP) for muscle protein turnover is largely derived from the oxidation of fat; the preferred energy substrate of resting muscle (Rasmussen & Wolfe, 1999.

Muscle protein turnover can occur at a greater rate if there is higher amino acid availability, possibly through an increase in protein intake (Paddon-Jones *et al.*, 2005). Studies using testosterone based injections to increase MPS have demonstrated that the increase in lean body mass that occurred was accompanied by a decrease in fat mass (Ferrando *et al.*, 2002). These data lead to the suggestion that a kilocalorie (kcal) restricted diet with a high percentage of protein would be expected to alter tissue composition favoring the loss of fat and the growth of muscle. This may go some way in aiding obese individual's loss of fat mass (Wolfe, 2006).

Although many different factors such as body composition, muscle mass, hormonal status, genetic variability and environmental factors will affect energy expenditure and therefore energy requirements of an individual, it is possible to predict the energy requirements of an individual needed to maintain body weight homeostasis based on established regression equations. Determining an individual's basal metabolic rate (BMR) is normally the first step in obtaining an estimate of energy requirements. BMR is the minimum rate of expenditure for a conscious person. BMR accounts for the energy required to maintain organ and systemic function. Level of activity will determine how much of a person's energy needs are accounted for by their BMR.

The Schofield equations (Schofield, 1985) are one common set of regression equations used to establish estimates of BMR values (Table 3.1).

Table 3.1 Schofield equations for calculating basal metabolic rate (BMR)

Schofield BMR Equations		
Age	**Male**	**Female**
10-17 years	17.7 x W + 657 SEE = 105	13.4 x W + 692 SEE = 112
18-29 years	15.1 x W + 692 SEE = 156	14.8 x W + 487 SEE = 120
30-59 years	11.5 x W + 873 SEE = 167	8.3 x W + 846 SEE = 112

W = Body weight in Kilograms; SEE = Standard error of estimation

Adapted from: (Schofield, 1985)

The number achieved from the equations should be altered within the standard error range shown. Subjects who are lean and/ or more muscular require more kcal than average. Obese subjects require less. Subjects at the young end of the age range for a given equation require more kcal. Subjects at the high end of the age range for a given equation require fewer kcal.

Once BMR has been calculated an individual's activity must also be accounted for to determine their energy demands, this is known as their physical activity level (PAL). Following a committee meeting in 1991 (COMA-Committee on Medical Aspects of Food Policy, 1991), the following table was produced accounting for leisure time and occupational activity on which to base PAL when calculating energy requirements (Table 3.2).

Table 3.2 Guide for the prediction of physical activity levels

Non-occupational activity	***Occupational activity***					
	Light		**Moderate**		**Moderate/ Heavy**	
	M	**F**	**M**	**F**	**M**	**F**
Non-active	1.4	1.4	1.6	1.5	1.7	1.5
Moderate	1.5	1.5	1.7	1.6	1.8	1.6
Very active	1.6	1.6	1.8	1.7	1.9	1.7

M= Male; F= Female

Based on: COMA committee meeting (COMA-Committee on Medical Aspects of Food Policy, 1991)

BMR is multiplied by PAL to give an estimate of total energy expenditure (TEE) and therefore the energy requirement to maintain body weight homeostasis.

For example:

- 25 year old female
- Body weight 60 kg
- Moderate occupational activity
- Very active leisure time

BMR = 14.8 x 60 + 487 (with no SEE correction for age or body composition)

BMR = 1375

PAL = 1.7

TEE = 1375 x 1.7

TEE = 2337 kcal/day

Often the roles of muscle in relation to energy balance and/ or the prevention of obesity are overlooked. TEE is the sum of resting energy expenditure (REE), the thermic effect of food and energy expenditure due to activity. Generally REE is the largest component of TEE and it is muscle metabolism variation that may alter this considerably as the metabolism of other tissues and organs are relatively constant. As large variations in muscle mass are possible, often achieved through training, the rate of MPS and MPB can vary as well and it is these variations that are principally responsible for the energy expenditure of resting muscle.

The average 24 hour FSR of muscle protein is ~0.075%/h^{-1}, including response to three mixed-meals (Tipton *et al.*, 1999). With the average muscle mass of young healthy males ranging from 35 to 50 kg and elderly women often having a muscle mass of <13 kg, calculated muscle protein synthesis can range from ~0.23-0.90 kg/d^{-1} (Tipton *et al.*, 1999). This large variation means that because 4 mol of ATP are utilized per mole of amino acids incorporated into protein and the hydrolysis of 1 mol ATP releases 20 kcal energy (Newsholme, 1978), the energy released per day as a result of muscle protein synthesis may range from 485 kcal in a well muscled young male to only 120 kcal in an active elderly female (Wolfe, 2006).

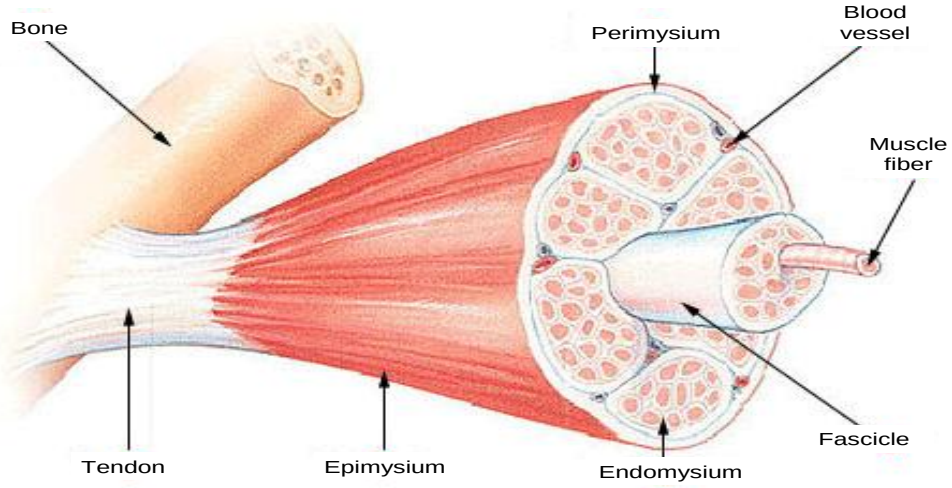
With regard to weight loss, if the above difference of 365 kcal/ day^{-1} was positive or negative energy balance this would lead to a loss or gain of 47g fat mass/ day^{-1} (as 1kg fat = 7700kcal), which over a sustained period would lead to a loss or gain of 1.4 kg fat mass per month. The difference in muscle mass need not be as large as shown in the example above to have a significant effect on energy balance. A relatively small difference of 5 kg

in muscle mass could have a significant effect. An extra 5 kg of muscle mass translates to an additional energy expenditure of ~50 kcal.day^{-1}, which in turn translates to a loss of 2.35 kg fat mass per year. With obesity developing over a time period that is often many years, such a seemingly small fat mass loss per year may contribute both to the prevention of obesity and aid in the treatment of it where necessary.

3.1.2 Muscle

For any action the body performs, whether it is movement, digestion or the heart beating, muscle is involved. There are three main classifications of muscle: cardiac, smooth and skeletal.

Skeletal muscle is the term for the muscles of the body required for every conscious coordinated movement of the body. There are more than 600 skeletal muscles in the body widely varied in size, shape and use. The structure of skeletal muscle is based around individual muscle fibers; the basic contractile units of skeletal muscles. They are individually surrounded by a connective tissue layer known as the endomysium and grouped into bundles known as fascicles which are surrounded by a layer known as the perimysium (Figure 3.1).

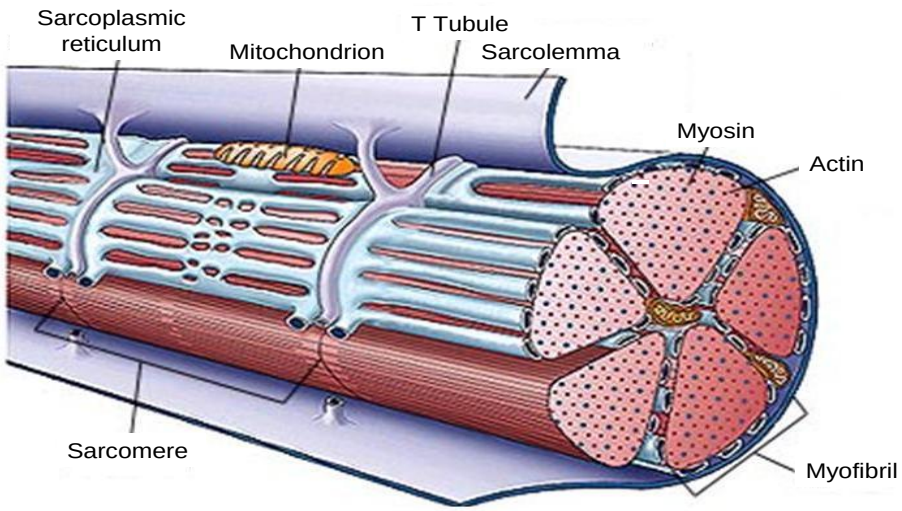


Adapted from:

http://training.seer.cancer.gov/module_anatomy/unit4_2_muscle_structure.html

Figure 3.1 Skeletal muscle structure

Individual muscle fibres themselves also house a complex structure (Figure 3.2). Each individual muscle fibre is surrounded by a plasma membrane called the sarcolemma which fuses with the tendon attaching that muscle to bone. Within the sarcolemma a muscle fibre is composed of successively smaller subunits. The largest of these units are myofibrils. Sarcoplasm surrounds the myofibrils and contains dissolved proteins, minerals, glycogen, fats and myoglobin. The sarcoplasm also contains a complex network of transverse tubules (T tubules); extensions of the sarcolemma that pass laterally through the muscle fibre. T tubules are interconnected and pass among the myofibrils allowing an action potential to be rapidly transmitted to the sarcoplasmic recticulum (SR). These tubules also allow substances to be transported into the inner parts of the muscle fiber. The SR is a network of tubules than run roughly longitudinally through the muscle fibre. These tubules loop around the myofibrils and serve as a storage site for calcium which is essential for muscle contraction.



Adapted from: www.mhhe.com/biosci.htm

Figure 3.2 Skeletal muscle fibre structure

Myofibrils are the contractile components of skeletal muscle composed of sarcomeres, the smallest functional units of a muscle. Within each myofibril numerous sarcomeres join end to end at the Z-discs. Within each sarcomere there is:

- An I band
- An A band
- An H zone (in the middle of the A band)

Surrounding the Z-disc towards the end of the sarcomere is the region of the isotropic-band (I-band). Following the I-band inwards is the region of the anisotropic-band (A-band) and within this band is the (H-zone) (Figure 3.3).

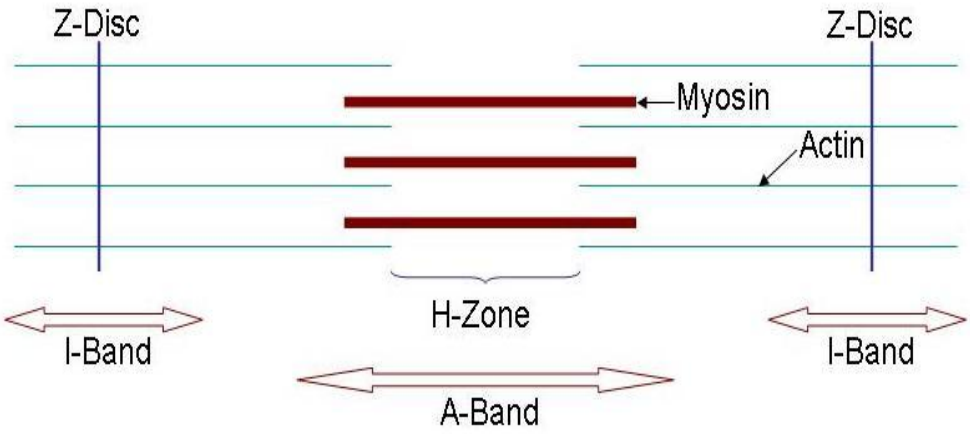


Figure 3.3 Outline of a sarcomere

Actin protein filaments are the major component of the I-band and extend into the A-band. Myosin protein filaments extend throughout the A-band and overlap into the H-zone. It is the interaction of actin and myosin filaments that cause muscle fibres to shorten resulting in muscular contraction and movement with myosin being the actual motor.

In addition to being rich in connective tissue, skeletal muscle is highly vascularised to provide essential nutrients for muscle function.

3.1.3 Fat

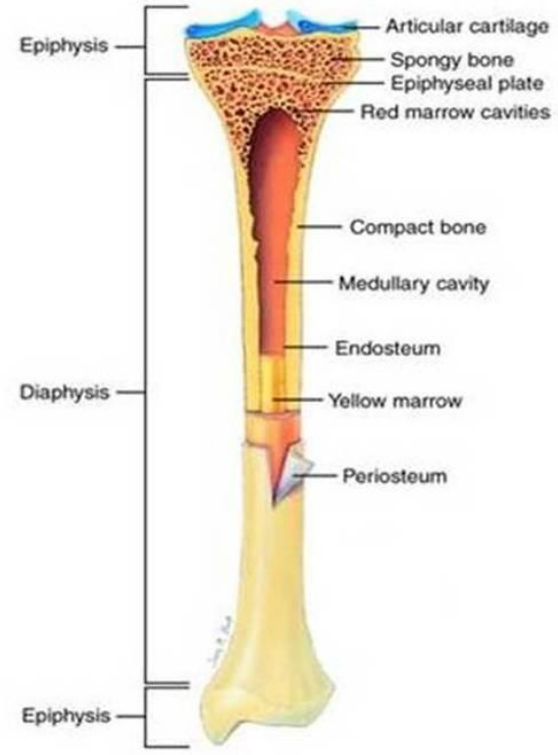
Adipose tissue is no longer viewed, as it had been previously, as a passive organ for triglyceride storage. As developing pre-adipocytes differentiate to become mature adipocytes they acquire the ability to secrete various proteins, many of which are released as cytokines or adipokines and are

discussed later in this chapter. The autocrine and paracrine effect of interleukin 6 (IL-6) and tumor necrosis factor-α (TNF-α) confirms adipose tissue to be a pro-inflammatory organ (Van Gaal *et al.*, 2006). Adipose tissue is not only associated with local, but also more generalized systemic inflammation, involving circulating pro-inflammatory proteins, although these are linked to not only adipocyte but also hepatic origin. These findings have further implicated adipose tissue in the progressive development of a number of diseases, further supporting the benefits of RET as a method by which body composition can be improved.

3.1.4 Bone

As is true for muscle, bone is a fundamental part of the human body with numerous functions to play. Two hundred and six bones make up the human skeletal system which provide support and protection for other systems of the body and provide attachments for muscles to allow movement to occur. Bone is also required to maintain posture and for mineral storage and hemopoiesis; blood cell formation in the red bone marrow.

Bones can be categorized into 5 main categories: Long bones, short bones, flat bones, irregular bones and sesamoid bones. Long bones possess, in general features that are typical of all bones (Figure 3.4).



Adapted from: www.baileybio.com

Figure 3.4 Long bone structure

The shaft or diaphysis is the long cylindrical portion of the bone. The diaphysis wall is made of hard, dense compact bone and this is known as the cortex. The outer surface of the diaphysis is covered by a dense, fibrous membrane known as the periosteum and a similar fibrous membrane known as the endosteum covers the inside of the cortex. Between the walls of the diaphysis lies the medullary cavity, which contains fatty marrow. At each end of a long bone is the epiphysis, which is usually specifically shaped to join with the epiphysis of an adjacent bone at a joint. The epiphysis is formed from trabecular bone.

Cortical (or compact) bone is harder than trabecular (cancellous) bone and more compact with only a small proportion of its volume being porous, non-mineralized tissue. Because of its make-up cortical bone can withstand greater stress but less strain than trabecular bone. In contrast, trabecular bone is spongy with up to 90% of its volume being porous meaning that is can withstand greater strain than cortical bone before fracturing.

Although bones only continue to grow longitudinally until the epiphyseal plates close, growth in diameter continues throughout life. Bone is continually remodeled to repair micro-damage that occurs as a result of activities of daily living. This remodeling occurs as new concentric layers are built on top of old layers with new bone being formed by specialist cells known as osteoblasts. As we age an imbalance of osteoblasts and osteoclasts result in age-related bone losses of ~0.5% per year (Brewer *et al.*, 2011).

Bone is made up of calcium carbonate, calcium phosphate, collagen and water. About 60-70% of bone weight is made up of calcium with water accounting for ~25-30%. The role of collagen in bone is to provide some flexibility and also strength in resisting tension. As we age progressive losses in collagen occur (Bailey *et al.*, 1999) and these result in an increased likelihood of fractures.

3.1.4.1 Osteoporosis

Osteoporosis is a metabolic bone disease that results in bone fragility and increases susceptibility to bone fractures due to a reduction in the density of bone tissue. The underlying mechanism of osteoporosis is an imbalance between bone resorption and bone formation (Raisz, 2005). Osteoporosis is a condition, like sarcopenia which is associated with ageing and effects a large proportion of our ageing population; approximately 1 in 2 women and 1 in 4 men over the age of 50 years will experience an osteoporosis related fracture in their lives (Brewer *et al.*, 2011). Bone remodeling occurs in response to physical stress, although conversely physical inactivity can lead to significant bone loss. Not all exercise modalities have the same positive effects on bone mass; unloaded exercise, such as swimming for example, has no impact on bone mass while walking has limited positive effects (Guadalupe-Grau *et al.*, 2009). Bone mineral density (BMD) determines fracture risk and is most commonly measured by DEXA (Brewer *et al.*, 2011). RET has been shown to maintain or even increase bone mineral density in older individuals (Bonaiuti *et al.*, 2002; Cochrane

et al., 2005; Chilibeck *et al.*, 2005) and the Bone-Estrogen Strength Training (BEST) project identified six specific RET exercises that yielded the largest improvements in BMD in middle-aged to older females, 4 of which were identical to the ones used in this study and 2 of which were very similar in the muscle groups used (squat compared to seated leg press and military press compared to seated chest press) (Lohman *et al.*, 1995). In addition, in the Mediterranean Intensive Oxidant Study (MINOS) skeletal muscle mass, improved by RET, was positively correlated with BMD and those with the lowest skeletal muscle mass had an increased risk of falls due to impaired balance and lower muscular strength (Szulc *et al.*, 2005).

3.1.5 Body composition and ageing

Ageing is associated with the loss of FFM and an increase in FM (Cohn *et al.*, 1976). The loss of FFM can be attributed to decreases in bone mass (Heaney *et al.*, 1982), skeletal muscle mass and body water (Fulop, Jr. *et al.*, 1985). Ageing is also associated with the preferential deposition of body fat in the trunk region (Borkan *et al.*, 1985), potentially leading to android obesity, shown to be associated with CHD and related mortality (Donahue *et al.*, 1987).

As we age FM increases and relatively more fat is deposited internally as visceral fat. Visceral fat is associated with all diseases linked to a centralized pattern of fat distribution, such as CV disease, type 2 diabetes and hypertension. The marked decline in FFM that occurs with age is primarily due to a loss of skeletal muscle mass (sarcopenia), as already mentioned, and a loss in bone mineral (Kuczmarski, 1989; Heymsfield *et al.*, 1989; Roubenoff, 2000). Both of these losses increase the risk of disability and frailty in an ageing population.

When looking at body composition, especially anthropometry, in an ageing population changes in height may need to be accounted for if using tools such as BMI. Decreased height with age may be attributable to a loss of

muscle tone, alterations in inter-vertebral discs and changes in posture (Barbosa *et al.*, 2005) and may give 'false' body composition estimates.

3.1.6 Body composition and gender

It is not only age but also gender that has significant effects on body composition. However the effect of gender, unlike ageing is present from puberty throughout life and is evident in the majority of the population.

Pre-menopausal women frequently develop peripheral (often gynoid) obesity with subcutaneous fat accumulation whereas men and post-menopausal women are more prone to central (android) obesity. Central/ android obesity is associated with increased cardiovascular disease mortality and the development of type 2 diabetes and is cited as a key feature of the metabolic syndrome. Visceral adipocytes which often feature in android obesity are a major source of circulating free-fatty acids (FFA's) and cytokines, which may then be directly delivered via the portal vein to the liver inducing insulin resistance and an atherogenic lipid profile. Inflammation of this nature increases cardiovascular risk particularly when present in women (Regitz-Zagrosek *et al.*, 2006).

Waist circumference; a measure of central obesity is recognized as a predictor of cardiovascular disease and the National Cholesterol Education Program have determined waist circumference measures of >88cm for women and >102cm for males predictive of increased risk for CHD and metabolic diseases (National Cholesterol Education Programme., 2001). Circumference measures allow calculation of the waist: hip ratio, an indirect measure of upper and lower-body fat distribution where a high ratio indicates greater upper body or central adiposity and is related to risk factors associated with cardiovascular and metabolic disease (Ohrvall *et al.*, 2000). In 1988 Bray and Gray reported waist: hip ratio values of above 0.82 for women and 0.94 for men as indicative of high risk for adverse health consequences (Bray & Gray, 1988). A heightened waist: hip ratio seemingly occurs in both sexes with age.

It is not just fat deposition that is different between males and females but differences in muscle are also apparent. In women the decline of muscle mass is greatest after menopause, which may be explained by the reduction of oestrogen to testosterone (Messier *et al.*, 2011). However, older men lose appendicular limb mass more rapidly than women (Zamboni *et al.*, 2003; Hughes *et al.*, 2002), despite much higher concentrations of testosterone. If not testosterone-related, it may be the heightened rates of basal MPS in women (~+30%) that account for this sexual dimorphism (Smith *et al.*, 2008). Overall the prevalence of sarcopenia is two-fold greater in women than in men (Janssen *et al.*, 2004), this is likely related to the fact that the risk of morbidity due to muscle loss is greatest in those with the lowest pre-morbid muscle mass and women have a smaller peak muscle mass. This principle is exemplified by ex-weightlifters who lose muscle mass at the same rate as their sedentary age-matched peers but due to their higher peak muscle mass, they retain strength for longer and report less incidence of disability (Pearson *et al.*, 2002).

Gender differences in body composition are evident throughout adulthood with males over 80 years old presenting a greater loss of muscle mass than females, while females undergo a greater reduction in subcutaneous fat in the decade leading up to this age (Barbosa *et al.*, 2005).

Discussed in more detail later in this thesis adiposity is shown to have a strong association with systemic inflammation, this relationship between adiposity and certain pro-inflammatory cytokines is significantly stronger in women (Thorand *et al.*, 2006) and may indicate that weight reduction may be more effective in preventing sub-clinical inflammation in females.

3.1.7 Body composition and resistance-exercise training

Most forms of exercise training will result in moderate weight loss, moderate-to-large losses in body fat and small-to-moderate gains in FFM (Wilmore, 1983). The degree of alteration to an individual's body composition will depend on the mode of exercise, frequency, intensity, duration and concomitant nutritional status. Training, in particular

exercises which involve impact work also have the potential to increase BMC and BMD (Mullins *et al.*, 2001). Mechanical force on bone is essential for the modeling and re-modeling of bone which in turn increases both bone strength and mass (Frost, 1997). Weight bearing exercises (body weight and/ or additional weight) provide a direct mechanical force on bones but it is muscle contractions that place bones under the largest voluntary loads (Frost, 1997). Correlations between hand-grip strength and bone area, BMC and BMD in both athletes (Ducher *et al.*, 2005) and sedentary individuals (Pang & Eng, 2005) support the notion that muscle contractions can significantly benefit bone strength and mass.

Resistance training is a form of strength training; a blanket term for all exercise that develops the strength and size of skeletal muscles. Resistance training occurs when each effort is performed against a specific opposing force. Resistance training may be defined as isotonic if the body is moving against set force or isometric if holding still against force at a constant tension. Performed properly, following established guidelines based on the desired goal resistance training can provide significant functional benefits, increase bone, muscle, ligament and tendon strength, improve joint function, improve cardiac function and most importantly in relation to body composition increase metabolic rate. This increase in metabolic rate associated with resistance training is due to an increase in skeletal muscle; a metabolically active tissue which requires energy at all times, even at rest. At rest skeletal muscle consumes 13.0 kcal/kg per day, considerably more than adipose tissue at 4.5 kcal/kg per day (Heymsfield *et al.*, 2002).

As RET increases muscle mass, weight loss will not occur without caloric restriction, however even without caloric restriction RET can have favorable effects on body composition by reducing fat mass, including abdominal fat (Hunter *et al.*, 2002; Tresierras & Balady, 2009). A study from over 15 years ago reported that in older women who performed a RET regime that involved training three times a week for 16 weeks intra-abdominal adipose was reduced despite no change in weight due to increased muscle mass (Treuth *et al.*, 1995). A more recent study again

using a three times per week RET intervention reported no change in body weight but decreased fat-mass and increased muscle mass following 12 weeks of training (Iglay *et al.*, 2007). Decreases in limb fat mass, even with no change in limb circumference have also been reported following a RET program (Treuth *et al.*, 1994).

Evidence is available that RET increases both regional and total lean tissue mass and decreases both regional and total fat tissue mass in people of all ages. These changes in BC suggest that RET and the effects it has on BC can play an important role in the prevention of age-associated losses in strength and muscle mass and may affect fat deposition alleviating the decline in functional abilities and health status suffered by many as they age (Treuth *et al.*, 1994).

3.2 Methodology

3.2.1 Dual-energy x-ray absorptiometry

Each subject received two DEXA scans (DEXA; GE LUNAR II), one at screening and one when they returned for the second of their two acute studies. As well as analyzing whole body estimates of different tissues and measuring BMC and BMD (Figure 3.5) DEXA measurements also allowed us to obtain data on selected regions of interest (ROI). Subject positioning on the DEXA bed was optimized to allow the ROI body compartments to be analyzed separately i.e. ensuring a space between the legs and between the arms and torso whenever possible. The "upper leg" ROI for body composition analysis was selected as the area inferior to the lowest visible point of the coccyx to the mid-point of the patella (Figure 3.6a). The "leg" ROI was defined as the area inferior to the lowest visible point of the coccyx. For analysis of abdominal composition the "abdominal" ROI was selected as the lowest visible point of the coccyx upwards to the highest visible point of the pelvic girdle (Figure 3.6b) with the "trunk" ROI assigned by the DEXA software.

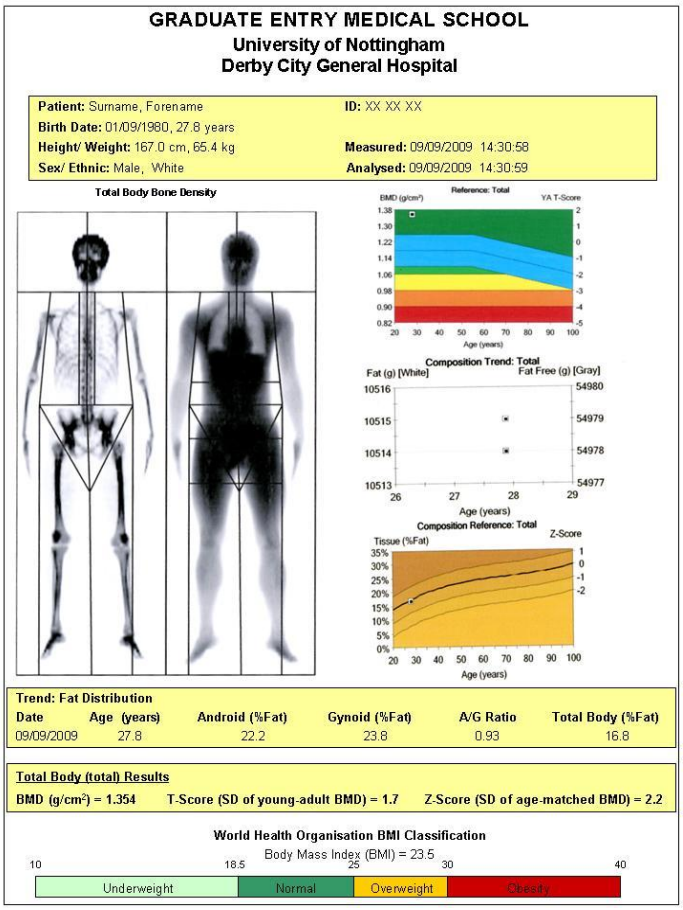
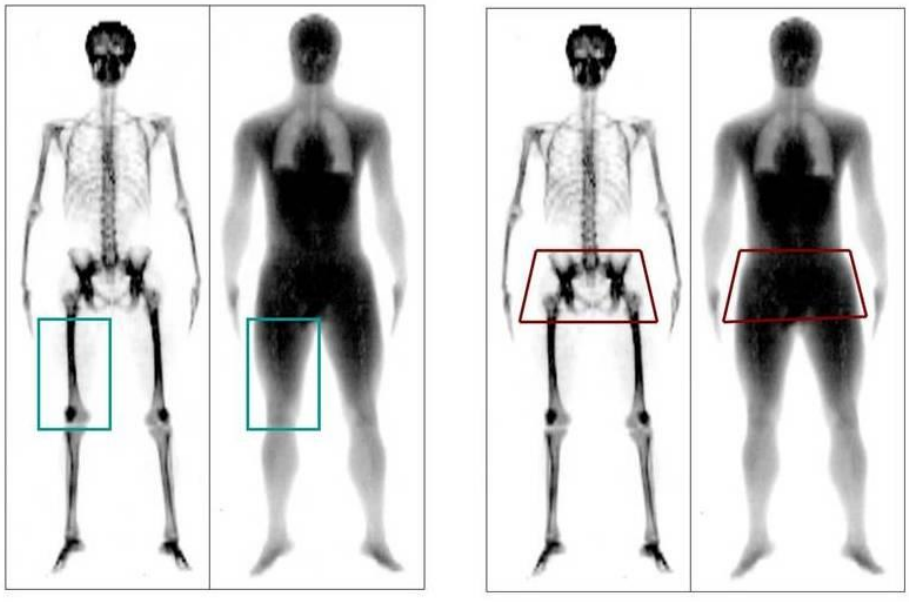


Figure 3.5 Total body standard DEXA report (example)



A. **B.**

Figure 3.6 Customized DEXA regions of interest
A. Upper Leg region of interest
B. Abdominal region of interest

3.2.2 Anthropometric indexes

3.2.2.1 Body mass index

Body mass index (BMI) is commonly used in both clinical and alternative settings to offer an assessment of someone's weight. Calculated using the formula of weight (kg)/ height $(m)^2$ the limitations of BMI as an assessment tool have already been discussed earlier in this chapter. Used mainly in this study as an assessment tool against exclusion criteria we have also recorded subject BMI both before and after the RET.

3.2.2.2 Relative skeletal muscle mass index

The relative skeletal muscle mass index (RSMI) is derived from total lean mass (kg) divided by height (m). The RSMI can be an advantageous way of assessing lean mass, especially in the elderly as it uses height as one of its calculation factors which is known to decrease with advancing age (Sorkin *et al.*, 1999; Hirani & Aresu, 2012). The title of relative skeletal muscle mass index is slightly misleading in that it is not only lean muscle mass that is accounted for as all non-bone lean tissues will feature, including organ mass. Nonetheless, decreased RSMI is associated with narrower bones, thinner bone cortices, lower bending strength, impaired balance and increased risk of falls in the elderly (Szulc *et al.*, 2005).

3.2.2.3 Appendicular lean body mass index

Appendicular lean body mass index (ALBMI) is a value derived from appendicular (arms and legs) lean mass (kg) divided by height (m) and has been shown to be more strongly associated with bone mineral content than total lean mass (Goulding *et al.*, 2009). As with the RSMI, ALBMI can be advantageous in an ageing population due to the inclusion of stature. Age has been shown to be an independent determinant of appendicular lean body mass when corrected for stature as featured in the ALBMI calculations (Gallagher *et al.*, 1997).

3.3 Results

3.3.1 Changes in body composition analyzed by dual-energy x-ray absorptiometry after resistance-exercise training

3.3.1.1 Percentage Body Fat

All three age-groups reduced their percentage body fat following the RET (Y, *P*=<0.05; M, *P*=<0.001; O, *P*=<0.01) (Figure 3.7).

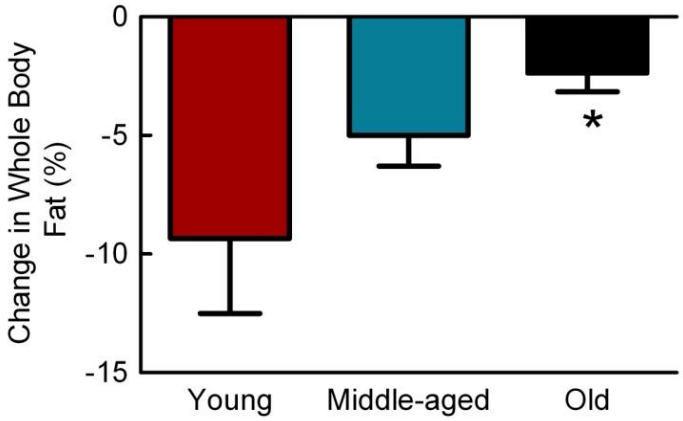


Figure 3.7 Percentage change in whole-body fat in young, middle-aged and older subjects after RET. Values are means±SEM. Statistical analysis via ANOVA with Bonferroni post analysis. *=*P*<0.05 vs. young.

Neither before or after RET was there a significant difference in percentage body fat between the age groups even though the young reduced their percentage body fat by a greater amount that the old (*P*=<0.05). When dividing the groups by gender the males in all three age groups reduced their percentage body fat after RET (Y, *P*=<0.001; M, *P*=<0.05; O, *P*=<0.01) whereas only the middle-aged females experienced a reduction (*P*=<0.01). Young males had a lower percentage body fat than young females both before and after RET (*P*=<0.001), and this gender disparity was also the case in the middle-aged (*P*=<0.001) and older groups (*P*=<0.05). Young males had a lower percentage body fat than older males before (*P*=<0.01) and after RET (*P*=<0.001) that was also lower than middle-aged males after RET (*P*=<0.05). These age differences were not apparent in the females where there were no significant differences between the three age-groups either before or after RET (Figure 3.8).

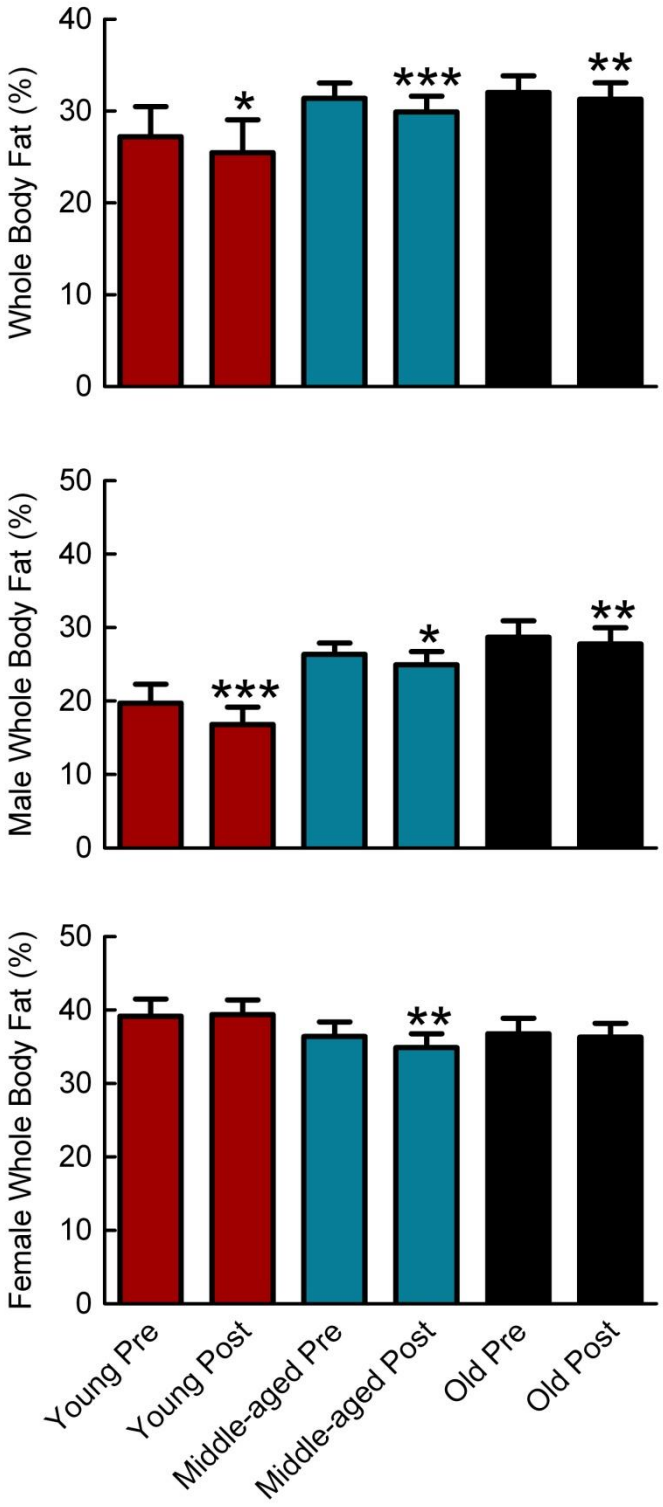


Figure 3.8 Whole body fat percentages in young, middle-aged and older male and female subjects before and after RET. Values are means±SEM. Statistical analysis via ANOVA with Bonferroni post analysis. *=P<0.05 vs. pre-training in the same age-group, **=P<0.01 vs. pre-training in the same age-group, ***P<0.001 vs. pre-training in the same age-group.

3.3.1.2 Whole-Body Lean Mass

Whole-body lean mass was increased in the young (*P*=<0.01) and middle-aged groups (*P*=<0.05) after RET and there was a trend for it to increase in the older group (*P*=0.08). The increase in the young tended to be greater than in the older group (*P*=0.1) (Figure 3.9).

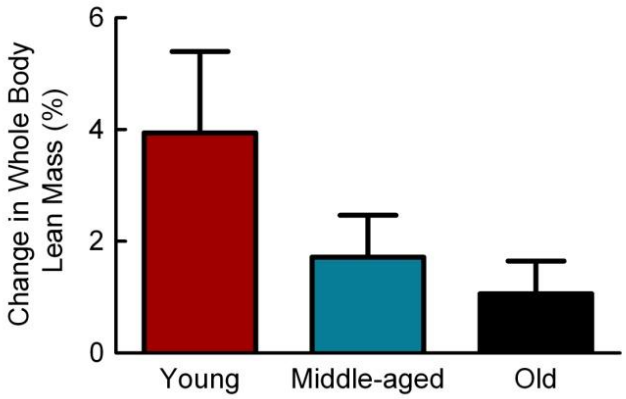


Figure 3.9 Percentage change in whole body lean mass in young, middle-aged and older subjects after RET. Values are means±SEM. Statistical analysis via ANOVA with Bonferroni post analysis.

There were no significant differences in the whole-body lean mass of the three age-groups either before or after RET. When analysing the data divided also by gender only the young males increased their whole body lean mass following RET (*P*=<0.01), although there was a trend for an increase in the older males (P=0.09). In all three of the ages-groups the males had significantly more whole-body lean mass than the females both before and after RET (Y, *P*=<0.01; M, *P*=<0.001; O, *P*=<0.001). There were no differences between the age groups when divided by gender (Figure 3.10).

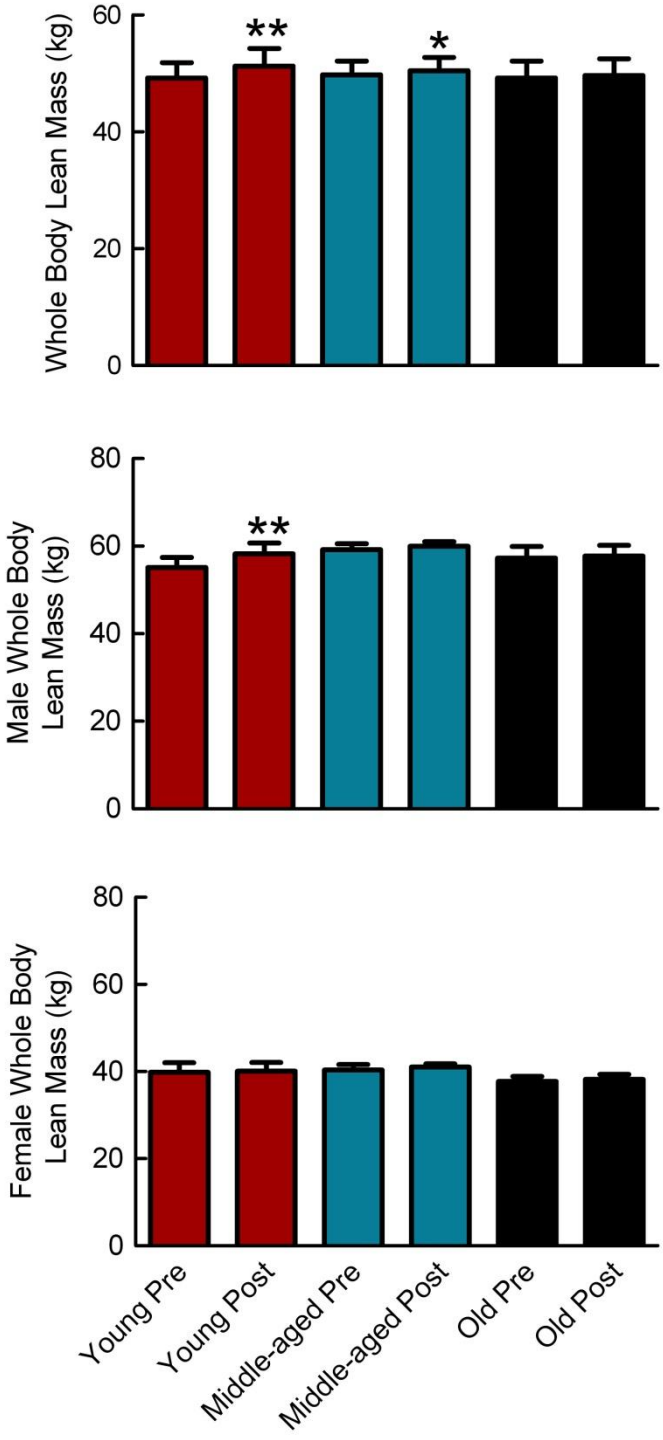


Figure 3.10 Whole body lean mass in young, middle-aged and older male and female subjects before and after RET. Values are means±SEM. Statistical analysis via ANOVA with Bonferroni post analysis. *=P<0.05 vs. pre-training in the same age-group, **=P<0.01 vs. pre-training in the same age-group.

3.3.1.3 Dominant Leg Lean Mass

As with the whole-body lean mass, there was no significant difference in dominant leg lean mass between the age groups either before or after RET. When the data was separated by gender both the young males and females increased their dominant lean leg mass after RET (P=<0.05). Mirroring the trend seen in whole-body lean mass the males in all three age-groups had greater dominant lean leg mass than the females (P=0.001) and there were no differences between the age groups within gender (Figure 3.11).

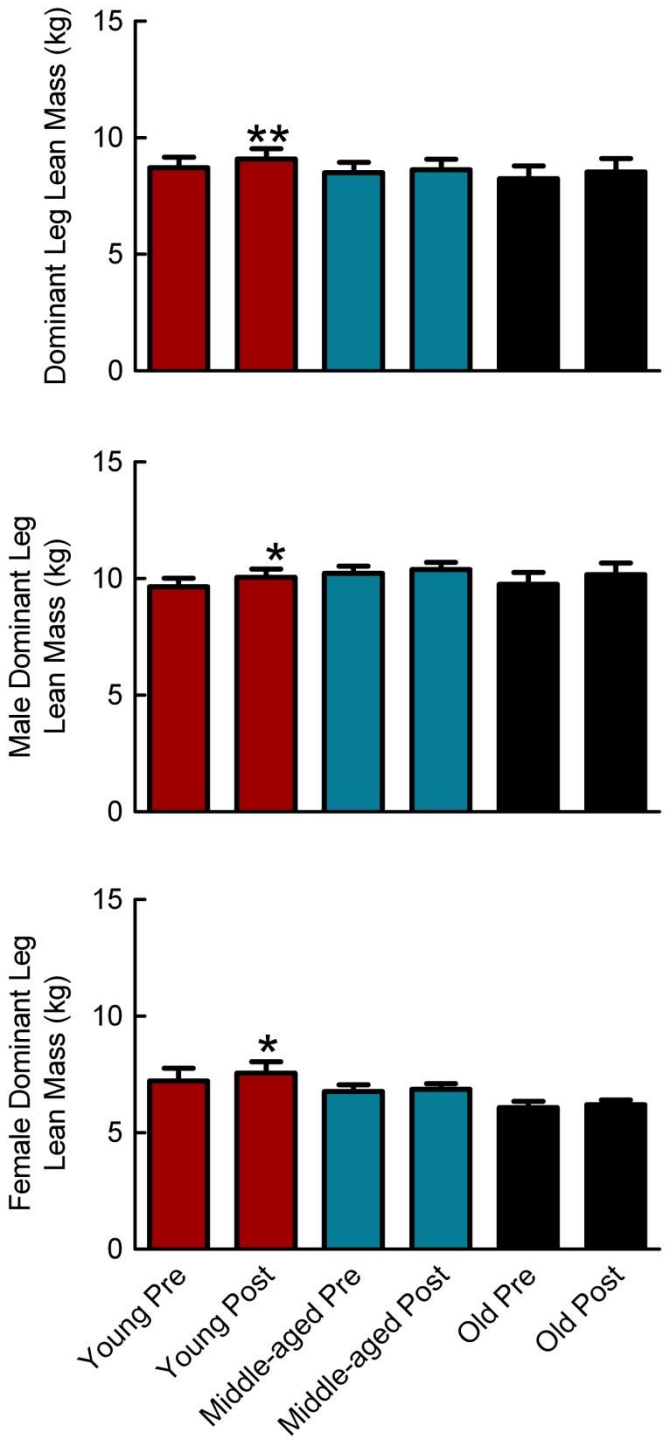


Figure 3.11 Dominant leg lean mass in young, middle-aged and older male and female subjects before and after RET. Values are means±SEM. Statistical analysis via ANOVA with Bonferroni post analysis. *=P<0.05 vs. pre-training in the same age-group, **=P<0.01 vs. pre-training in the same age-group.

The young were the only group to significantly increase their dominant leg lean mass (*P*=<0.01) although the older group did increase their dominant leg lean mass more than the middle-aged (Figure 3.12).

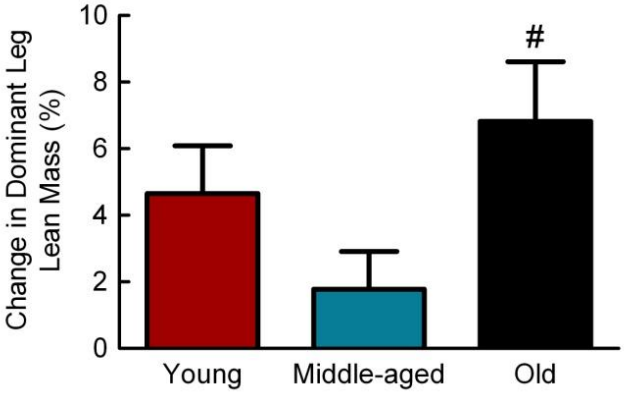


Figure 3.12 Percentage change in dominant leg lean mass in young, middle-aged and older subjects after RET. Values are means±SEM. Statistical analysis via ANOVA with Bonferroni post analysis. #=*P*<0.05 vs. middle-aged.

3.3.1.4. Dominant Upper Leg Lean Mass

Upper dominant leg lean mass was increased in the young (*P*=<0.01), middle-aged (*P*=<0.01) and older age group (*P*<0.05) after RET. The increase in the young tended to be greater than that in the older group (*P*=0.1) (Figure 3.13). The change in upper leg lean mass did not mirror the change shown in whole leg lean mass with no significant differences between the age-groups percentage increase.

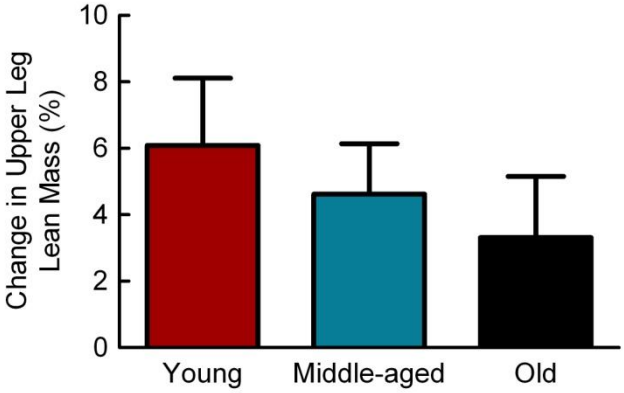


Figure 3.13 Percentage change in upper dominant leg lean mass in young, middle-aged and older subjects after RET. Values are means±SEM. Statistical analysis via ANOVA with Bonferroni post analysis.

There were no significant differences in the upper leg lean mass of the three age-groups either before or after RET. When analysing the data divided also by gender the young and middle-aged males increased their upper leg lean mass following RET (*P*=<0.05 and <0.01, respectively), with a trend for an increase in the older males (P=0.06). In all three of the ages-groups the males had significantly more upper leg lean mass than the females both before and after RET (*P*=<0.001). There were still no differences in absolute upper lean leg mass between the age groups when divided by gender (Figure 3.14).

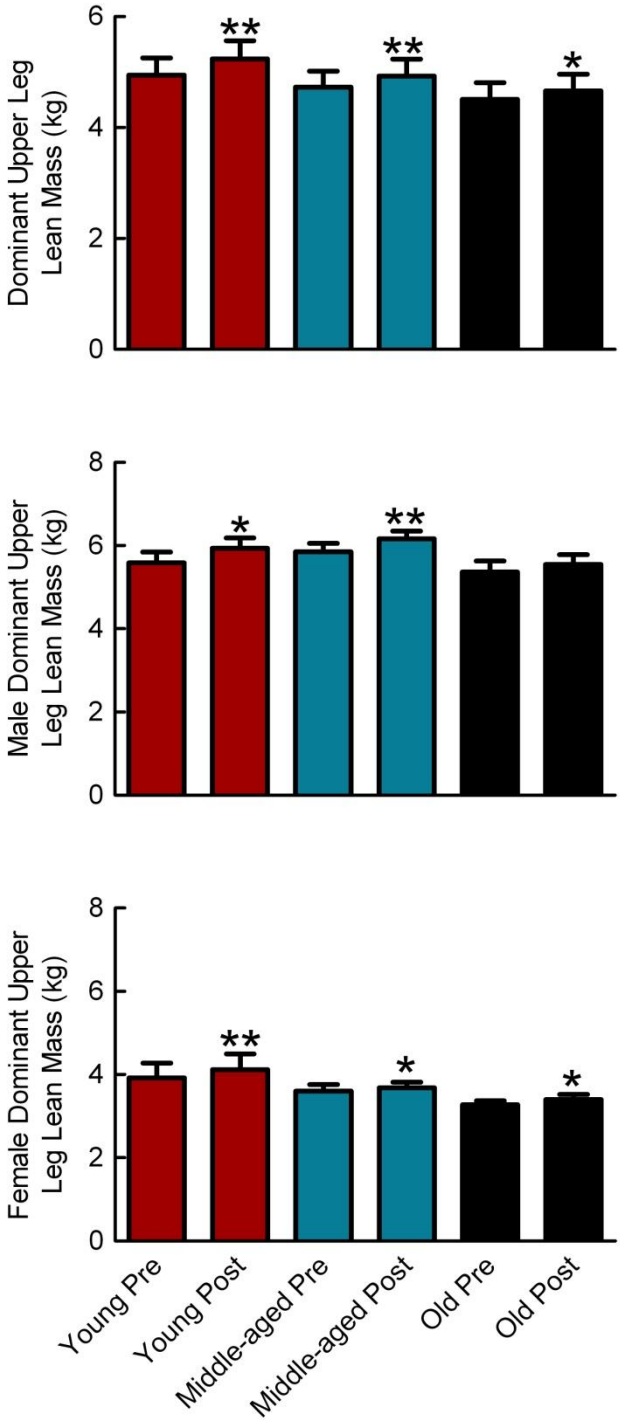


Figure 3.14 Dominant upper leg lean mass in young, middle-aged and older male and female subjects before and after RET. Values are means±SEM. Statistical analysis via ANOVA with Bonferroni post analysis. *=P<0.05 vs. pre-training in the same age-group, **=P<0.01 vs. pre-training in the same age-group.

3.3.1.5 Body Mass Index

BMI was not changed by RET in any age-group and there was no significant difference in BMI between the age groups either before or after RET. When the data was analysed with the results divided by gender the only change following RET was that the young females had a significantly higher BMI. Before RET, there was no difference between the BMI of young males and females, however after RET the young males BMI was significantly lower than that of the young females (P=<0.05). There was no gender difference in BMI in the middle-aged and older groups. Young males had a significantly lower BMI than middle aged and older males both before and after RET (P=<0.001). There was no age difference in the females BMI either before or after RET (Figure 3.15).

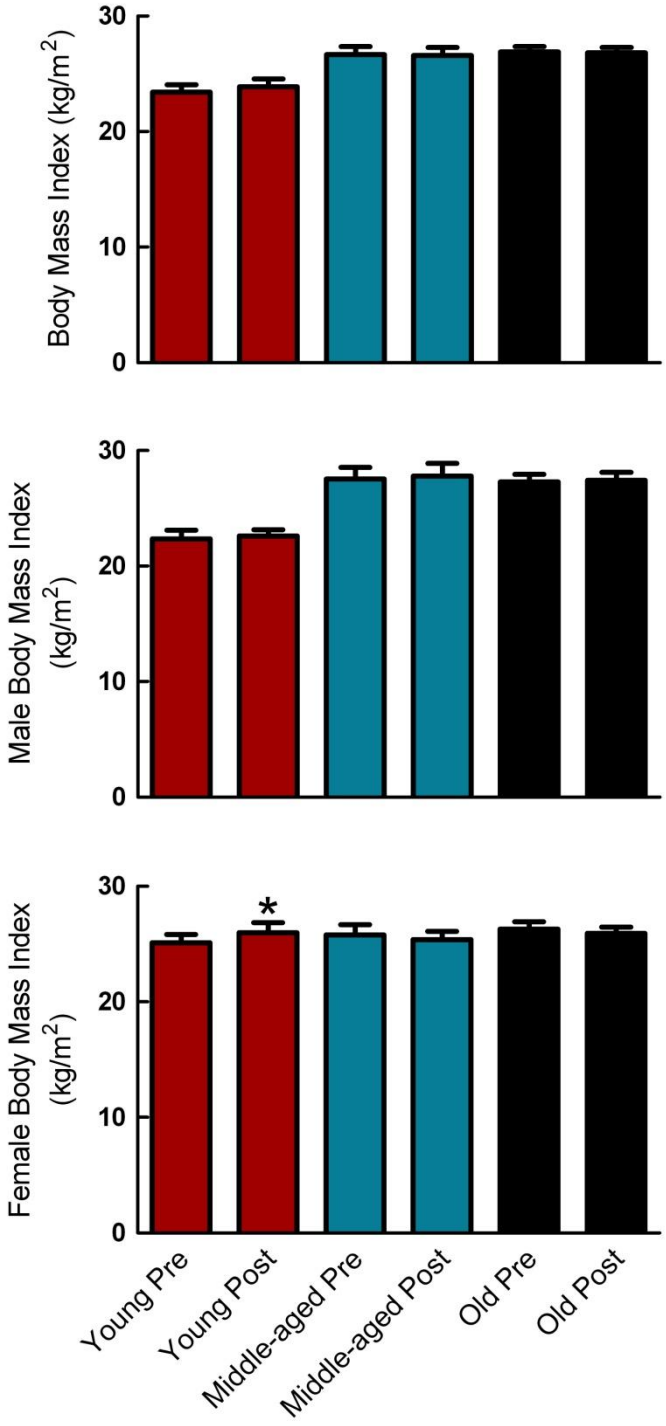


Figure 3.15 Body mass indexes in young, middle-aged and older male and female subjects before and after RET. Values are means±SEM. Statistical analysis via ANOVA with Bonferroni post analysis. *=P<0.05 vs. pre-training in the same age-group.

Often cited as poor marker of body composition, comprising only of height and weight measurements, BMI is the tool most commonly used in a healthcare setting, with many practitioners failing to evaluate its

limitations. Even so despite its seemingly crude method using only height and weight as indices of body composition, for the subjects in this study there was a significant correlation between BMI and % body fat (Figure 3.16).

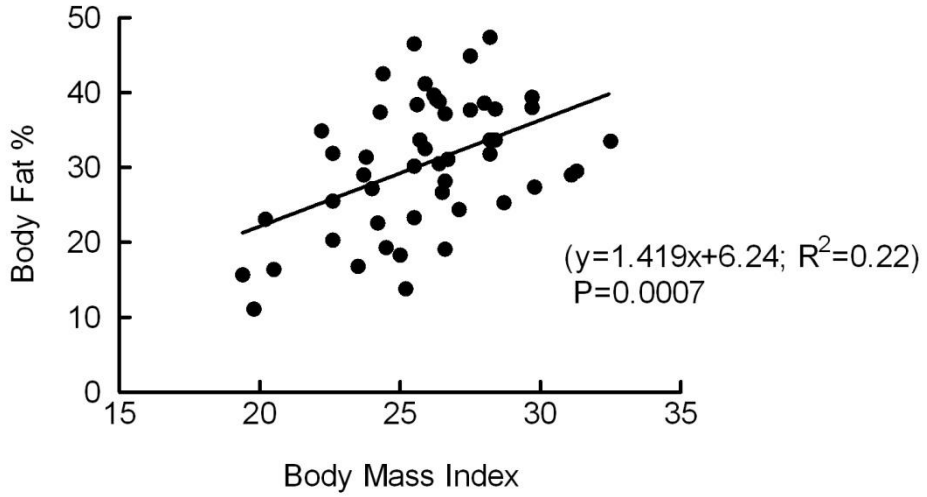


Figure 3.16 Correlation between body fat% and BMI in young, middle-aged and older subjects before RET. Statistical analysis via Pearson's correlation.

3.3.1.6 Trunk Fat Mass

Trunk fat mass was reduced in young (*P*=<0.001), middle-aged (*P*=<0.01) and older subjects (*P*=<0.01) after RET, with no significant difference in the level of change experienced by the three age-groups (Figure 3.17).

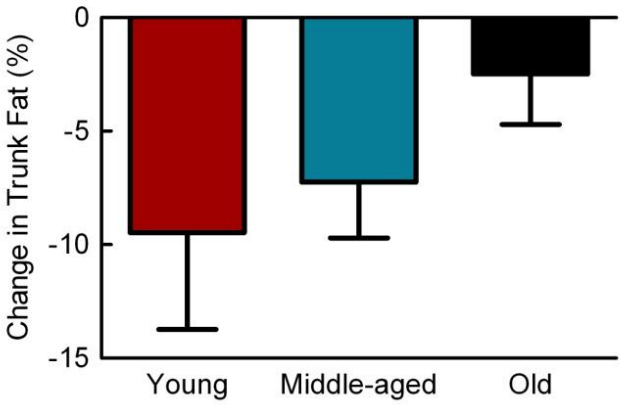


Figure 3.17 Percentage change in trunk fat in young, middle-aged and older subjects after RET. Values are means±SEM. Statistical analysis via ANOVA with Bonferroni post analysis.

Trunk fat mass in the young was lower than that in the older group both before and after RET (P=<0.05). When the groups were divided by gender the young males had a significant reduction in trunk fat mass after RET (P=<0.05) and there was a trend for this reduction in the middle-aged (P=0.08) and older males (P=0.1). Only the middle-aged females had reduced trunk fat mass after RET (P=<0.01), with no change in the young and older females. Both before and after RET the young males had lower trunk fat mass than the young females and both before and after RET they had lower trunk fat mass than the middle-aged (P=<0.01 pre and post) and older males (P=<0.01 before, P=<0.001 after). There were no differences in the trunk fat mass of females in the three different age-groups either before or after RET, and in the middle-aged and older groups there were no differences between male and females trunk fat mass either before or after RET (Figure 3.18).

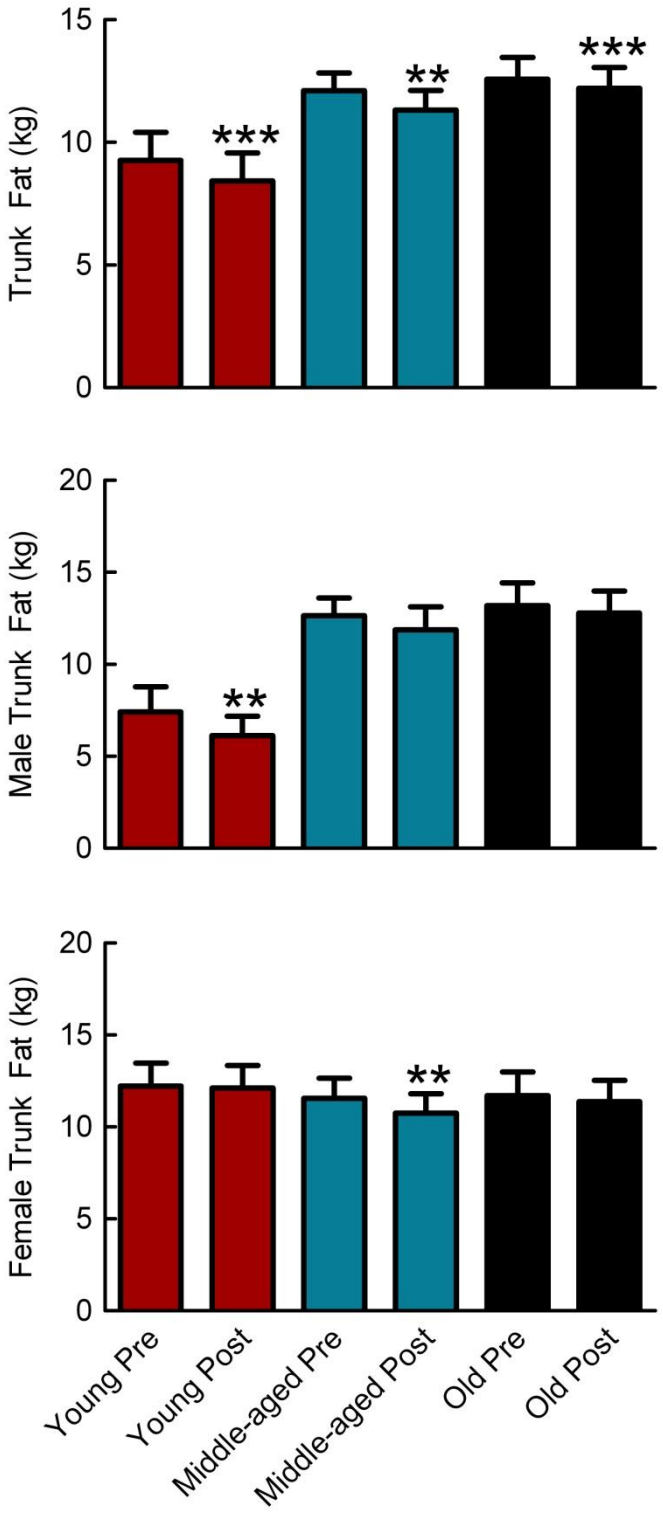


Figure 3.18 Trunk fat in young, middle-aged and older male and female subjects before and after RET. Values are means±SEM. Statistical analysis via ANOVA with Bonferroni post analysis. **=P<0.01 vs. pre-training in the same age-group, ***P<0.001 vs. pre-training in the same age-group.

3.3.1.7 Bone Mineral Density

There were no significant differences in the BMD of the three age-groups, contrary to what may have been expected, either before or after RET. RET did not change the BMD in any of the age groups. When the age-groups were also divided by gender there was no difference in the BMD of females according to age before RET, however after RET the young and middle-aged females had significantly higher BMD than the older females (P=<0.05). There was no difference in BMD between the young males and females, but in the middle-aged and older groups the males had significantly higher BMD than the females both before and after RET (P=<0.001) (Figure 3.19).

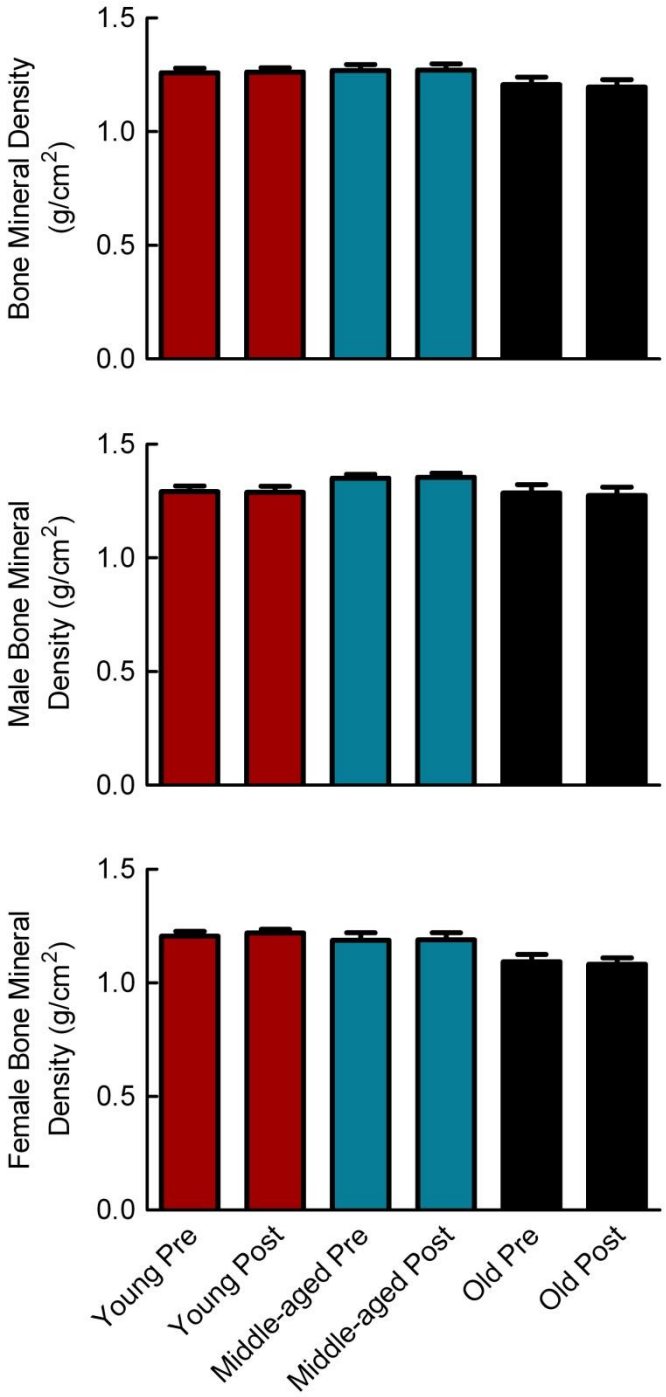


Figure 3.19 Bone mineral density in young, middle-aged and older male and female subjects before and after RET. Values are means±SEM. Statistical analysis via ANOVA with Bonferroni post analysis.

3.3.1.8 Relative Skeletal Mass Index

There were no significant differences in RSMI between the age-groups either before or after RET. The RSMI increased in the young (P=<0.01) and middle-aged groups (P=<0.05) after RET, with a trend for it to increase in the older group (P=0.1), therefore following the pattern shown in whole-body lean mass. When divided by gender only the young males increased their RSMI after RET (P=<0.01). The young males had significantly higher RSMI after RET than young females (P=<0.05), although there were no differences before. The middle-aged males had higher RSMI than the middle-aged females both before and after RET (P=<0.001) but there were no differences between the older males and females either before or after RET. There were no significant differences between the age-groups, in either males or females before or after RET (Figure 3.20).

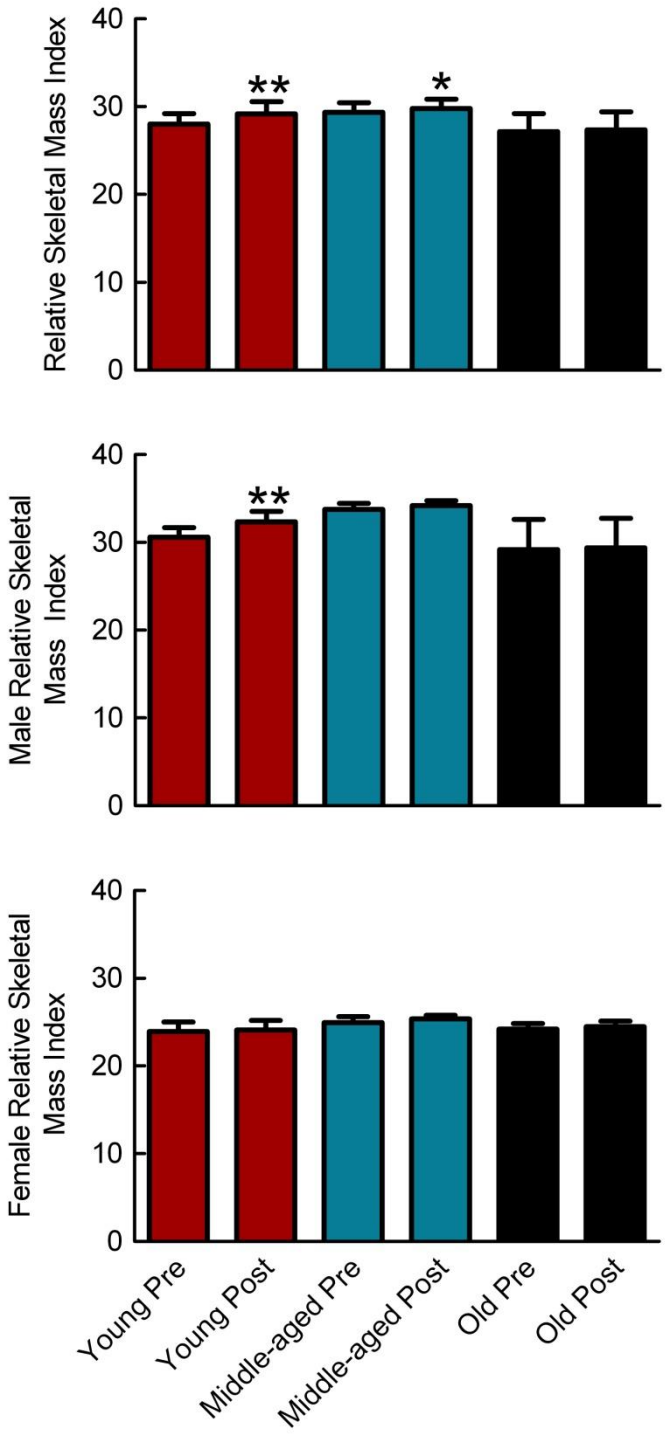


Figure 3.20 Relative skeletal mass indexes in young, middle-aged and older male and female subjects before and after RET. Values are means±SEM. Statistical analysis via ANOVA with Bonferroni post analysis. *=P<0.05 vs. pre-training in the same age-group, **=P<0.01 vs. pre-training in the same age-group.

3.3.1.9 Appendicular Lean Body Mass Index

The ALBMI, as with the RSMI and the whole-body lean mass was not significantly different between the age-groups either before or after RET, although the young (*P*=<0.001), middle-aged (*P*=<0.05) and older groups (*P*=<0.05) all significantly increased their ALBMI after RET. The ALBMI of both the young males and females increased significantly after RET (*P*=<0.01), with no changes in the middle-aged or older males or females when the age-groups were also divided by gender which may have been due to reduced statistical power when the *n* was reduced. There was no difference between the ALBMI of the young males and females before RET, however the males did demonstrate a higher ALBMI after RET (*P*=<0.05). In the middle-aged and older groups the ALBMI was higher in males than females both before and after RET (M, *P*=<0.001; O, *P*=<0.05). There was no difference in ALBMI according to age either before or after RET in males or females (Figure 3.21).

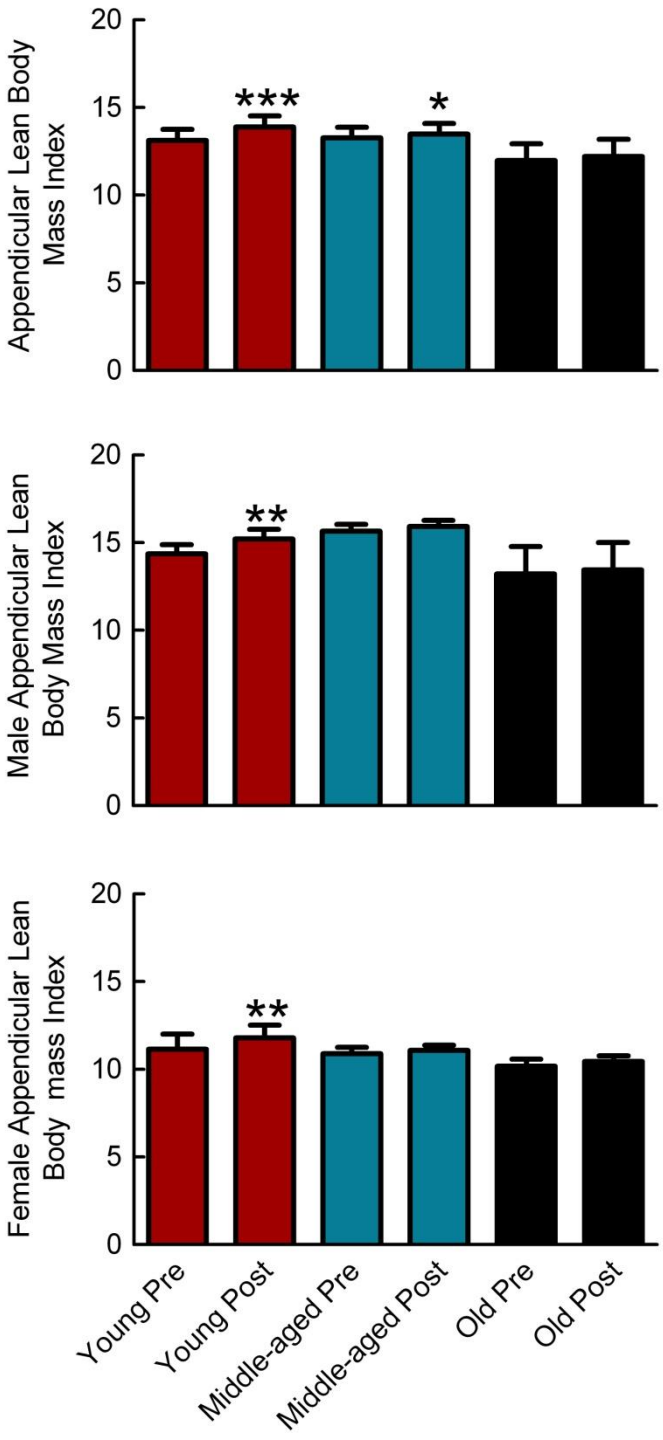


Figure 3.21 Appendicular lean body mass indexes in young, middle-aged and older male and female subjects before and after RET. Values are means±SEM. Statistical analysis via ANOVA with Bonferroni post analysis. *=P<0.05 vs. pre-training in the same age-group, **=P<0.01 vs. pre-training in the same age-group, ***P<0.001 vs. pre-training in the same age-group.

3.3.1.10 Android: Gynoid Ratio

There were no significant differences in android: gynoid (A:G) ratio between the age groups before or after RET, although the middle-aged group did decrease their A:G ratio after RET (*P*=<0.05). As a data set that in which you would expect to observe gender differences it was surprising that there were no differences in the A:G ratios between males and females in the young or older age-groups. The middle-aged males had a significantly higher A:G ratio than the middle-aged females both before and after RET (*P*=<0.001). The young and middle-aged males both had a lower A:G ratio after RET (*P*=<0.05), while the young females had a higher A:G ratio after RET. The young males had a lower A:G ratio than the middle-aged (*P*=<0.001) and older males (*P*=<0.01) both before and after RET. There were no significant differences in the A:G ratios of the females from different age-groups either before or after RET (Figure 3.22).

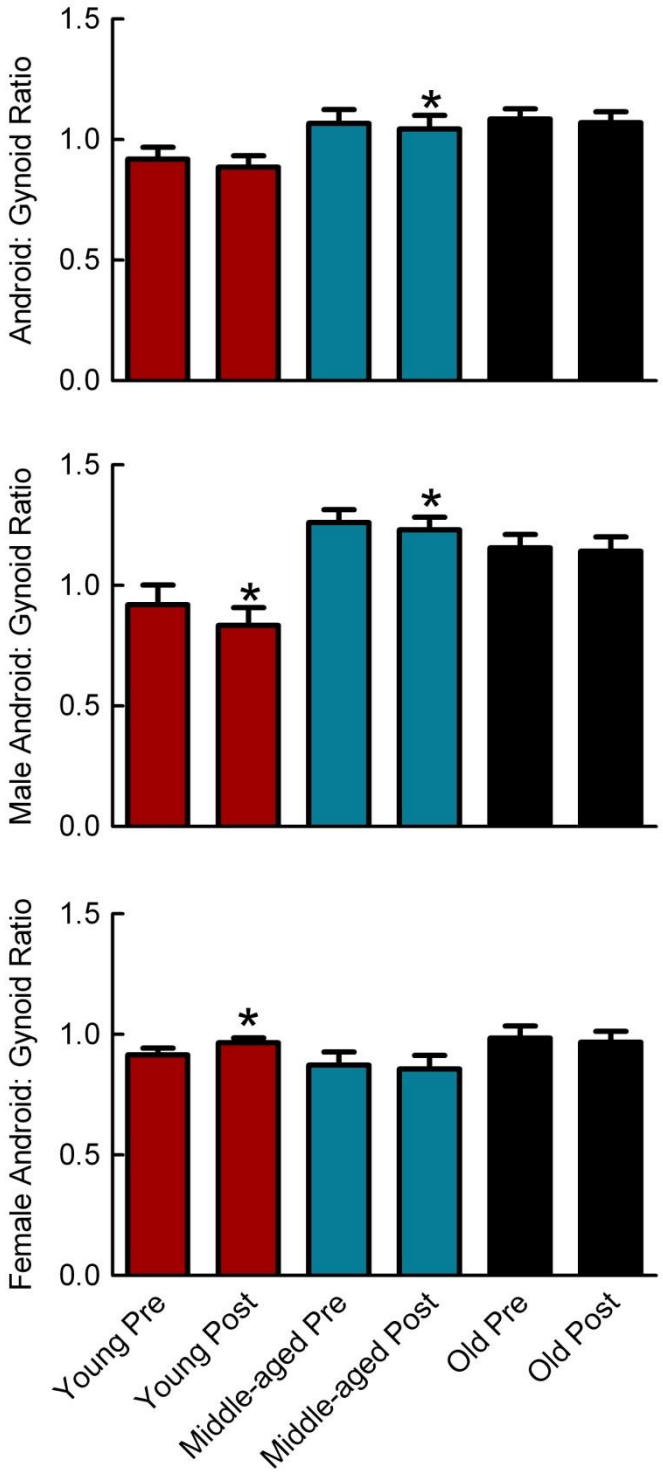


Figure 3.22 Android: gynoid ratios in young, middle-aged and older male and female subjects before and after RET. Values are means±SEM. Statistical analysis via ANOVA with Bonferroni post analysis. *=P<0.05 vs. pre-training in the same age-group.

3.3.1.11 Abdominal Fat Percentage

The young and the middle-aged group showed a reduction in abdominal fat percentage after RET (P<0.01; Figure 3.23).

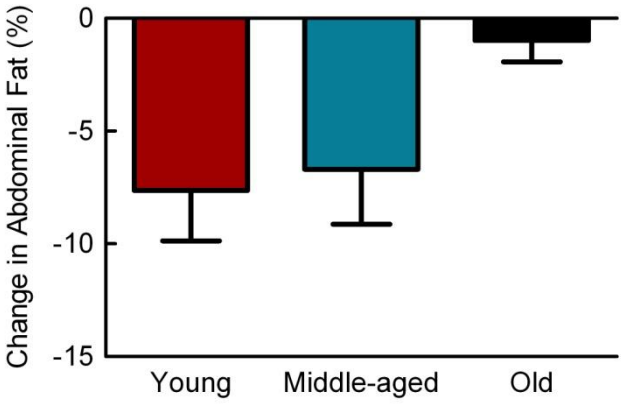


Figure 3.23 Percentage change in abdominal fat in young, middle-aged and older subjects after RET. Values are means±SEM. Statistical analysis via ANOVA with Bonferroni post analysis.

There were no significant differences in abdominal fat percentage between the age groups either before or after RET. When the age-groups were also divided by gender, the young and middle-aged males displayed a reduction in abdominal fat percentage after RET (Y, P<0.01; M, P<0.05) but the older males did not. Only the middle-aged females showed a reduction after RET, mirroring what was seen in the female data regarding whole-body fat percentage. In all three age-groups males had a significantly lower abdominal fat percentage than females both before and after RET (P<0.001). Young males had a significantly lower abdominal fat percentage than older males both before (P<0.05) and after (P<0.01) RET, with no difference between the young and middle-aged males or the middle-aged and older males. Neither before or after RET were there any significant differences in abdominal fat percentage between the females in the different age-groups (Figure 3.24).

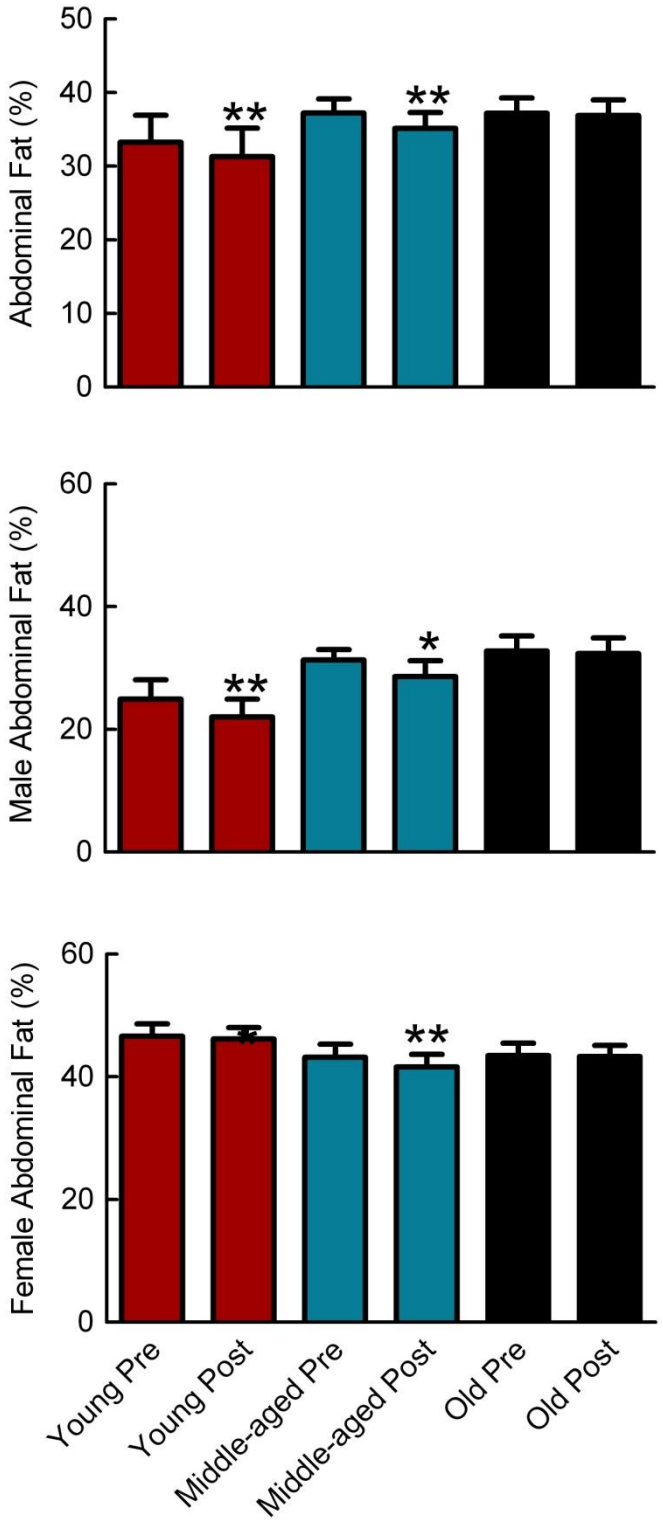


Figure 3.24 Abdominal fat in young, middle-aged and older male and female subjects before and after RET. Values are means±SEM. Statistical analysis via ANOVA with Bonferroni post analysis. *=P<0.05 vs. pre-training in the same age-group, **=P<0.01 vs. pre-training in the same age-group.

3.4 Discussion

Contrary to our hypothesis neither the whole-body lean mass, the total lean leg mass nor the upper lean leg mass of our older individuals was significantly different to that of our middle-aged or young subjects, suggesting that our older individuals were pre-sarcopenic. This may be due in some part to a degree of self-selection, in that the older individuals who applied to participate in this study (advertised as "Nottingham and Derby Active Ageing") may likely be a cohort who, although did not participate in any formal exercise regime prior to their inclusion in this study, may amongst their age-matched peers score highly in ranks of daily physical activity and independence, although this is data that we did not collect.

Use of DEXA as our method of body composition analysis may be another reason why we were unable to report any significant differences between the lean mass of our young and older subjects. In older individuals there will be larger proportions of intramuscular fat, also known as myosteatosis (Kent-Braun *et al.*, 2000) and connective tissue (Macaluso *et al.*, 2002) which the DEXA will not be able to distinguish and may therefore result in an over-reporting of lean mass in our older subjects.

Although we were not able to report any significant differences in lean mass between our ages groups we did observe a significant increase in whole body lean mass of 2.07±0.53% as hypothesized, when all of subjects were grouped together, with the increase not significantly different between the young (3.94±1.45%), middle-aged (1.72±0.75%) and older (1.06±0.58%) groups; although there was a trend for it to be higher in the young. There were no significant gender differences in this increase in any age-group. The overall increase of 2.07±0.53 % is not too dissimilar to other literature which reports 5-17% increases in muscle size with ~4 months resistance training (Narici & Maganaris, 2006). The 17% value, was reported using cross-sectional area via CT after 16 weeks training, 3 times per week, performing 4 sets of 10-20 repetitions at 70-90 % 1-RM (Brown *et al.*, 1990), while the lower value of 5% was obtained after 16

weeks training, 3 times per week at 80% 1-RM performing single sets of 10 repetitions (Ferri *et al.*, 2003) which is closer to our adopted protocol although the intensity was still 10% 1-RM higher.

In addition to absolute lean mass values there are also two widely accepted indices which can be used for the assessment of lean mass, especially when including older individuals, derived from DEXA data. The relative skeletal mass index (RSMI) is calculated as total lean mass (kg), divided by height (m). The RSMI can be an advantageous way of assessing lean mass, especially in the elderly as it uses height as one of its calculation factors and this is known to decrease with advancing age (Sorkin *et al.*, 1999; Hirani & Aresu, 2012). The title of relative skeletal muscle mass index is slightly misleading in that it is not only lean muscle mass that is accounted for, but all non-bone lean tissues will feature, including organ mass. Nonetheless, decreased RSMI is associated with narrower bones, thinner bone cortices, lower bending strength, impaired balance and an increased risk of falls in the elderly (Szulc *et al.*, 2005a), all of which when combined with the widely reported problems of decreased metabolic rate, reduced BMD, decreased insulin sensitivity and lower maximal aerobic capacity which are reported with age (Evans, 1995; Dinenno *et al.*, 1999) and contribute to age-related health declines.

The appendicular lean body mass index (ALBMI) is calculated as appendicular lean mass (kg), divided by height (m). ALBMI has been shown to be more strongly associated with bone mineral content than total lean mass, making it favorable for assessment of bone health (Goulding *et al.*, 2009) and as declines in physical function with age are often attributable to losses in muscle mass and strength at the extremities evans (Evans, 1995) it may also be favorable for assessing function and independence in the elderly (Sugawara *et al.*, 2002). As with the RSMI, the ALBMI can be advantageous in an ageing population due to the inclusion of stature in the calculation. In older individuals where lean mass and bone mass decrease, the ALBMI is frequently used to assess sarcopenia (Goulding *et al.*, 2009; Baumgartner *et al.*, 1998) and low values are

associated with both muscle weakness and reduced BMD (Waters *et al.*, 2010). Although age has been shown to be an independent determinant of appendicular lean body mass when corrected for stature (Gallagher *et al.*, 1997), it is still proposed by many groups that strength and muscle function are better measure of muscle health with age than lean mass or lean mass related indices (Lauretani *et al.*, 2003).

The few but notable discrepancies between the changes observed in RSMI and ALBMI demonstrate the importance of method selection when assessing BC in a given population. Neither index showed age-related differences before nor after RET, but while all age-groups demonstrated increases in ALBMI with RET, only the young and middle-aged groups showed significant increases in RSMI. When divided into gender groups the young males showed an increase in RSMI with RET, placing their value significantly higher than that of young females, something that was also seen for ALBMI. The RSMI was higher in the middle-aged males compared to middle-aged females both before and after RET, as was ALBMI which was also higher in the older males compared to age-matched females. Only the young males and females showed an increase in ALBMI when the gender groups were divided suggesting that this may be due to statistical power from a reduced *n* as all groups showed the increase when categorised by age only. Neither index showed an age-related difference within gender.

With regard to our hypothesis of age-related differences in fat mass this proved correct for males but not for females, with young males having a lesser percentage body fat than older males both before and after RET and also less than the middle-aged males after RET. In all of the age-groups, both before and after RET, males had lower body fat percentage than females with no differences in body fat percentage between the female age-groups before or after RET. Only the middle-aged females reduced their percentage body fat after RET while all of the male age-groups demonstrated a significant reduction. This is similar to the results shown by Donges and colleagues in which males showed a significantly greater

decrease in FM compared to females and also a greater increase in total body FFM. The work of Donges indicated a gender disparity in the relationship between functional gains and body composition changes following RET as females experienced greater increases in strength than males albeit with lesser changes in body composition (Donges & Duffield, 2012). When the gender sets were grouped together there were no significant differences in percentage body fat values between the three age-groups before or after RET, although all three age-groups showed significant reductions in body fat percentage, as has been previously demonstrated (Hunter *et al.*, 2002; Cuff *et al.*, 2003; Treuth *et al.*, 1995; Tresierras & Balady, 2009); with reductions significantly higher in the young than in the old, inversely mirroring the lean mass gains.

The heightened response in the young group both in terms of lean mass gains and reductions in body fat percentage may be explained by greater total energy expenditure (Carbone *et al.*, 2012). Although all of the subjects performed the same relative workload (given as a percentage of 1-RM) the young group performed work against significantly higher loads which would have increased the energy expenditure of their activity above that of the other age-groups. Taken in combination with the diet-diary data presented in Chapter 2 where the young significantly reduced their reported energy intake during RET it is not surprising that they elicited the greatest BC changes based on the principle of simple energy balance .This theory may also hold true in explaining why the males experienced more favorable BC changes compared to the females as for each age-group they also performed exercise against a greater resistance.

The small but significant changes in body fat percentage in all three age-groups highlights the positive role RET can have on body composition, especially when taken in combination with the changes in lean mass. The gender differences in changes, at least in this study, suggest RET to be more effective in altering the BC profile of males but this is independent of improvements in functional outcome where females are equal.

Another method for assessing body composition changes without the need for DEXA, which requires specialist equipment and operators, is that of BMI. BMI (or Quetelet's Index as it is sometimes known) is simply height in metres, divided by weight in kilograms squared and derived from a liner regression including weight to the power of 1.82 (later was rounded up to 2). In large-scale epidemiological studies increased BMI has been associated with increased all-cause mortality (Calle *et al.*, 1999), although it has been questioned if this is true in elderly people or if an ageing-obesity paradox exists (Allison *et al.*, 1997; Uretsky *et al.*, 2007; Diehr *et al.*, 1998). The use of BMI has also been questioned in those with high FFM and/ or those with postural deviations (Garrido-Chamorro *et al.*, 2009; Camhi *et al.*, 2011). Despite the many assumptions made by the use of BMI and the limitations, in this study in a group of mixed sex, healthy volunteers there is a significant relationship between BMI and percentage body fat as measured by DEXA, although no changes in BMI were evidenced either with age or RET, despite significant changes in FM and FFM being observed.

Regardless of measurement method it is accepted that in all ages central adiposity is predictive of negative health outcomes, including morbidities and premature mortality. In combination with reduced FFM, increased central adiposity is more predictive of future health outcomes in an elderly population than BMI (Zamboni *et al.*, 2005; Srikanthan *et al.*, 2009), with mortality increasing by 13% for every standard deviation increase in waist circumference (Janssen *et al.*, 2000). Based on this knowledge our hypothesis surrounding age-related changes in body composition and fat distribution being linked to increased risk of cardiovascular health risks was developed. We assessed central adiposity by examining both trunk fat (given as a pre-defined ROI by the DEXA software) and more specifically abdominal fat mass, identified from the lowest visible point of the coccyx upwards to the highest visible point of the pelvic girdle. We also assessed changes (with RET) and differences (age and gender) in the android: gynoid (A:G) ratio, a ratio of fat distribution where a lesser value (ideally <1.0) is positive for future health outcomes.

Trends in the differences and changes were not greatly different between trunk fat and abdominal fat, which is likely expected as the abdominal region is part of the trunk ROI, although they were not identical. All age-groups reduced their trunk fat following RET, while only the young and middle-aged groups reduced abdominal fat. Trunk fat only was lower in the young compared to the old when males and females were grouped and this did support our hypothesis of age-related differences in fat distribution being associated with increase CV disease risk.

When the age-groups were divided by gender the young males showed reduced trunk fat after RET with a trend for the same in the middle-aged and old groups, while the old group showed no change in abdominal fat after RET compared to all of their younger male counterparts who did. Only middle-aged females reduced either their trunk or abdominal fat. Only the young males had less trunk fat than the females, while this was true for abdominal fat in all of the age-groups again supporting our hypothesis. For both of the measurements the young males had less fat than the older males, with no age-related difference in the females, suggesting that our hypothesis involving total fat mass was correct in hypothesizing age-related fat distribution patterns linked to CV disease in males, but not in females.

The greater extent of significant changes in trunk fat compared to abdominal fat, especially for the older group where there was no change regardless of gender grouping, suggests that the positive changes in trunk fat are predominantly a result of changes in the upper trunk and limbs opposed to the abdominal region itself. There were very few differences in the A:G ratio with RET and those observed were not consistent between age and/ or gender groups. Surprisingly the middle-aged group demonstrated an increased A:G ratio after RET, as did the young females. When divided into gender groups the young and middle-aged males showed a reduced A:G ratio after RET, with the young males having a lower ratio than the middle-aged and old groups both before and after

RET. The lower ratio in the young group is expected as evidence suggests that male accumulation of android obesity begins at about 30 years of age. This also supports the higher ratio shown in middle-aged males compared to females both before and after RET.

Although few significant changes were seen in the 'markers' of central obesity, combining the increases in LMM that were seen in all age-groups with the reductions in body fat percentage and trunk fat suggests that RET can improve BC. Further support for combining information about FM and FFM in predicting future health is given by Wannamethee and colleagues who found that obesity markers such as waist circumference, BMI and waist: hip ratio were only predictive if corrected for a marker of muscle mass (Wannamethee *et al.*, 2007). This was also supported by Kim *et al* with data from the Korean Sacropenic Obesity study, where those experiencing sarcopenic obesity have a 3.2 times greater prevalence of the metabolic syndrome than non-sarcopenic obese subjects and 8.2 times more than healthy weight controls (Kim *et al.*, 2010).

It is not only muscle and fat that are affected by age and/ or activity, bone health is also a crucial component of healthy independent ageing. We hypothesized that RET would improve measures of bone quality, assessed as changes in BMD. Although we found no significant difference in BMD with age or RET when the age-groups were mixed-sex, we did find in females that there was no age-related reduction in BMD before RET but the young and middle-aged groups had higher BMD than the old after RET, suggesting that younger females have the propensity to improve their BMD by RET. An increase in BMD after RET has been previously reported by other groups in both males and females, young and old (Tsuzuku *et al.*, 1998; Bonaiuti *et al.*, 2002; Chilibeck *et al.*, 2005; Sundell, 2011). Both the middle-aged and older males had higher BMD than the age-matched female subjects both before and after RET, while this gender disparity was not evident in the young. These trends are not surprising given previous reports of reduced BMD in post-menopausal

female (Raisz, 2005) due to reductions in oestrogen after the onset of menopause.

In addition to examining muscle mass, structure and function, fat mass and distribution and bone quality, a more complete picture of the benefits of RET could have been sought by the inclusion of measurements to assess tendon mechanical properties such as tendon length, pennation angle and Young's modulus (Narici & Maffulli, 2010).

CHAPTER 4- RISK FACTORS FOR CHRONIC DISEASE

CHAPTER HYPOTHESES:

i. There are age-related declines in insulin sensitivity and glucose tolerance which can be improved by resistance-exercise training.

ii. Bio-markers for cardiovascular disease are increased with age and can be reduced by resistance-exercise training.

4.1 Introduction to chronic disease

Chronic diseases are defined as diseases that last for a long time; over 3 months as defined by the US National Centre for disease statistics. In general chronic diseases cannot be prevented by vaccination or cured by medication. Chronic diseases such as heart disease, stroke, cancer, diabetes and arthritis are among the most common, costly and preventable of all health problems in the western world (www.cdc.gov.chronicdisease). In the USA, 70% of deaths are from chronic diseases, with heart disease, cancer and stoke accounting for more than 50%. In 2005, just fewer than 1 in 2 adults in America had at least one chronic illness with this number even higher in an elderly population as the incidence of chronic diseases heightens with advancing age (Martin *et al.*, 2010; Moore *et al.*, 2012). Approximately 25% of people with chronic disease have at least one daily activity that is limited by their condition, with arthritis the most common cause (Freedman *et al.*, 2007). Physical activity is commonly cited as one of the 4 key modifiable behaviors' that can help prevent chronic disease, alongside good nutrition, smoking cessation and alcohol limitation.

4.1.1 Chronic disease and resistance-exercise training

The benefits of physical activity for reducing chronic disease risk are plentiful and it is not only body weight and/ or composition that can be affected by a net energy imbalance. When energy in exceeds energy out, the substrate induced increase in tricarboxylic acid cycle activity generates an excess of mitochondrial nicotinamide adenine dinucleotide (NADH)

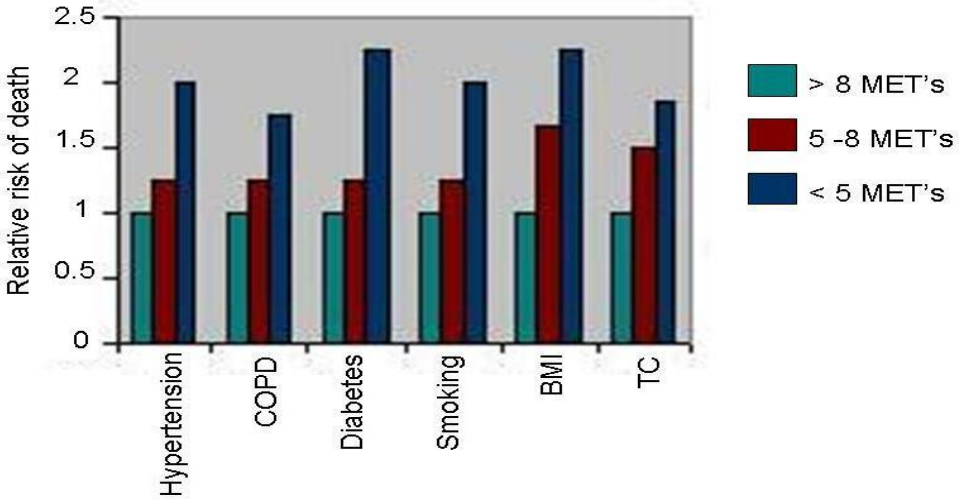
and reactive oxygen species (ROS) causing oxidative stress (Maddux *et al.*, 2001). Oxidative stress is considered to be one common factor underlying insulin-resistance, type 2 diabetes, and CVD and it may also go some way to explain the presence of inflammation in all of these conditions (Van Gaal *et al.*, 2006). Therefore, RET may be beneficial in maintaining energy balance and alleviating this problem.

With the increase in life expectancy occurring in industrialized nations, the leading causes of death have shifted dramatically from infectious diseases to non-communicable diseases and from younger to older individuals, in the main. In developed countries 75% of deaths in persons older than the age of 65 are now from CVD and cancer, two of the most common chronic diseases (Knoops *et al.*, 2004).

Physical inactivity is a modifiable risk factor for cardiovascular disease and a wide variety of other chronic diseases including type 2 diabetes, cancer (colon and breast), obesity, hypertension, osteoporosis, osteoarthritis and depression (Blair *et al.*, 1989; Lee & Skerrett, 2001; Puett & Griffin, 1994; Paffenbarger, Jr. *et al.*, 1986).

Since the early works of Morris in the 1950's (Morris *et al*, 1953; Morris & Heady, 1953) and Paffenbarger and colleagues in the 1970's (Paffenbarger, Jr. *et al.*, 1978; Paffenbarger & Hale, 1975), numerous studies have illustrated the decreased relative risk of death, both from all-cause mortality and from specific diseases, associated with increased physical activity (Blair *et al.*, 2001; Macera & Powell, 2001; Macera *et al.*, 2003; Oguma *et al.*, 2002; Lee & Paffenbarger, Jr., 2000; Lee *et al.*, 1995; Kohl, III, 2001). More recently, The HALE Project, reported increased physical activity (as defined by being in the highest tertile for physical activity according to the Voorips questionnaire) as lowering the risk of all-cause mortality by 37%, coronary heart disease by 28%, CVD by 35%, cancer by 36% and other causes by 48% (Knoops *et al.*, 2004).

Other than just lowering risk factors for chronic disease and other causes of mortality, exercise may actually have a 'protective effect'. People who have risk factors for cardiovascular disease yet who are physically active may be at lower risk of premature death than sedentary people with no risk factors for cardiovascular disease (Warburton *et al.*, 2006) (Figure 4.1).



Adapted from: Myers et al, 2002 (Warburton *et al.*, 2006b)

Figure 4.1 Relative risk of death from any cause among participants with risk factors. Chronic obstructive pulmonary disease (COPD), High total cholesterol (TC). Comparison of subjects who achieved a physical activity level of less than 5 MET's (metabolic equivalents), 5-8 MET's, or whose exercise capacity was more than 8 MET's.

Physical activity independently acts to reduce the risk of premature death and each hour of activity at one metabolic expenditure value (MET; where 1 MET = 1 kcal.kg body weight^{-1}) higher than normal is associated with an 8% decrease in CHD risk.

In the pre-face to the USDHHS Surgeons General Report, Dr. A. Manley concluded physical activity to be so directly related to preventing disease and maintaining a high quality of life, that it should be accorded the same level of attention as other physical health practices and join the front ranks of essential health objectives (US Department of Health and Human Services, 1996).

Physical activity also has the ability to have a positive effect on body composition which may further reduce this risk. Many studies have recognized that physical activity alone can only moderately attenuate, but not eliminate the effect of adiposity on health, while at the same time demonstrating that being lean cannot counteract the increased disease risk associated with physical inactivity, leading to the conclusion that a combination of both is required to offset chronic disease risk as much as possible (Hu *et al.*, 2004; Stevens *et al.*, 2002; Li *et al.*, 2006; Manson *et al.*, 1995).

Increased adiposity is an independent cardiovascular disease risk factor and also increases the incidence of other risk factors which may lead to CVD or cause premature mortality themselves, notably type 2 diabetes, dyslipidaemia, hypertension and inflammation (both local and systemic) (Manson *et al.*, 1995). Much of the disease risk associated with increased adiposity is related to the link between adipocytes and inflammation. Both adipocytes and resident macrophages generate local inflammation, synergistically stimulating the inflammatory activity of the other (Lau *et al.*, 2005).

It is not however only being overweight that has been associated with increased risk of disease and/or premature mortality, extremely low levels of body fat have also been found to be detrimental to human health, with many associated physical signs, symptoms and abnormalities (Table 4.1):

Table 4.1 Physical features of extremely low levels of body fat.

Physical features of extremely low levels of body fat
Physical symptoms: • heightened sensitivity to cold • dizziness and syncope • amenorrhea • low sexual appetite • poor sleep with early morning wakening
Physical signs: • emaciation; stunted growth • dry skin • cold hands and feet (hypothermia) • bradycardia, hypotension and cardiac arrhythmias • weak proximal muscles
Physiological abnormalities: *Endocrine* • low concentrations of leutenising and follicle stimulating hormone • low concentrations of thyroid stimulating hormone • mild increase in plasma cortisol • raised growth hormone concentration *Cardiovascular* • ECG abnormalities (prolongation of the Q-T interval) *Gastrointestinal* • Delayed gastric emptying *Hematological* • Moderate anemia *Metabolic* • Hypercholesterolaemia • Dehydration *Bone* • Osteopenia and osteoporosis (due to low bone mineral density)

Adapted from: Fairburn and Harrison, 2003 (Fairburn & Harrison, 2003)

Although the above table of features associated with extremely low levels of body fat may appear vast most are reversed by the restoration of a healthy body weight and level of fatness, which is normally achievable through healthy eating. The possible exception to this is bone mineral density and the associated risk of osteopenia and osteoporosis which if caused by sustained periods of malnourishment or extreme underweight the damage may be irreversible (Fairburn & Harrison, 2003).

Based on fat topography it has been suggested that although visceral fat correlates with CV risk factors (Despres *et al.*, 2001) and ectopic fat in organs can impair their function (Van Gaal *et al.*, 2006), subcutaneous fat, especially gluteal fat may in some circumstances have a protective effect against CV disease (Yusuf *et al.*, 2005).

Achieving a healthy body weight through lifestyle interventions is usually endorsed as the first step in improving CV health, reinforcing the prominent role of obesity in the development of metabolic syndrome and CVD. Individuals with a central deposition of adipose tissue can experience elevated morbidity and mortality from stroke, congestive heart failure, myocardial infarction and CVD (Lakka *et al.*, 2001; Kenchaiah *et al.*, 2002). Reduction of total fat mass and/or visceral fat should theoretically reverse most, if not all of the metabolic and vascular abnormalities that excess adiposity can cause (Lau *et al.*, 2005).

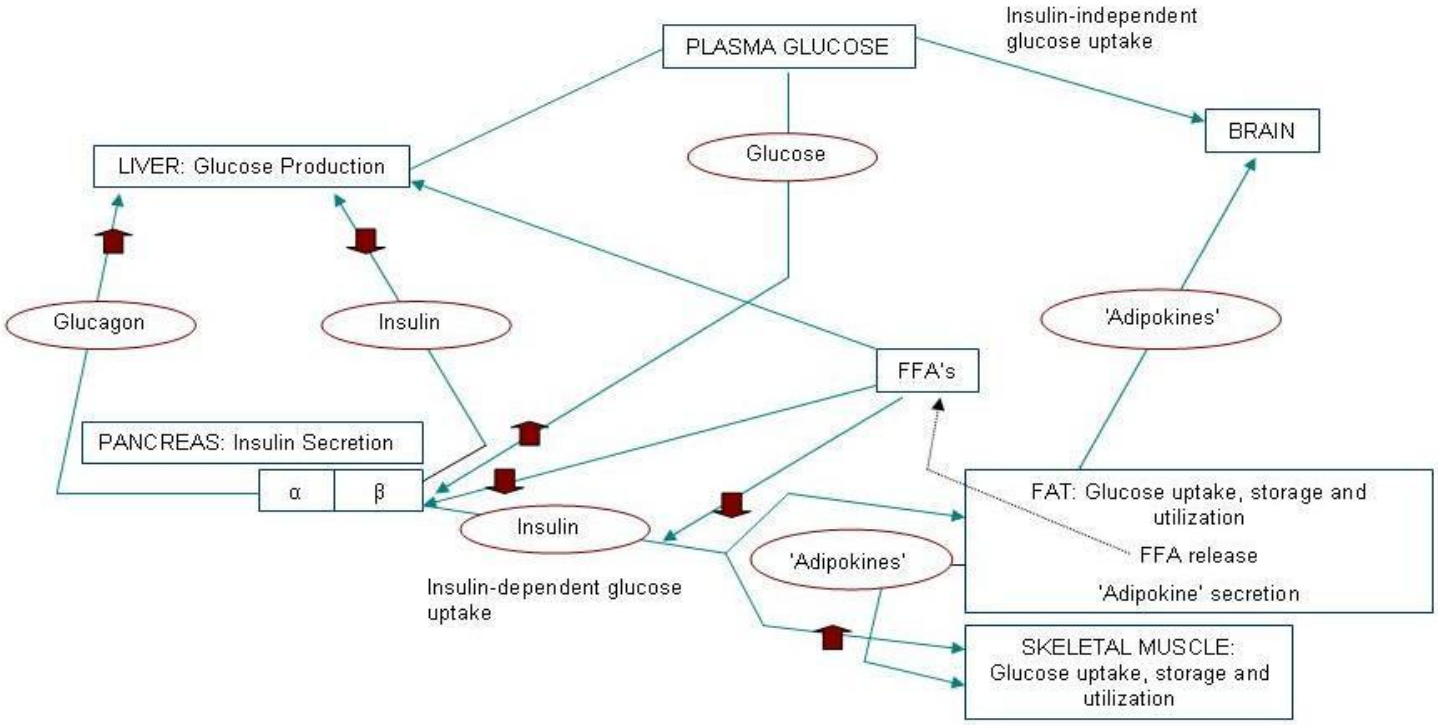
4.1.2 Type 2 diabetes

Type 2 Diabetes Mellitus (also known as non-insulin dependent diabetes (NIDDM) and adult-onset diabetes) is now one of the main threats to human health (Zimmet, 2000), accounting for 90-95% of all diabetes and affecting an estimated 6% of the adult population in Western society. Pronounced changes in the human environment, in human behavior and in human lifestyle have accompanied globalization and have resulted in escalating rates of both obesity and diabetes (Zimmet *et al.*, 2001). The pairing and mirrored increase in both of these health problems led to the adoption of the term ‘diabesity’ (Astrup & Finer, 2000), first suggested by Shafrir in 1996 (Shafrir, 1996).

Insulin sensitivity decreases with ageing (Boirie *et al.*, 2001; Pagano *et al.*, 1996) and an increase in body fat is often seen as an appealing explanation for the decline in insulin sensitivity in both the obese and in elderly individuals where body composition changes often see muscle mass diminishing and body fat increasing. In general an increase in body fat is paired with an increase of non-estrified fatty acids (NEFA’s) in plasma

which since the "glucose-fatty acid cycle" was proposed by Randle in 1963 (Randle *et al*, 1963) has been associated with insulin resistance. New research is now suggesting however that the "glucose-fatty acid cycle" was inadequate and that alterations in the metabolic function of muscle are more likely to affect insulin sensitivity within the muscle. Triacylglycerol deposition in muscle has been found to be associated with insulin resistance in a variety of subjects (Pan *et al.*, 1997; Ferrannini *et al.*, 1996; Goodpaster *et al.*, 2003) and obesity without insulin resistance is not associated with increased muscular triacylglycerol deposition suggesting that increased triacylglycerol deposition within muscle is likely to be an indicator of insulin resistance due to dysfunctional muscle lipid metabolism and independent of total body fat mass (Wolfe, 2006). Rather than an increase in FFA delivery the increased triacylglycerol deposition is more likely to be due to impaired disposal via oxidation, likely due to a decline in mitochondrial oxidative function (Petersen *et al.*, 2003). This decline may be caused by numerous factors including genetics and/ or physical inactivity.

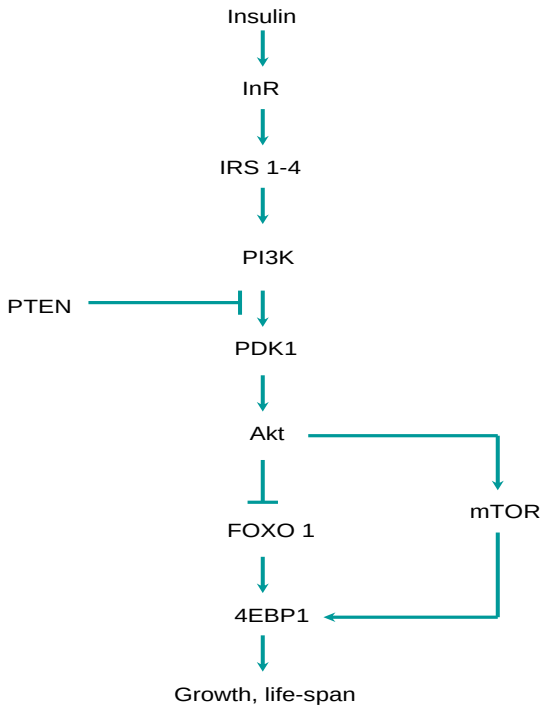
Type 2 Diabetes may occur when not enough insulin is produced and/ or when cells of the body are resistant to insulin. Daily environmental and dietary stresses overload and ultimately destroy the body's systems for regulating blood glucose (Figure 4.2). Insulin resistance correlates with a degree of excess adipose tissue, notably abdominal adiposity, and this is a strong predictor for the development of type 2 diabetes (Lau *et al.*, 2005). As outlined above adipose tissue is not the only precursor for insulin resistance and the metabolic function of muscle is central to the development of insulin resistance and ultimately type 2 diabetes (Wolfe, 2006).



Adapted from: Saltiel and Kahn, 2001 (Saltiel & Kahn, 2001)

Figure 4.2 The regulation of glucose metabolism.

When adipocytes exceed their storage capacity and the process of fat cell proliferation fails, fat begins to accumulate in tissues not suited for lipid storage, leading to the formation of specific metabolites that inhibit insulin signal transduction (Van Gaal *et al.*, 2006). The majority of these metabolites will be ceramides; metabolites of saturated fats and one of the three forms of sphingolipids. Composed of one fatty acid and one sphingosine, ceramides are found in high concentrations in the lipid bi-layer of cell membranes. Once thought of as a purely structural element it is now known that ceramides can be released from the cell membrane by enzymes and act as a signalling molecule. It is in their role as a signalling molecule that ceramides have potential to disrupt the insulin signalling pathway- crucial in the development of type 2 diabetes (Figure 4.3). It has been proposed that ceramides may block insulin stimulated tyrosine phosphorylation of insulin receptor substrate-1 (IRS-1) and its subsequent activation and recruitment of PI3K (Zundel & Giaccia, 1998), and/ or block activation of Akt (Summers & Nelson, 2005). A 1.5-2 fold increase in endogenous ceramides is enough to inhibit insulin signalling (Chavez & Summers, 2003).



Adapted from: www.biocenter.helsinki.fi/bi/piig/research.htm

Figure 4.3 Basic insulin signalling pathway.
InR= Insulin receptor, IRS= Insulin receptor substrate, PI3K= Phosphoinositide 3-kinases, PTEN= Phosphate tensin homolog, PDK1= Phosphoinositide dependent protein kinase-1, FOXO 1= Forkhead box subgroup O-1, 4EBP1= 4E Binding protein-1, mTOR= Mammalian target of rapamycin. ↓= activation, ⊥= inhibition.

Excess subcutaneous fat and steatosis can cause insulin resistance in the liver, muscles and adipose tissue. This leads to an inability for the body to suppress hepatic glucose production, impairs glucose uptake and oxidation and disables the body's ability to suppress the release of NEFA's from adipose tissue (Van Gaal *et al.*, 2006).

Skeletal muscle is probably the most important tissue for insulin dependent glucose disposal in the body (Shulman, 2000). When functioning correctly insulin increases glucose uptake in muscle (up to 75% of insulin-dependent glucose disposal) and fat cells by stimulating the translocation of the glucose transporter, GLUT4 from intracellular sites to the cell surface and also inhibits hepatic glucose production, thus serving as a primary regulator of blood glucose concentration. Insulin also stimulates cell

growth and differentiation and promotes the storage of substrates in fat, liver and muscle by stimulating lipogenesis, glycogen and protein synthesis and by inhibiting lipolysis, glycogenolysis, gluconeogenesis and protein breakdown (Saltiel & Kahn, 2001). Unfortunately for our ageing population the process' of glucose transport, glycogen synthesis and glucose oxidation all appear to be susceptible to dysregulation with age (Shulman, 2000) resulting in a higher propensity for hyperglycemia and insulin insensitivity.

In normal individuals, despite periods of fasting and feeding, plasma glucose remains in a narrow range between 4 and 7 $mmol.l^{-1}$ (Saltiel & Kahn, 2001). If the body's system for regulating blood glucose becomes deficient, dysregulation of the above processes can result in elevations of both fasting and postprandial glucose and lipid levels above those classified as normal.

As well as improving body composition and lowering the obesity-related link with insulin-resistance, physical activity can independently improve glucose tolerance and sensitivity. Many research groups have reported the ability of RET to improve glucose tolerance and insulin action, especially in older people (Albright *et al.*, 2000; Miller *et al.*, 1994). This improvement may be due to the increased InR and Akt skeletal muscle content (Figure 4.3) that occurs with RET (Holten *et al.*, 2004), suggesting that RET positively alters the skeletal muscle insulin signalling pathway (Iglay *et al.*, 2007). Koopman and colleagues found that a single bout of resistance exercise improves insulin sensitivity by ~13% (Koopman *et al.*, 2005).

By improving non-insulin dependent glucose uptake; physical activity improves the ratio between high density lipoprotein (HDL) and low density lipoprotein (LDL) cholesterol because it increases the activity of lipoprotein lipase, decreases triglycerides, increases fibrinolysis, lowers platelet aggregation, increases oxygen uptake in the heart and in peripheral tissues, lowers resting heart rate (RHR) by increasing vagal tone and

lowers resting blood pressure (Sandvik *et al.*, 1993). Irrelevant to insulin-sensitivity but important to overall health and well-being, physical activity also directly improves myocardial O_2 supply, improving myocardial contraction and electrical stability (Van Gaal *et al.*, 2006).

Elevated plasma glucose and low HDL levels are just two components of the complex 'metabolic syndrome'. Originally hypothesized by Reaven (Reaven, 1988), it encompasses a common constellation of closely linked clinical features (Table 4.2) and is considered a precursor of type 2 diabetes (Reaven, 2005).

Table 4.2 Features of the metabolic syndrome.

Metabolic syndrome features
• Fasting plasma glucose ≥ 1.1g.L^{-1}
• Android obesity; waist circumference > 102cm males > 88cm females
• Resting blood pressure ≥ 130/85 mmHg
• Triglyceride level > 1.5g. L^{-1}
• High density lipoprotein cholesterol < 0.4g. L^{-1} males < 0.5g. L^{-1} females

Adapted from: NCEP Report (NCEP-Expert Panel on Detection, 2001)

In America, it has been estimated that the metabolic syndrome affects one in four adults, making it the leading public health issue associated with increased cardiovascular disease risk (Ford *et al.*, 2002).

In 2001, Roberts and colleagues examined the effects of diet on the clinical features which compose the metabolic syndrome in a rodent study involving Fischer rats. For 20 months half of the rats were fed a high fat diet rich in refined carbohydrates (HFS) while the others were fed a low fat diet consisting of mainly complex carbohydrates (LFCC). These diets were then switched for two months (Roberts *et al.*, 2001). Although energy intake was near to constant between the two diets conversion from LFCC to HFS decreased insulin stimulated glucose transport signalling which in turn increased levels of plasma insulin, plasma triglycerides, LDL cholesterol, very low density lipoprotein (VLDL) cholesterol and total

cholesterol (TC). Body weight also increased as did blood pressure and the LDL: HDL cholesterol ratio. Conversely, conversion from HFS to LFCC led to normalization of glucose transport, blood pressure, plasma insulin and VLDL cholesterol. There was also a significant reduction in obesity. Similar findings were observed by Roberts and colleagues later, this time in human subjects where a ~50% decrease in metabolic syndrome and type 2 diabetes occurred following treatment albeit without the reduction in obesity. The treatment for this study was intense lifestyle and diet modification. The LFCC diet was light on fat, medium on protein and high in unrefined carbohydrates and fibre although not calorie restricted. Exercise was 45-60 minutes per day of treadmill walking at 70-85% of maximal heart rate. All of this was undertaken as a residential program with adherence essentially 100% (Booth & Chakravarthy, 2006). Results of these studies suggest 'syndrome X' or the 'metabolic syndrome' may be reversed with a LFCC diet when combined with regular exercise even if a significant change in body mass does not occur. This may prove a useful basis on which to develop treatment for numerous CV and metabolic disorder risk factors.

Obesity has been described as the most common cause of insulin resistance, and therefore type 2 diabetes in humans (Cavaghan *et al.*, 2000). In obese subjects, insulin levels typically increase to maintain normal glucose tolerance (Bell & Polonsky, 2001). One study observed 24-hr insulin secretion rates three to four times higher in obese subjects compared to lean controls (Cavaghan *et al.*, 2000).

The World Health Organisation (W.H.O) predicts a doubling in the incidence of type 2 diabetes in the next two decades, predominantly fuelled by affluent modern lifestyles and an increasing incidence of obesity. Epidemiological surveys suggest that indolent well-fed populations are two to twenty times as likely to develop type 2 diabetes as lean populations of the same race (Kumar & Clark, 1994).

Diabetes is caused by a failure to maintain a stable level of blood glucose in the face of normal fluctuations of supply and demand. Insulin is required for the cells to be able to utilize glucose; taking it from the blood into cells. Without the transportation of glucose from blood to the cells, the cells may be starved of energy and/ or high blood glucose levels may damage the eyes, kidneys, nervous system and diabetes-accelerated atherosclerosis leads to increased risk of myocardial infarction, stroke and limb amputation (Brownlee, 2001).

Insulin resistance, the cause of type 2 diabetes happens when the bodies tissues do not respond fully to the actions of insulin, so cannot make use of glucose in the blood. The pancreas responds by producing more insulin and the liver, where glucose is stored, releases more glucose to try and increase the amount of glucose available for the cells. As the cells become more resistant to insulin, levels of surplus glucose circulating in the blood increase.

Different factors, both sociological and physiological can increase the risk of developing type 2 diabetes. These include an android patterning of fat distribution, resulting in a high waist: hip ratio, a high BMI, a high body fat percentage, elevated blood pressure and high cholesterol levels, all factors associated with a sedentary lifestyle (Zimmet, 1999) and obesity (Fagot-Campagna *et al.*, 2001).

Apart from heightened genetic susceptibility of certain ethnic groups it is clear that environmental and behavioral factors such as a sedentary lifestyle, nutrition and obesity are very important risk factors for type 2 diabetes (Zimmet, 1992). With changes in these factors a new aspect of the type 2 diabetes epidemic is emerging; that of its increasing incidence in children, teenagers and adolescents (Fagot-Campagna *et al.*, 2001). Type 2 diabetes has already been identified in children from Japan, United States, Pacific Islands, Hong Kong, Australia and the United Kingdom (Rosenbloom *et al.*, 1999;Ehtisham *et al.*, 2000). In Japan, type 2 diabetes is now more common than type 1 in children, accounting for 80% of

childhood diabetes; an incidence that almost doubled between 1976 and 1995 (Kitagawa *et al.*, 1998).

The many aetiological determinants and risk factors of type 2 diabetes are outlined below (Table 4.3).

Table 4.3 Aetiological determinants and risk factors of type 2 diabetes.

Aetiological determinants and risk factors of type 2 diabetes
Genetic factors • Genetic markers • Family history
Demographic characteristics • Sex • Age • Ethnicity
Behavioural and lifestyle related factors • Obesity (including duration and fat distribution) • Physical inactivity • Diet • Stress • Westernisation/ urbanisation/ modernisation
Metabolic determinants • Impaired glucose tolerance • Insulin resistance
Pregnancy-related determinants • Gestational diabetes • Diabetes in offspring of women with diabetes during pregnancy • Intra-uterine mal or over nutrition

Adapted from: Zimmet et al, 2001 (Zimmet *et al.*, 2001)

An excellent model of the phenomenon that has accompanied modernization exists with the Israeli sand rat (*Psammomys obesus*). When this animal was removed from its natural environment and given an abundant high-kcal diet, it developed both type 2 diabetes and obesity (Zimmet, 1999). The changed lifestyle experienced by the rat emulates the lifestyle that is now experienced by humans as we progress further into the 21st century, as we now consume diets with an excess of energy, simple carbohydrates and saturated fats while at the same time performing less physical activity both leisure-based and related to occupation (Dowse & Zimmet, 1993).

The completed Diabetes Prevention Program (1999) in the United States showed that, over three years, lifestyle intervention targeting both diet and exercise reduced the risk of progression from impaired glucose tolerance (IGT) to type 2 diabetes by 58%. Similar results for other lifestyle interventions have also been shown (Pan *et al.*, 1997; Tuomilehto *et al.*, 2001). Although the results of these studies have been positive, several studies have provided evidence that approaches aimed at high risk individuals (for example, those with IGT) may not be enough to prevent all type 2 diabetes (Zimmet *et al.*, 2001), with data from the UK Prospective Diabetes Study Group (UKPDS) indicating that pancreatic β-cell function is already substantially reduced prior to clinical diagnosis of type 2 diabetes (UK Prospective Diabetes Study Group, 1995; 1998).

IGT is an intermediate category between 'normal' and type 2 diabetes relative to glucose levels. This is diagnosed by an oral glucose tolerance test where fasting blood glucose is below 6.7 $mmol.l^{-1}$ and when the sample taken 2-hours after ingestion of 75g of glucose is between 6.7 and 10.0 $mmol.l^{-1}$. This is compared to the diagnosis of type 2 diabetes where the fasting values are over 6.7$mmol.l^{-1}$ and when the 2-hour value is over 10.0$mmol.l^{-1}$ (Kumar & Clark, 1994).

Both aerobic and resistance exercise have been shown to be associated with a decreased risk of type 2 diabetes (Helmrich *et al.*, 1994; Manson *et al.*, 1992; Helmrich *et al.*, 1991). In one large prospective study (Helmrich *et al.*, 1991) each increase of 500kcal in energy expenditure per week was associated with a decreased incidence of type 2 diabetes of 6%. This benefit was particularly evident in those suffering some of the risk factors for type 2 diabetes (Table 4.2).

Resistance training may be of benefit in relation to diabetes as high levels of adipose tissue and low skeletal muscle mass will decrease the bodies' ability to handle an exogenous glucose load; potentially diverting glucose which could have been oxidized by skeletal muscle into fat and liver stores. Resistance exercise training which stimulates skeletal muscle

hypertrophy may be able to prevent and/ or reverse this situation allowing the body to better cope with exogenous glucose. In a study by Cuff et al., the addition of RET to an aerobic training regime enhanced glucose disposal (that was not improved by aerobic training alone) and was associated with a loss of abdominal subcutaneous and visceral adipose and increased muscle density (Cuff *et al.*, 2003).

Glucose deposition in skeletal muscle in older adults is determined by decreased amounts and requirement of the GLUT-4 glucose transporters, decreased insulin receptors, decreased glycogen synthase activity and decreased glucose oxidation. Even a small amount of exercise (endurance or resistance training) increases the storage capacity of skeletal muscle and the activities of the fore mentioned factors regulating glucose deposition. These changes have been associated with increased glucose tolerance, meaning the body has a greater ability to metabolize glucose, and decreased insulin resistance.

This study may go some way to illustrate the beneficial effect that one type of exercise training can have on a primary risk factor for type 2 diabetes, however, with the changes in work patterns from heavy labour to sedentary, the increase in computerization and mechanics, improved transport (Zimmet, 2000), high energy, high-fat diets and the lack of leisure time activity there is an urgent need to address these problems both socially and politically to aid in the prevention of type 2 diabetes (McKinlay & Marceau, 2000).

In conclusion, it must be accepted that type 2 diabetes is not just a disease but a symptom of a much larger global problem- the effect environmental and lifestyle changes are having on human health (Zimmet, 2000).

4.1.2.1 Pathophysiology of type 2 diabetes

Skeletal muscle is the tissue responsible for the majority of insulin-dependent glucose disposal in the body (Shulman, 2000). Glucose transport, glycogen synthesis and glucose oxidation all appear to

deteriorate with advancing age (Krishnan *et al.*, 2003; Houmard *et al.*, 1995; Gumbiner *et al.*, 1992), resulting in hyperglycaemia, insulin resistance and/ or insulin insensitivity. It is not however ageing *per se* that may be responsible for the age-related decline in insulin-sensitivity that is observed, it may be the decreased physical activity (Ryan, 2000), changed body composition (Boden *et al.*, 1993) or chronic low-grade inflammation (Bruunsgaard & Pedersen, 2003), all of which are related to ageing, that contribute the insulin resistance experienced (Festa *et al.*, 2000).

4.1.2.1.1 Glucose

The importance of glucose in the pathogenesis and development of type 2 diabetes has been outlined above. As a monosaccharide, glucose is an important carbohydrate and is used by living cells as a source of energy (Figure 3.2). Commonly referred to as blood sugar in lay literature, blood glucose level is normally controlled between 4- 6 mmol.L^{-1}, equating to a level in the circulating blood of ~3.3-7g if an average adult blood volume of 5 liters is assumed. Insulin is not the only anabolic hormone that can affect blood glucose; human growth hormone (HGH), glucagon and catecholamine's can all increase blood glucose levels. Fasting blood glucose levels, especially when combined with fasting insulin levels and entered into the homeostasis model assessment (HOMA) equation can serve as a good predictor of insulin sensitivity, and even independently can serve as a guide to the risk of IGT and/ or type II diabetes.

4.1.2.1.2 Insulin

Insulin resistance may be calculated using the homeostasis model assessment (HOMA) equation (Matthews *et al.*, 1985) and/ or the more recent quantitative insulin sensitivity check index (QUICKI) (Katz *et al.*, 2000) (see below), suitable for use in subjects with no baseline symptoms of IGT (i.e. Normal fasting blood glucose levels >4, <7mmol.l^{-1}). QUICKI and HOMA have been described as the most accurate surrogate methods of assessing insulin sensitivity, with QUICKI described as having excellent linear correlation with insulin sensitivity assessed by means of a glucose clamp (Chen *et al.*, 2005). Insulin sensitivity and insulin resistance may be

used as precursors of type 2 diabetes, a disease commonly associated with adults, and in particular the elderly, but now growing more prevalent in all age-spectrums of the population.

$$\textbf{HOMA} = \frac{\text{Glucose (mmol.L}^{-1}\text{) x Insulin (µU.mL}^{-1}\text{)}}{22.5}$$

$$\textbf{QUICKI} = \frac{1}{\text{Log fasting insulin (µU.mL}^{-1}\text{) + Log fasting glucose (mg.dL}^{-1}\text{)}}.$$

To obtain glucose in $mg.dL^{-1}$, the conversion from the common measurement unit of $mmol.L^{-1}$ is:

$$\textbf{mg.dL}^{-1}\textbf{ x 0.55 = mmol.L}^{-1}$$

HOMA equation values above 2.35 for men and 1.88 for women are deemed to be indicative of the metabolic syndrome and therefore detrimental to human health (Balkau & Charles, 1999). Lower HOMA scores are positive as the score is representing insulin resistance while QUICKI scores represent insulin sensitivity so elevated values should be interpreted as positive. Hrebicek and colleagues found QUICKI values below 0.357 to be distinctly linked to the identification of patients with manifestations of the metabolic syndrome and insulin resistance (Hrebicek *et al.*, 2002); although they did recommend that values would be lab dependent as formal reference values were not available.

4.1.2.2 Implications of type 2 diabetes

The International Diabetes Federation reports that 366 million people worldwide have diabetes (~8.5% of the global population) and that this figure will have increased to 522 million by 2030 (www.diabetes.org/). In the UK alone, 2.9 million people have been diagnosed as diabetics with an estimated 500'000 undiagnosed sufferers (www.diabetes.org.uk/). Type 2 diabetes can have considerable negative effects on the vasculature and can lead to devastating microvascular complications (He & King, 2004) such as retinopathy (Kempen *et al.*, 2004; Saaddine *et al.*, 2008), nephropathy

and neuropathy, which in the most serious of cases may lead to blindness, renal disease/ failure and amputation, respectively (Natarajan *et al.*, 2012). Type 2 diabetes also increases cardiovascular mortality risk 5 times in males and 8 times in females, associated with coronary artery disease, atherosclerosis, hypertension and stroke (Beckman *et al.*, 2002) as well as being linked to increased infection susceptibility (Natarajan *et al.*, 2012). The increased incidence of these complications in type 2 diabetics has been linked to hyperglycemia, hyperlipidemia, advanced glycation end products, growth factors and pro inflammatory cytokines (Natarajan *et al.*, 2012; Natarajan & Nadler, 2004; Brownlee, 2001; Libby, 2002).

4.1.2.3 Resistance-exercise training and type 2 diabetes

Skeletal muscle is an important determinant of insulin sensitivity (Shulman, 2000) and in many studies RET has enhanced insulin sensitivity and improved glucose tolerance (Tresierras & Balady, 2009). In a meta-analysis of 9 randomized control trials containing 372 subjects with type 2 diabetes, progressive RET led to absolute reductions of HbA1c (Irvine & Taylor, 2009), something that was also shown by Bweir et al., (Bweir *et al.*, 2009) using a training regime not dramatically different from the one used in this study. Many studies assessing the efficacy of different exercise regimes use HbA1c as a marker of improvement in diabetic status.

HbA1c is the abbreviation for glycated hemoglobin and is a useful tool for assessing average blood glucose in the past 2-3 months (Sigal *et al.*, 2007). HbA1c is a form of hemoglobin produced in a non-enzymatic glycation pathyway by hemoglobins exposure to plasma glucose. The American Diabetes Association define diabetic HbA1c levels as >48mmol/l or 6.5%. A 1% increase in HbA1c is associated with a 15-20% increase in major cardiovascular events and a 1% decrease with a 37% reduction in microvascular complications (Sigal *et al.*, 2007).

Whilst there are no studies that show adverse effects of RET on Type 2 diabetes the literature is not entirely consistent. Based on the self-scoring SF-36 scale RET was found to be better than aerobic training at improving

scores of health status in a multi-ethnic Asian diabetic cohort (Ng *et al.*, 2011) and Minges and colleagues reported reduced obesity and improved physical function in diabetics after RET (Minges *et al.*, 2011). Studies using the HbA1c marker are less unanimous in their support for RET alone. In 2007 Sigal *et al* reported that 6 months RET significantly reduced HbA1c (by 0.38%), waist circumference and abdominal subcutaneous fat but concluded that greater results were achieved through a combination of RET and aerobic training (Sigal *et al.*, 2007). These results are however difficult to interpret as the combined group performed the full aerobic and RET regimes, therefore doubling the workload and exercise time of either of the single-mode interventions. These improvements in HbA1c were also found by Castaneda and colleagues following 16 weeks progressive RET where not only was HbA1c reduced by 1.1% but muscle glycogen stores were increased by ~20mmol glucose per kg muscle and the prescribed dose of diabetic medication was reduced in 72% of exercisers (Castaneda *et al.*, 2002). Also in agreement with the work of Sigal was an increase in lean muscle mass and a decrease in trunk fat mass with a further reported benefit of reduced systolic blood pressure. In older diabetics HbA1c was also reduced with RET and this was again paired with an increase in lean muscle mass, the benefits of which have already been discussed earlier in this work (Dunstan *et al.*, 2002). Church and colleagues however, found that only a combination of RET and aerobic exercise was able to reduce HbA1c and that this combination-training also improved VO_2 max. They did conclude that in relation to body composition both modes of exercise and the combination were equivocal in reducing waist circumference and fat mass.

Improved glucose tolerance and/ or uptake after RET appears not to be simply a consequence of increased muscle mass but also a result of qualitative changes in resistance-trained muscles. A study using a unilateral model of RET found that RET enhanced insulin action in both type 2 diabetics and healthy subjects independent of increases in lean muscle mass. They concluded that increases in GLUT-4 content and insulin pathway signalling protein expression were at least part of the

mechanism behind improved insulin action following RET (Holten *et al.*, 2004).

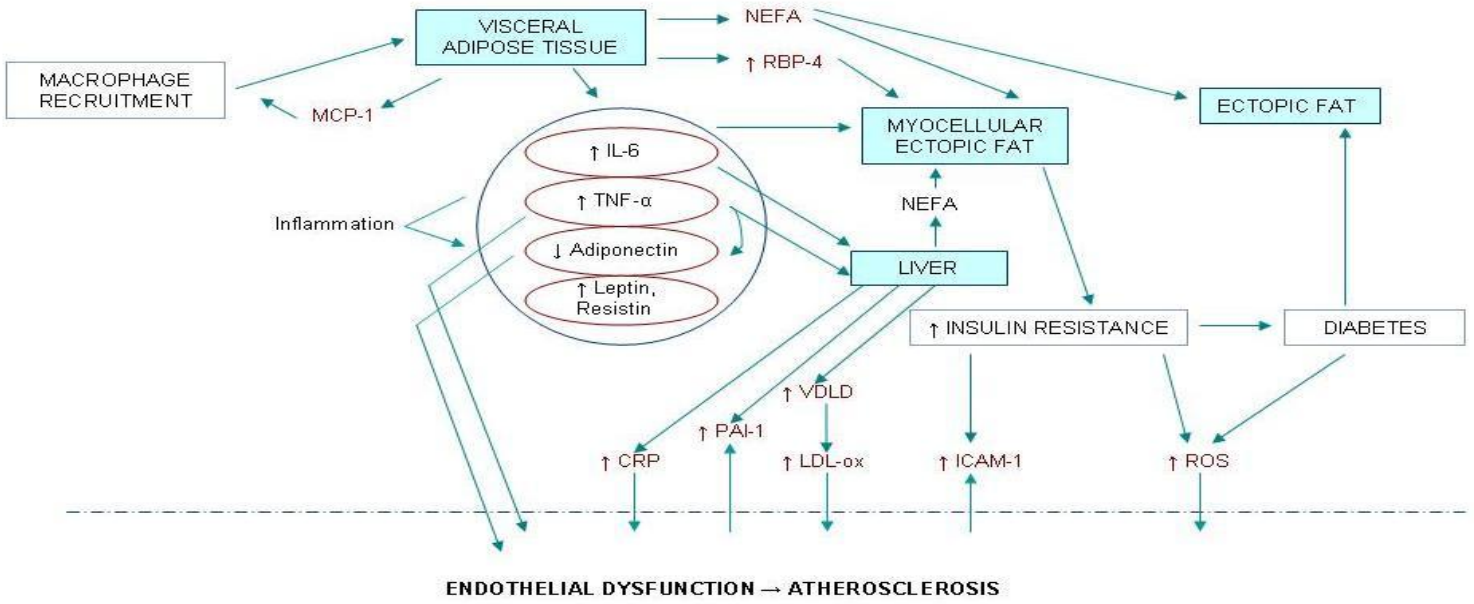
4.1.3 Cardiovascular disease

Atherosclerotic disease, often cited as the major cause of CVD remains the leading cause of death in industrialized nations despite major advances in its diagnosis, treatment and prevention. The increasing epidemic of obesity, insulin resistance and type 2 diabetes is likely to add to this burden if the lifestyle changes associated with westernization are not halted or at best reversed (Lau *et al.*, 2005).

Different mechanisms linking adiposity to CVD have been postulated; adding support to already established classical risk factors and increasing the proposition of less conventional risk factors such as adipokine involvement (Van Gaal *et al.*, 2006).

Adipose tissue is an active endocrine and paracrine organ that releases large numbers of cytokines such as leptin, adiponectin, IL-6 and TNF-α for example. These products not only influence body weight homeostasis but also insulin resistance, diabetes, lipid levels, tension, coagulation, fibrinolysis, inflammation and atherosclerosis (Lau *et al.*, 2005).

As further examined later in this chapter (Figure 4.5) atherosclerosis is an inflammatory process that initially begins with endothelial dysfunction. Evidence is beginning to accumulate that suggests that adipokines may directly influence endothelial function through their pro-inflammatory properties thus suggesting a reason for the link between body composition and CVD disease risk (Figure 4.4).



Adapted from: (Van Gaal *et al.*, 2006)

Figure 4.4 Mechanisms linking adiposity with CV disease.
MCP-1: Monocyte chemo attractant protein; NEFA: Non-estrified fatty acid; RBP-4: Retinol binding protein four; IL-6: Interleukin six; TNF-α: Tumor necrosis factor alpha; CRP: C-reactive protein; PAI-1: Plasminogen activator inhibitor one; VLDL: Very low density lipoprotein; LDL-ox: Low density lipoprotein oxidation; ICAM-1: Intracellular adhesion molecule one; ROS: Reactive oxygen species.

Myocardial infarction (MI) (commonly referred to as a heart attack) is often an end stage of CVD and nine measurable and modifiable factors which could act as predictors have been identified including abdominal adiposity and the absence of regular physical activity (Yusuf *et al.*, 2004) (Table 4.4).

Table 4.4 Modifiable predictors of myocardial infarction.

Predictors of Myocardial infarction
• Low level of exercise/ physical activity
• Abdominal obesity
• Hypertension
• High ApoB: ApoA-1 ratio
• Diabetes
• Low dietary intake of fruit/ vegetables
• High dietary intake of alcohol
• Smoking
• Low psychosocial status

Adapted from: (Yusuf *et al.*, 2004)

4.1.3.1 Cytokines

Cytokines are a family of proteinaceous substances produced by various cells and critical to the functioning of immune responses, including the involved inflammatory responses. Chronic inflammation is thought to play a role in disease development and in functional decline during ageing (Jankord & Jemiolo, 2004).

TNF-α is a cytokine involved in systemic inflammation and the acute phase response of the immune process. TNF-α is released by white blood cells, endothelial cells and several other cells in the course of damage. Its release is stimulated by several other mediators such as IL-1 and it generally works with other cytokines such as IL-6 and IL-1 in various organ systems. IL-1 is a cytokine that forms part of the inflammatory response and increases the expression of adhesion factors on endothelial cells.

TNF-α can suppress appetites often causing cachexia in times of disease states, it can stimulate the liver leading to increased levels of C-Reactive

Protein (CRP) and as well as stimulating phagocytosis, TNF-α can stimulate macrophages leading to the production of IL-6, oxidants and the inflammatory lipid; Prostaglandin-E2 (PGE2). On some tissues TNF-α can lead to an increased insulin resistance, increasing the propensity of affected individuals to develop type 2 diabetes (Zhang *et al.*, 2003a).

Ageing is associated with chronic small increases in basal systemic circulating levels of inflammatory markers and many pro-inflammatory cytokines (Paolisso *et al.*, 1998; Hager *et al.*, 1994; Morin *et al.*, 1997; Cohen *et al.*, 1997) with multi-functional cytokines such as TNF-α and IL-6 associated with mortality and morbidity in the elderly (Bruunsgaard & Pedersen, 2003). In cohorts of frail, older individuals levels of TNF-α and IL-6 may be considered as disease markers. Circulating levels of TNF-α seem to be the best predictor of mortality in frail, elderly populations with a high mortality rate while IL-6 appears a better marker in healthy, elderly populations (Hager *et al.*, 1994).

High levels of serum inflammatory mediators have been associated with numerous disease states including atherosclerosis, type 2 diabetes, hypertension, depression and overall mortality (Kohut *et al.*, 2006). Evidence supports the direct role of TNF-α in the development of atherosclerosis and type 2 diabetes in older individuals (Bruunsgaard & Pedersen, 2003). TNF-α also induces a catabolic state that causes frailty for which increased levels of circulating IL-6 also seems to be a risk factor. Recent research suggests that patients with elevated basal levels of C-reactive protein (CRP) are at increased risk of hypertension and cardiovascular disease. In 2004 Erlinger and colleagues proposed CRP also had a role to play in cancer development with significantly higher levels of serum CRP in those who went on to develop cancer compared to those who did not (Erlinger *et al.*, 2004).

Lifestyle factors such as diet and exercise may modulate levels of circulating cytokines and other inflammatory markers such as CRP. Exercise has been shown to reduce Toll-like receptor (TLR) expression,

which can reduce the release of pro-inflammatory cytokines and depress the levels of acute phase proteins (including CRP) released by the liver (McFarlin *et al.*, 2004). IL-6 and TNF-α are the main cytokines responsible for inducing CRP secretion in the liver. CRP is a marker of low grade inflammation and has been suggested to play a role in the pathogenesis of atherosclerotic lesions in humans (Libby *et al.*, 2002). CRP is an acute phase plasma protein produced by the liver and a member of the pentraxin family of proteins. Increased levels of serum CRP are found in those suffering acute inflammation and measuring serum CRP is a recognized method of marking inflammation. Elevation of CRP enhances the CHD prognosis in men with four or more features of the metabolic syndrome (see Table 4.2) (Libby *et al.*, 2002b). Some research groups have shown that aerobic exercise, but not flexibility or resistance training, reduces levels of serum TNF-α, IL-6, CRP and IL-8 independent of BMI and psychosocial factors in older adults (Kohut *et al.*, 2006), with strength training lowering TNF-α only. These findings are however contradictory to those of other groups who have shown resistance training to also have an effect on lowering serum levels of multiple pro-inflammatory cytokines including IL-6, something which is further examined by this study. A general consensus does exist to advocate exercise and dietary interventions as possible strategies to lessen inflammatory activity and improve the health status of the elderly (Bruunsgaard & Pedersen, 2003).

TNF-α has marked effects on whole body lipid and glucose metabolism (Zhang *et al.*, 2003a) and over-expression of TNF-α in adipose tissue and muscle of both humans and animals may contribute to the development of insulin resistance. Elevated levels of TNF-α mRNA and protein have been associated with both obesity and insulin resistance (Hotamisligil *et al.*, 1993) and data is accumulating to suggest that TNF-α plays a direct role in the metabolic syndrome (Petersen & Pedersen, 2005). Lowering the active levels of TNF-α and the deletion of TNF-α receptors have both been shown to improve insulin sensitivity (Hotamisligil, 1999).

TNF-α is an inflammatory cytokine released in greater quantities by obese human patients and patients with insulin resistance. TNF-α not only initiates but also supports atherosclerotic lesion formation, impairs endothelium-dependent vasodilation and promotes endothelial dysfunction which could lead to impaired blood flow, the consequences of which are discussed in detail later.

TNF-α is also thought to play a role in the skeletal muscle wasting that occurs with chronic infections, rheumatoid arthritis, cancer, ageing and other chronic diseases (Greiwe *et al.*, 2001).

Infusion of TNF-α decreases muscle protein synthesis (Garcia-Martinez *et al.*, 1993) and in cultured human myoblasts inhibits the increase in protein synthesis rate mediated by growth factors (Frost *et al.*, 1997). In vivo, TNF-α suppresses protein synthesis rate in dogs and induces skeletal muscle protein breakdown in rats (Goodman, 1991).

Numerous studies, including one by Greiwe and colleagues have found both TNF-α mRNA and levels of TNF-α protein to be higher in the elderly compared to the young (Greiwe *et al.*, 2001). In the elderly Greiwe showed that with resistance training TNF-α levels decreased, and that both strength and muscle protein synthesis rates in the elderly (aged 81+/-1) were inversely related to levels of TNF-α protein (Greiwe *et al.*, 2001). The results of this study suggests that TNF-α contributes to age associated muscle wasting and that resistance training may go some way to attenuate this process by suppressing skeletal muscle TNF-α expression.

The mechanism by which TNF-α may lead to muscle loss is not fully understood, though numerous suggestions have been proposed. One proposal is that as TNF-α has been shown to activate transcription factor NF-κB (Li *et al.*, 1998), which in turn increases inducible nitric oxide synthases (iNOS) gene expression, leading to the production of highly reactive free radicals that may cause oxidative damage to the muscle. A second proposal is that TNF-α can itself induce cell death. TNF-α has two

ubiquitously expressed cell surface receptors: Tumour necrosis factor receptor (TNFR) I and TNFR-II. TNFR-I contains an 80 amino acid intracellular 'death domain' that interacts with a series of adaptor proteins capable of triggering either apoptosis or necrosis (Ledgerwood *et al.*, 1999).

The above suggestions try to explain why TNF-α may contribute to muscle loss with ageing, although it is not obvious why this cytokine is elevated in the muscle with age. There is however consensus supporting the notion that chronic low-grade inflammation accompanies ageing (Petersen & Pedersen, 2005).

Increased skeletal muscle TNF-α may reflect a decreased ability for ageing skeletal muscle to control oxidative stress. In murine studies active NF-κB has been shown to be present in the tissues of ageing mice whilst correlating with TNF-α expression (Spencer *et al.*, 1997). Ageing humans may respond the same to oxidative stress and this may affect TNF-α levels in aged human muscle.

Successful ageing probably cannot occur without exercise, the main function of skeletal muscle. Exercise studies have shown that RET decreases skeletal muscle TNF-α and increases protein synthesis, suggesting that resistance training may reduce the inhibitory effect of TNF-α on the production of new protein in the elderly. Data from Greiwe and colleagues showed that TNF-α is transcribed by human myocytes, elevated in the muscle of the elderly but decreased by exercise (Greiwe *et al.*, 2001). This study involved a frail elderly cohort where TNF-α has been shown to be the best predictor of mortality (Bruunsgaard & Pedersen, 2003).

Antagonising the effects of TNF-α through RET may delay some of the inevitable decline in skeletal muscle function faced by an ageing population. It may also help improve insulin resistance, as shown in

rodents (Ventre *et al.*, 1997), although the mechanistic details of how this improvement may occur in humans are still debated (Ofei *et al.*, 1996).

IL-6 is one of the cytokines, along with CRP most strongly associated with increased CVD risk and the prediction of future CVD or Type 2 diabetes (Lau *et al.*, 2005). The release of IL-6 mainly from abdominal adipocytes has been suggested to play a pivotal role in the relationship between oxidative stress, as discussed earlier in this chapter (see 1.3), and endothelial dysfunction (Van Gaal *et al.*, 2006). IL-6 not only contributes to CRP elevation and low grade inflammatory states but also has a close relationship with coagulation, insulin-resistance, dyslipidaemia and the fore mentioned endothelial dysfunction (Van Gaal *et al.*, 2006).

The role of IL-6 with regard to insulin resistance remains controversial; infusion of recombinant human IL-6 into resting healthy humans does not impair glucose production or uptake (Steensberg *et al.*, 2003), but when diabetic patients were given this same infusion, plasma concentrations of insulin decreased to levels comparable with age and BMI matched healthy controls, suggesting that IL-6 enhanced insulin sensitivity (Petersen *et al.*, 2005). Again controversially one recent study showed that IL-6 had no inhibitory effect on insulin action and glycogen synthesis (Weigert *et al.*, 2004), while IL-6 knock-out mice developed IGT reversed by IL-6 infusion (Wallenius *et al.*, 2002).

The majority of cytokine work is related to sepsis/ infection in which the cytokine cascade is different to that induced by exercise, especially with regard to IL-6 (Table 4.5). In the exercise induced cytokine cascade IL-6 is the first cytokine present, followed by an increase in IL-10 and IL-1ra, stimulated by IL-6 (Steensberg *et al.*, 2003). IL-6 also stimulates the release of soluble TNF-α receptors, but not TNF-α (Tilg *et al.*, 1997).

Table 4.5 Cytokine cascades in response to sepsis and exercise.

Cytokine cascades in response to sepsis and exercise	
SEPSIS	**EXERCISE**
TNF-α	IL-6
IL-1β	IL-1ra
IL-6	TNF-R
IL-1ra	IL-10
TNF-R	CRP (8-10 hrs later)
IL-10	

Adapted from: Petersen et al, 2005 (Petersen & Pedersen, 2005)

Not described as a pro or anti-inflammatory cytokine IL-6 responds to acute exercise. IL-6 is produced by muscle fibres (Petersen & Pedersen, 2005), leading it to be defined as a myokine; a cytokine produced and released by contracting skeletal muscle fibres and that exerts its effects in other organs of the body (Pedersen *et al.*, 2003). The discovery that skeletal muscle is a endocrine organ which can produce and release cytokines may be of great importance as skeletal muscle is the largest organ in the human body (Petersen & Pedersen, 2006).

There is a marked increase in circulating levels of IL-6 after exercise (Petersen & Pedersen, 2005), with increases in plasma IL-6 occurring in an exponential trend relative to exercise intensity, duration and muscle mass recruited (Febbraio & Pedersen, 2002; Pedersen & Hoffman-Goetz, 2000). The release of IL-6 post exercise is amplified by the presence of low muscle glycogen (Petersen & Pedersen, 2005).

Even moderate exercise can have large effects on IL-6 and the exercise induced increase in plasma IL-6 seems greater still in elderly individuals (Pedersen *et al.*, 2004). Data suggests that IL-6 has inhibitory effects on the previously discussed TNF-α production and that the anti-inflammatory role of IL-6 is continued by its stimulation of IL-10 production (Petersen & Pedersen, 2005).

IL-10 is often cited as the most important anti-inflammatory cytokine found within the human immune response (Opal & DePalo, 2000). Synthesized by Cluster of differentiation 4 (CD4+), T-helper 2 (Th2), monocytes and beta (β) cells, it acts as an inhibition factor against pro-inflammatory cytokines and can deactivate monocyte/ macrophage pro-inflammatory cytokine synthesis; inhibiting cytokine production by neutrophils and inhibiting NF-κB nuclear translocation (Opal & DePalo, 2000). IL-10 inhibits the production of IL-1α, IL-1β and TNF-α, all cytokines which play an important role in the activation of granulocytes, monocytes/ macrophages, natural killer cells and T and B cells (Moore *et al.*, 1993).

The possibility exists that with regular exercise, the anti-inflammatory effects of an acute bout of exercise may act to protect again chronic systemic low-grade inflammation. Although this link has not yet been proven this would offer one explanation as to how regular exercise protects against type II diabetes, atherosclerosis and other clinical conditions related to chronic systemic low-grade inflammation (Petersen & Pedersen, 2005). Decreases in TNF-α may be indicative of this and may also be representative of increased IL-10, an anti-inflammatory cytokine.

4.1.3.2 Blood pressure and heart rate

Blood pressure (or vascular pressure) is the term used to refer to the force exerted by circulating blood on the walls of the blood vessels. As circulating blood moves away from the heart through the arteries, arterioles, capillaries and veins the pressure decreases. In general the term blood pressure is referring to the pressure in the arteries and is reported in millimeters of mercury (mmHg). Two values are given to compose a blood pressure reading and are scribed as $^{A}/_{B}$ or pronounced as "A over B". "A" refers to the systolic arterial pressure (SBP), sometimes known as the peak pressure; this is the pressure occurring near the beginning of the cardiac cycle. "B" is the diastolic arterial pressure (DBP), and is the lower of the two values representing the arterial pressure at the resting phase of

the cardiac cycle. A single value, mean arterial pressure is sometimes given, this is calculated as:

Mean Arterial Pressure = ⅓ × SBP + ⅔ × DBP

Typical resting values for a healthy human adult are ~ $^{120}/_{80}$, although large individual variation occurs and even within an individual this value can significantly vary due to numerous factors including circadian rhythm, stress, nutrition, and hydration. Those values deemed normal are also age dependent with children's values often lower and higher values in the elderly.

During the actions involved in RET, be they isometric or isotonic hemodynamic responses will occur causing an increase in HR, systolic blood pressure (SBP) and systolic volume. If high intensity repetitions are being performed there is also likely to be an increase in diastolic blood pressure (DBP), with these responses greatest during the last repetitions of a set that is designed to elicit maximal exertion (Umpierre & Stein, 2007).

Chronic exercise, including RET has however been shown to help in both the short term and long term control of BP and has been advocated as a mechanism in manageing hypertension (Umpierre & Stein, 2007; Fagard, 2006). It is not only BP that chronic RET has been shown to have a positive effect on, but also on RHR. HR is used to describe the frequency of the cardiac cycle and is usually calculated as the number of contractions of the heart per minute and expressed as beats per minute (bpm). RHR for the average human heart is ~70 bpm, but as with BP this can vary greatly between individuals based on age and training status. A recent study found that a RET program of 10 weeks in duration had a significant effect on lowering RHR (Poelkens *et al.*, 2007).

Resistance training is progressively gaining recognition in CVD prevention and rehabilitation (Umpierre & Stein, 2007) and this may be due to directly lowering RHR and BP, both risk factors for CVD if elevated, or related to

the changes in BC that RT can induce, as visceral fat reduction is directly associated with lowered resting blood pressure (BP) (Van Gaal *et al.*, 2006).

4.1.3.3 Cholesterol

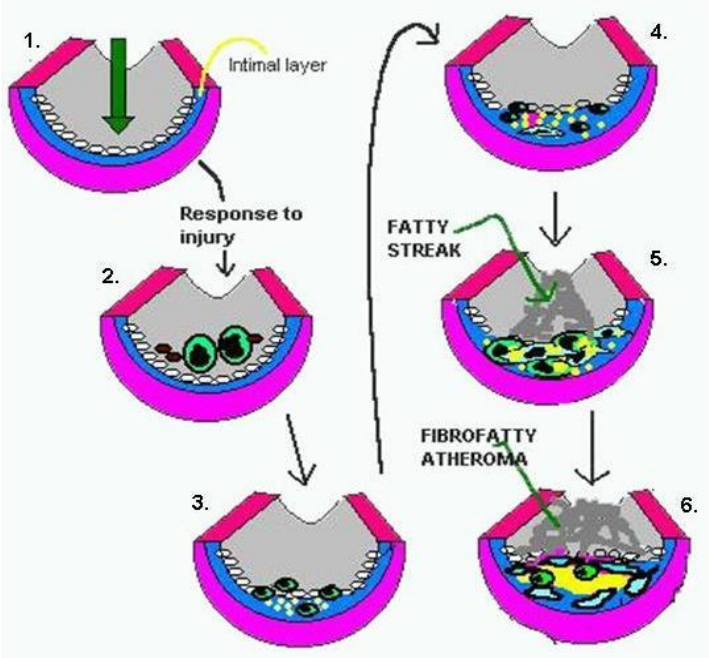
Dyslipidaemia, often associated with increased adiposity is characterized by high levels of VLDL cholesterol, triglycerides and TC. This is normally accompanied by an increase in small dense LDL particles and lowered HDL cholesterol (Howard *et al.*, 2003). Several mechanisms have been proposed as an explanation for the link between increased adiposity and low HDL cholesterol:

- Impaired lipolysis of triglyceride-rich lipoproteins (TRL) lowers HDL concentration by decreasing transfer of apolipoproteins and phospholipids from TRL to HDL.
- Delayed clearance of TRL facilitates the cholesterol ester transfer protein (CETP) mediated exchange between cholesterol esters in HDL and triglycerides in VLDL.
- Increased activity of hepatic lipase in insulin resistance states, such as that which occurs with increased adiposity, produces smaller HDL particles, leading to HDL elimination (Frenais *et al.*, 2001).
- Insulin resistance could have a direct affect on the production of ApoA-I and/ or secretion of nascent HDL from the liver (Van Gaal *et al.*, 2006).

Familial hypercholesterolaemia is generally believed to stand independently as a risk factor for CHD and this risk is substantially higher if in the presence of insulin resistance caused by abdominal adiposity (Gaudet *et al.*, 1998).

In insulin resistant states the dyslipidaemia is characterized by a different composition and distribution of LDL particles which results in an increased concentration of small dense LDL particles. LDL particles are enriched in

triglycerides, which when rapidly lipolysed by hepatic lipase (HL) leave smaller, denser LDL particles. It is the activity of both HL and CETP, both found to be increased with the metabolic syndrome, that lead to these small dense particles which are more prone to oxidation and glycation. These small dense LDL particles can move through endothelial fenestrations, entering the sub endothelial space where inflammation and transformation into plaque can occur (Kwiterovich, Jr., 2002). This modified LDL is then mostly taken up by macrophage scavenger receptors, rather than the normal LDL receptor (LDLR) pathway and it is this that induces atherosclerosis (Van Gaal *et al.*, 2006), for which the process is outlined below (Figure 4.5).



Adapted from: http://hsc.usf.edu/CLASS/jennifer/atherogenesis.htm

Figure 4.5 The atherogenic process.

1. Chronic endothelial injury leads to increased permeability and adhesion molecules.
2. Monocytes and later platelets adhere to the injured area. Lipids begin to be deposited in the area.
3. Intimal aggregation of monocytes, macrophages and lipid molecules.
4. Chemotactic and growth factors are released resulting in proliferation of smooth muscle cells by which connective tissue is formed.
5. Macrophages and smooth muscle cells engulf lipid particles and become laden with them.
6. Smooth muscle cells proliferate, some foam cells die and lipid debris is left within in the plaque. This process results in a much decreased lumen diameter.

There is conflicting data regarding the association between serum cholesterol levels and mortality from CHD. Although general consensus supports the association between high serum TC and the risk of CHD (Zhang *et al.*, 2003; Cui *et al.*, 2006; Iso *et al.*, 1989) Russian Lipid Research Clinics state that there is no association between the two, while US Lipid Research Clinics have stated that they believe there to be a strong negative association between HDL cholesterol and CHD mortality (Perova *et al.*, 1995). Much US work and policy stems from four prospective studies carried out in the 1980's in which the association between HDL

cholesterol and mortality was inverse and significant (Gordon *et al.*, 1989) results which were mirrored by Perova in 1995 when comparing HDL cholesterol and CHD in groups of both American and Russian subjects over a 12 year period (Perova *et al.*, 1995). In both the Russian and American sample of this study Perova found high levels of HDL cholesterol to have a negative association with CHD mortality, although the association was lower for the Russian sample.

Elevated levels of serum cholesterol have also been found to be associated with thromboembolic stroke (Benfante *et al.*, 1994), although this association is often underreported because CHD, also associated with high serum TC levels, occurs earlier in life and with greater frequency than thromboembolic stroke. Although often underreported research has determined strong associations between TC and the risk of ischemic, but not hemorrhagic stroke (Zhang *et al.*, 2003b).

A recent study demonstrated that 12 weeks of whole-body RET significantly decreased total and LDL cholesterol, independent of diet and sex (Iglay *et al.*, 2007).

4.1.3.4 Blood Flow

Declining vascular function is a consequence of ageing which manifests as decreased compliance in arteries and arterioles, and ultimately leads to chronic high blood pressures and coronary disease (DeSouza *et al.*, 2002; Dinenno *et al.*, 1999; Lind & Lithell, 1993). A number of studies have reported age-related decrements in large artery blood flow to limbs. For instance, elderly humans exhibited 20-30% attenuation in resting PA leg blood flow, which was attributed to a 50% increase in leg vascular resistance, compared with younger individuals (Donato *et al.*, 2006).

Declines in large arterial blood flow are not only restricted to the PA state but are also observed when older individuals are challenged with vasodilatory stimuli, such as food intake (Skilton *et al.*, 2005) and exercise (Donato *et al.*, 2006; Poole *et al.*, 2003). As adults spend ~40% of their

diurnal cycle in the postprandial state deficits in limb blood flow in this condition may impact on both glucose transportation/ disposal and also on the anabolic responses to feeding as discussed in chapter two. Skilton and colleagues demonstrated that meal-related increased in forearm vascular reactivity were impaired in both older and diabetic adults with regression analysis demonstrating that age was the principal factor in impaired blood flow in these groups (Skilton *et al.*, 2005). Furthermore, compromised exercise hyperaemia has also been demonstrated, with elderly men displaying 20-30% lower leg blood flow (LBF) and leg vascular conductance (LVC) than younger men at various exercise workloads (Donato *et al.*, 2006) of knee-extensor or whole-body bicycling (Donato *et al.*, 2006; Poole *et al.*, 2003).

Both functional and metabolic consequences of declines in LBF responses to feeding and exercise have been suggested. First, decreases in LBF during/ after exercise may explain decrements in functional performance and recovery (e.g. O_2 delivery, metabolite clearance etc. (Vincent *et al.*, 2006)) in ageing skeletal muscles. Second, as LBF regulates delivery of insulin and AA for metabolism in skeletal muscles (Clark *et al.*, 2003; Timmerman *et al.*, 2010) reduced LBF can contribute to declines in insulin sensitivity and muscle protein anabolic responses to feeding and exercise with age (Barrett *et al.*, 2009; Clark, 2008; Vincent *et al.*, 2006; Bohe *et al.*, 2001; Chesley *et al.*, 1992; Gelfand & Barrett, 1987).

As already mentioned in earlier chapters sarcopenia, and associated dynapenia are strong predictors of all cause mortality (Metter *et al.*, 2004) and age-related declines in both muscle mass and strength are associated with co-morbidities such as falls, fractures and progressive disability (Rantanen *et al.*, 1999; Roubenoff, 2000). Although of unknown aetiology, there is increasing evidence that age-related declines in vascular function may contribute to metabolic dysregulation in skeletal muscles. For instance, exercise (Vincent *et al.*, 2006), insulin (Barrett *et al.*, 2009; Clark, 2008) and mixed-meal feeding (Vincent *et al.*, 2006) have each been shown to increase muscle perfusion and each of these stimuli, which are

synonymous with MPS (Bohe *et al.*, 2001; Chesley *et al.*, 1992; Gelfand & Barrett, 1987) have a blunted effects with ageing. Further evidence for this association is shown by increased AA availability during hyperinsulinameia, which improved the muscle protein anabolic effect of insulin with pharmacological enhancement of muscle perfusion (Timmerman *et al.*, 2010).

Exercise is an established intervention for the treatment and prevention of many clinical conditions such as cardiovascular disease (CVD), sarcopenia, the metabolic syndrome and type 2 diabetes and it may also be a promising intervention to prevent the age-related decline in limb blood flow (which may be implicated in the aforementioned conditions). Improvements in limb blood flow may be beneficial, especially to the elderly, who have reduced basal limb blood flow values (Dinenno *et al.*, 1999). Precise matching of blood flow and metabolism is required for virtually all living tissues, especially so for skeletal muscle where activity status can alter metabolism considerably (Clifford & Hellsten, 2004). The results of a study involving daily aerobic exercise showed that this mode of exercise training was unable to attenuate or prevent the age related reductions in limb blood flow (Dinenno *et al.*, 2001), but more positive results have been found following RET. A cross-sectional study of males showed that the decrease in limb blood flow with age was absent in those who habitually performed RET (Miyachi *et al.*, 2005). This absence was true even when leg blood flow (LBF) was expressed relative to leg muscle mass suggesting that RET induces changes other than a larger skeletal muscle mass with greater metabolic demands (Anton *et al.*, 2006). Evidence for the benefits of exercise on LBF is present even in the young as athletes have higher LBF values than inactive age-matched individual's (Ebeling *et al.*, 1993).

In summary, age-related changes in LBF are purported to contribute to the metabolic syndrome, a major precursor to atherosclerotic disease in humans that encompasses hyperinsulinaemia, dyslipidaemia and hypertension (Lind & Lithell, 1993) and in obese subjects compromised

microvascular flow in muscle leads to poor insulin action and therefore impaired glucose uptake and storage (Keske *et al.*, 2009; Baron *et al.*, 1990). Taking all this data together it is likely that methods for maintaining both resting PA LBF and 'acute' responses in LBF to vasodilatory stimuli, such as exercise and feeding represent an important avenue for preserving both muscle and CV health with ageing.

4.2 Methodology

4.2.1 Glucose concentrations

In keeping with most modern laboratories and recent reports plasma glucose levels were measured on a blood glucose analyzer (ILAB 300 Plus Clinical Chemistry System, UK). Bloods for this analysis were collected in Lithium Heparin tubes and then centrifuged at 4°C at 3200 rpm for 20 min before plasma collection.

4.2.2 Insulin concentrations

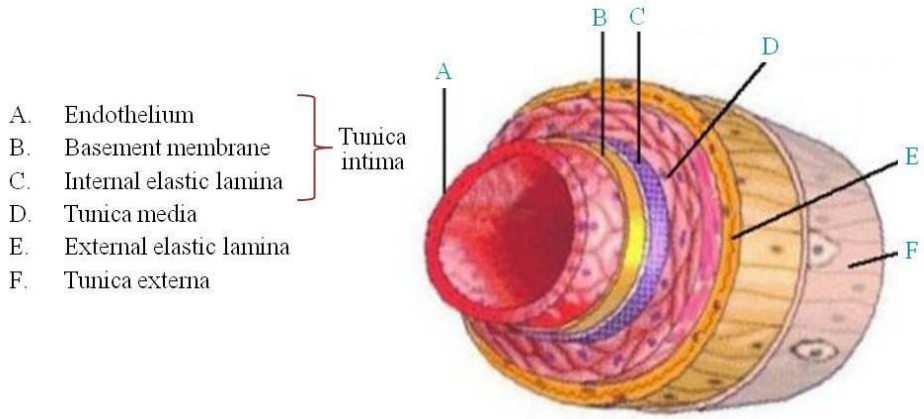
Insulin levels were measured using high sensitivity insulin Enzyme-Linked ImmunoSorbent Assay (ELISA) systems (Immunodiagnostic systems limited (IDS)). Bloods for this analysis were collected in Ethylenediaminetetraacetic Acid (EDTA) tubes, and then centrifuged at 4°C at 3200 rpm for 20 min before plasma collection.

All Enzyme-Linked ImmunoSorbent Assay (ELISA) and Enzyme Immunoassay (EIA) systems mentioned in this chapter were processed according to manufacturer's protocol guidance. In brief, monoclonal antibodies, directed against distinct epitopes on the test hormone, were pre-bound to a 96-well plate, to which the calibrators and samples were added prior to a secondary antibody labeled with horseradish peroxidase (HRP). Any unbound antibody was washed off the plate before a chromogenic solution, which reacts with HRP was added. The reaction was stopped with hydrochloric acid (HCL) and the optical density read at 450nm against a reference filter set at 650nm (Multiskan Ascent plate reader, Ascent

software V 2.6, Thermo Scientific, UK) to generate a 4 parameter logistic curve from which the sample values could then be read.

4.2.3 Leg blood flow measurements

Blood flow was measured by Doppler ultrasound (Toshiba Nemio-17). Performing Doppler ultrasound measurements on both legs (rested and exercised), for the duration of this study at various time-points allowed us to observe changes in basal leg blood flow (Basal) before and after the RET; leg blood flow responses to feeding (Fed) and leg blood flow response to the combination of exercise-plus-feeding (Ex+Fed). All Doppler ultra-sound measurements were taken with the subjects in the supine position, with dimmed lighting and no visual or audio stimuli. A single 5 MHz frequency probe (Toshiba Nemio-17) was used to measure common femoral artery mean blood velocity (MBV) and arterial lumen diameter (Figure 4.6).



Adapted from: http://www.fau.pearlashes.com/anatomy

Figure 4.6 Artery structure.

The common femoral artery extends from the external iliac artery, the artery from where the majority of blood supply to the lower limbs stems from with the exception of branches from the internal iliac artery which contributes to the supply of the gluteal region. The external iliac artery descends from the common iliac artery, which descends from the abdominal aorta and passes under the inguinal ligament (Figure 4.7a), at the mid-inguinal point to enter the thigh region, where it is re-named the femoral artery. The femoral artery lies first in an area known as the

‘femoral triangle’ (Figure 4.7b), which is defined by the inguinal ligament above, the medial border of the *sartorius* and the medial border of the *adductor longus*. In the femoral triangle the common femoral artery bifurcates, giving off a large branch (profunda femoral artery) which arises from the back of the femoral artery and then runs distally passing deeply between *adductor longus* and *adductor magnus* (Figure 4.7c). The deep femoral artery forms the principle supply to the thighs giving off medial and lateral circumflex arteries and perforating arteries. On leaving the ‘femoral triangle’ the femoral artery passes beneath *sartorius* and descends along the medial aspect of the thigh lying on *adductor magnus* and *adductor longus*. The femoral artery then passes through an opening between the part of *adductor magnus* attached to *linea aspera* and the part attached to the *adductor tubercle* to reach the popliteal fossa at the back of the knee where it is renamed the popliteal artery. Throughout its descent the femoral artery is accompanied on its medial side by the femoral vein (Figure 4.7d). Both lie together within a femoral sheath composed of a prolongation of the fascia lining the abdominal cavity.

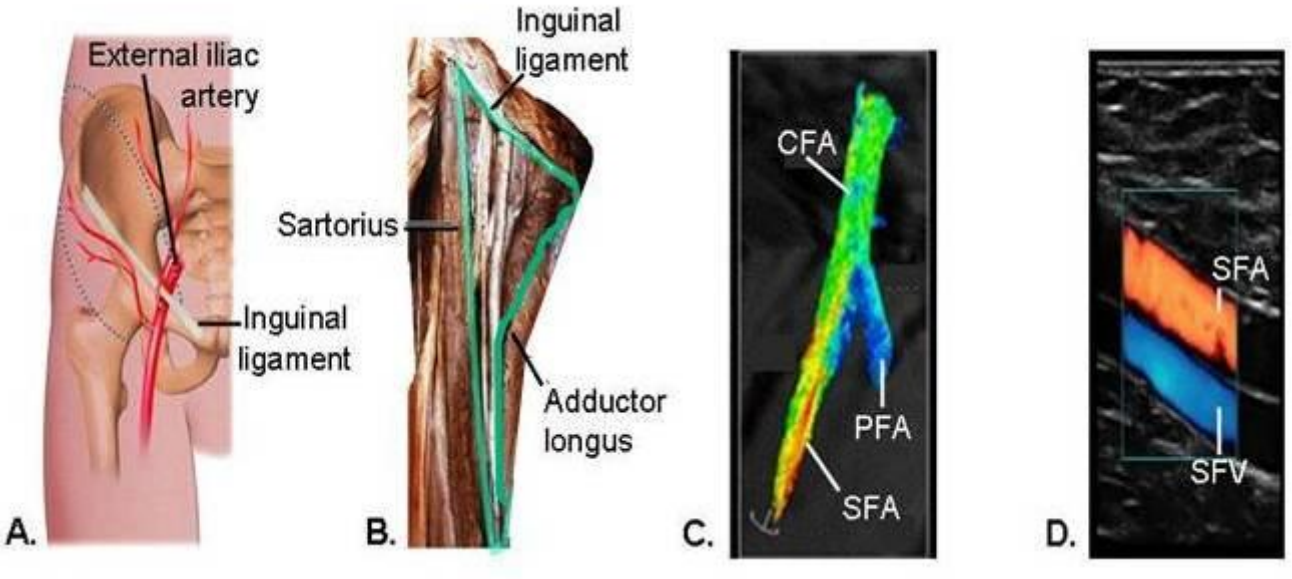


Figure 4.7 Femoral artery characteristics.

A. Relative position of the external iliac artery and inguinal ligament; Adapted from: www.maitrise-orthop.com
B. Boundaries of the 'femoral triangle'; Adapted from: www.gl.ac.uk/ibls/tutorial/anatomy/femoralt.html
C. A 3-dimensional contrast-enhanced magnetic resonance angiography showing the common femoral artery (CFA) bifurcation to superficial femoral artery (SFA) and profunda femoral artery (PFA); Adapted from: www.ensight.com/images/stories.html
D. An ultrasound image to show the relative proximity of the femoral artery and vein; Adapted from: www.altrasonix.com/vascular_04.jpg

Measurements were performed ~2-3cm proximal to the bifurcation of the femoral artery to minimize turbulence from the bifurcation. The insonation angle was <60° to allow a cosine >0 for the calculation of blood flow. Arterial lumen diameter was measured by video calipers for each measurement and defined as the distance between the media-adventitia interface of the near wall and the lumen-intima interface of the far wall of the vessel. Ultrasound images were printed (Mitsibushi P93), and calculated femoral leg blood flow values were analyzed. Femoral leg blood flow (LBF) was calculated as:

$$\textbf{LBF (l.min}^{-1}\textbf{) = MBV (cm.sec}^{-1}\textbf{) x } \pi \textbf{ x (femoral artery radius (mm))}^{2}\textbf{/1000 x 60}$$

Basal LBF measurements using the technique described above were taken at 70, 90 and 110 minutes after the study began, with a mean value from three measurements taken on each leg uses to obtain the basal value. There were no significant differences between the three measurements or between the legs. The subjects maintained a supine rested position from the beginning of the study until these time points and had been fasted for ~14 hours with the exception of water. They had not performed any exercise, other than activities of daily living in the 72 hrs prior to the study.

Fed blood flow measurements were taken 100, 120 and 140 minutes after feeding began. At the first fed blood flow measure subjects had consumed 5 of the 6 intermittent oral feeds which supplied energy at 4.25 times the subjects basal metabolic rate, calculated by standard equations (Schofield, 1985), with 15% of the total energy being supplied as protein. This included a 3-times bolus as the first feed and four further feeds. The leg that these measurements were taken from had been completely rested for the duration of the study with the exception of having to reposition themselves onto the leg extension machine.

Blood flow measurements to assess the response to the combination of exercise-plus-feeding were taken at the same time points as those for the

response to feeding alone; 100, 120 and 140 minutes after feeding began and completion of the exercise; 6 x 8 sets of unilateral leg extensions at 75% 1-RM, followed by 2 x 10 sets of calf-raises.

Using the Doppler ultrasound technique we found a coefficient of variation of 9% for three independent measures under each condition assessed, suggesting that we could reliably detect changes of ~18% of the basal value.

4.2.4 Muscle protein concentrations by immunoblotting

Muscle biopsies (~10-20 mg) were homogenized with scissors in ice-cold extraction buffer (10 μl/mg^{-1}) containing 50 mM Tris-HCl (pH 7.4), 0.1% Triton X-100, 1 mM EDTA, 1 mM EGTA, 50 mM NaF, 0.5 mM activated sodium orthovanadate (all Sigma Aldrich, Poole, UK) and a complete protease inhibitor cocktail tablet (Roche, West Sussex, UK). Homogenates were rotated on a Vibramax for 10 min at 4°C and centrifuged at 10'000 g for 10 min at 4°C before recovery of supernatants representing sarcoplasmic fractions. Bradford assays were used to determine sarcoplasmic protein concentrations after which samples were standardized to 1 mg/ml^{-1} by dilution with Laemmli loading buffer in order to measure relative protein concentrations of our proteins of interest. Samples were mixed and heated at 95°C for 5 min before fifteen μg of protein/lane was loaded on to Criterion XT Bis-Tris 12% SDS-PAGE gels (Bio-Rad, Hemel Hempstead, UK) for electrophoresis at 200 V for ~60 min. Gels were equilibrated in transfer buffer (25 mM Tris, 192 mM glycine, 10% methanol) for 30 min before proteins were electroblotted on to 0.2 μm PVDF membranes (Bio-Rad) at 100 V for 30 min. After blocking with 5% low-fat milk in TBS-T (Tris Buffered Saline and 0.1% Tween-20; both Sigma-Aldrich, Poole, UK) for 1 h, membranes were rotated overnight with primary antibody (all AbCam, Cambridge, UK) against our target proteins at a concentration of 1:2000 at 4°C. Membranes were washed (3×5 min) with TBS-T and incubated for 1 h at room temperature with HRP-conjugated anti-rabbit secondary antibody (New England Biolabs,

UK), before further washing (3×5 min) with TBS-T and incubation for 5 min with ECL reagents (enhanced chemiluminescence kit, Immunstar; Bio-Rad). Blots were imaged and quantified by assessing peak density after ensuring bands were within the linear range of detection using the Chemidoc XRS system (Bio-Rad, Hemel Hempstead, UK). Where required, phosphorylation of signalling proteins was corrected for loading anomalies to an appropriate loading control.

4.2.5 Cytokine concentrations

Circulating plasma levels of IL-6, IL-10 and TNF-α were measured using Luminex xMAP® technology. The Luminex system uses bead-based multiplexing to allow up to 100 analytes to be measured from a single sample in a traditional 96-well plate.

In brief, 5.6 micron polystyrene microspheres which are internally dyed with red and infrared flurophones, are colour coded into 100 distinct sets. Each bead set is coated with a reagent specific to a particular bioassay (e.g. IL-6), to allow the capture and detection of specific analytes from a sample. The Luminex analyser then emits lasers which excite the internal dyes that identify each microsphere particle, and also any reporter dye captured during the assay. Multiple readings are made on each bead set to improve the validity of the results.

4.2.6 Blood pressure and resting heart rate

Blood pressure and RHR were taken prior to both acute studies. Subjects were in a rested state for 20 minutes prior to the measurements and are un-stimulated throughout. Measurements were taken on an OMRON blood pressure machine (OMRON Healthcare Inc), with 3 measurements taken at each session and the mean value calculated. Mean arterial blood pressure (MAP) was calculated from these values using the formula:

MAP = (⅔ Diastolic blood pressure) + (⅓ Systolic blood pressure)

Blood pressure was also recorded during the acute studies at the same time as Doppler blood flow measurements were made. MAP calculated from blood flow measurements (as shown above) was used to calculate leg vascular conductance (LVC) and leg vascular resistance (LVR):

$$\mathbf{LVC\ (l.min^{-1}{\cdot}100mmHg^{-1}) = LBF\ (l.min^{-1})/MAP\ x\ 100}$$

$$\mathbf{LVR\ (l.min^{-1}{\cdot}100mmHg^{-1}) = MAP/Q/LBF\ (l.min^{-1})}$$

4.2.7 Cholesterol profiles

Fasted serum cholesterol profiles were produced by the clinical chemistry department at Derby City General Hospital, UK by analysis of blood samples collected in serum separator tubes (SST). Bloods for this analysis were collected and then centrifuged at 4°C at 3200 rpm for 20 min before serum collection. Profile results reported TC, LDL cholesterol and HDL cholesterol.

4.3 Results

4.3.1 Risk factors for type 2 diabetes

4.3.1.1 Glucose handling and uptake

There were no significant differences in the postprandial plasma glucose area under the curve (AUC) between the age groups either before or after RET. The young group had a reduced postprandial plasma glucose AUC after RET (43.64±1.31 vs. 39.82±1.20, $P<0.01$). The middle-aged and old groups AUC were unaffected by RET (Figure 4.8 and 4.9).

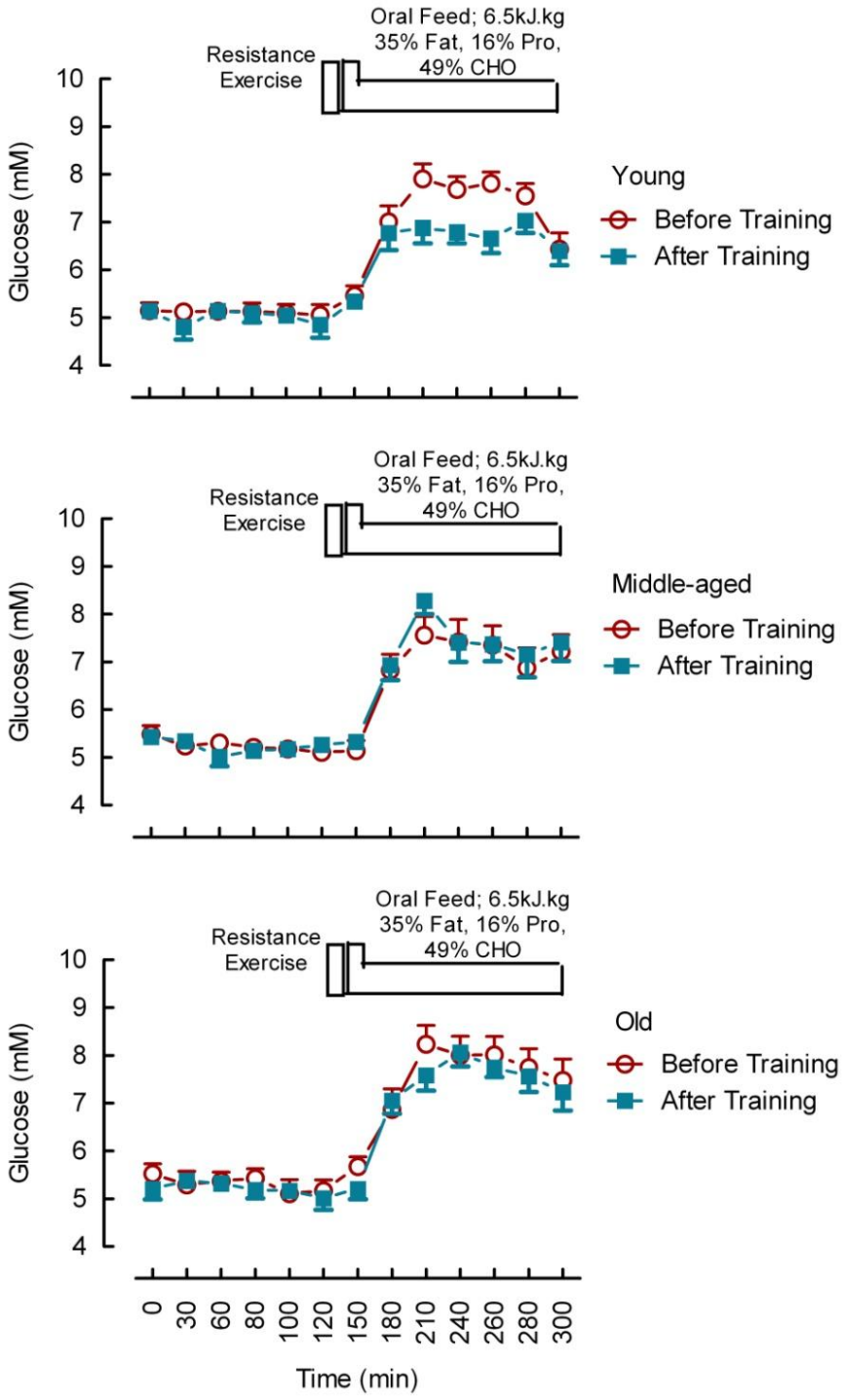


Figure 4.8 Time-course of postabsorptive and postprandial plasma glucose values in young, middle-aged and older subjects before and after RET. Values are means±SEM. Statistical analysis via ANOVA with Bonferroni post analysis on AUC values.

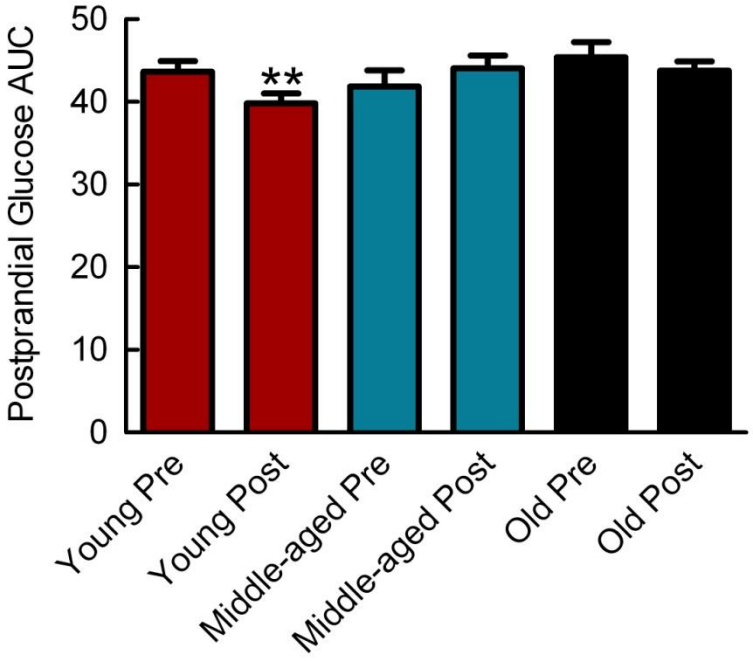


Figure 4.9 Postprandial glucose AUC in young, middle-aged and older subjects before and after RET. Values are means±SEM. Statistical analysis via ANOVA with Bonferroni post analysis and students t-test. **=P<0.01 vs. the same group before RET.

There were no significant differences in fasting glucose values between the age-groups either before or after RET. RET did not change the fasting glucose values in any of the age-groups (Figure 4.10).

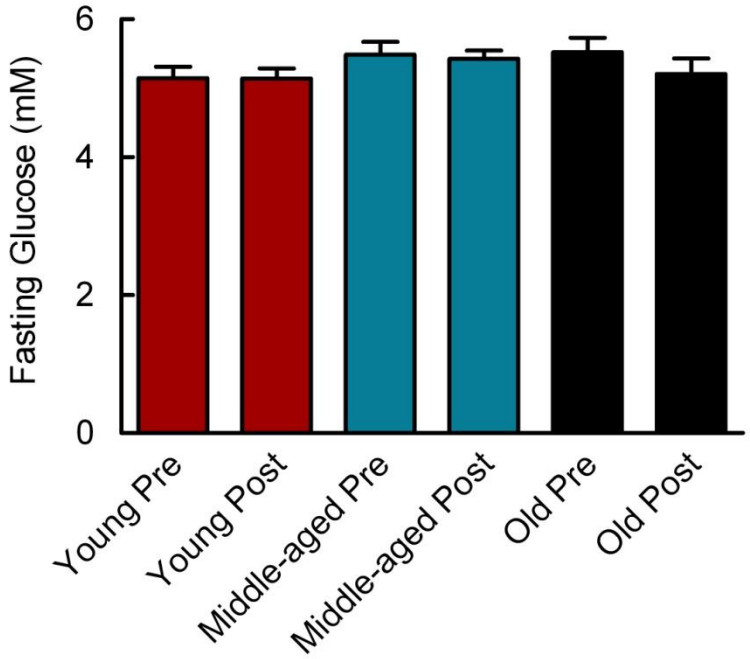


Figure 4.10 Fasting glucose values for young, middle-aged and old subjects before and after RET. Values are means±SEM. Statistical analysis via ANOVA with Bonferroni post analysis and student's t-test.

Peak glucose in the postprandial condition was no different between the groups before RET (Y, 7.93±0.29; M, 7.56±0.37; O, 8.23±0.40). After RET the young group displayed a significant reduction in their peak glucose (7.93±0.29 vs. 6.73±0.29, $P<0.05$), while the middle-aged group displayed a significant increase (7.56±0.37 vs. 8.28±0.28, $P<0.05$). The most marked change was in the old who also displayed a significant reduction in their peak glucose after RET (8.23±0.40 vs. 7.59±0.32, $P<0.01$). After RET the peak glucose of the young was significantly lower than that of the middle-aged (6.73±0.29 vs. 8.28±0.28, $P<0.05$) (Figure 4.11).

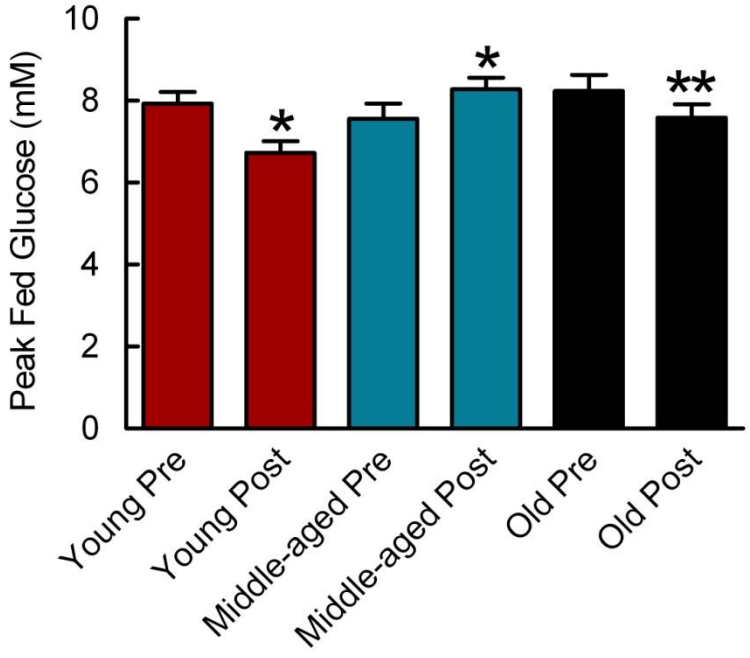


Figure 4.11 Peak glucose values in the postprandial condition for young, middle-aged and old subjects before and after RET. Values are means±SEM. Statistical analysis via ANOVA with Bonferroni post analysis and student's t-test. *=$P<0.05$ vs. pre-training, **=$P<0.01$ vs. pre-training.

4.3.1.2 Insulin action and sensitivity

Postprandial plasma insulin AUC was not different between the age groups either before or after RET. RET did not significantly affect the postprandial plasma insulin AUC in any of the age groups although there was a trend for it to be lower in the young after RET (181.95±21.06 vs. 146.19±17.11, *P*=0.05) (Figure 4.12 and 4.13).

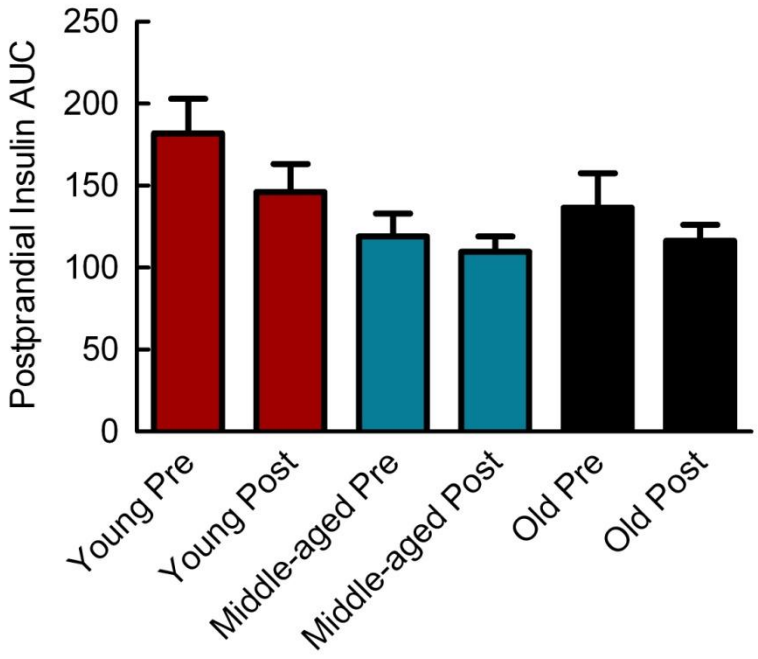


Figure 4.12 Postprandial insulin AUC in young, middle-aged and older subjects before and after RET. Values are means±SEM. Statistical analysis via ANOVA with Bonferroni post analysis and students t-test.

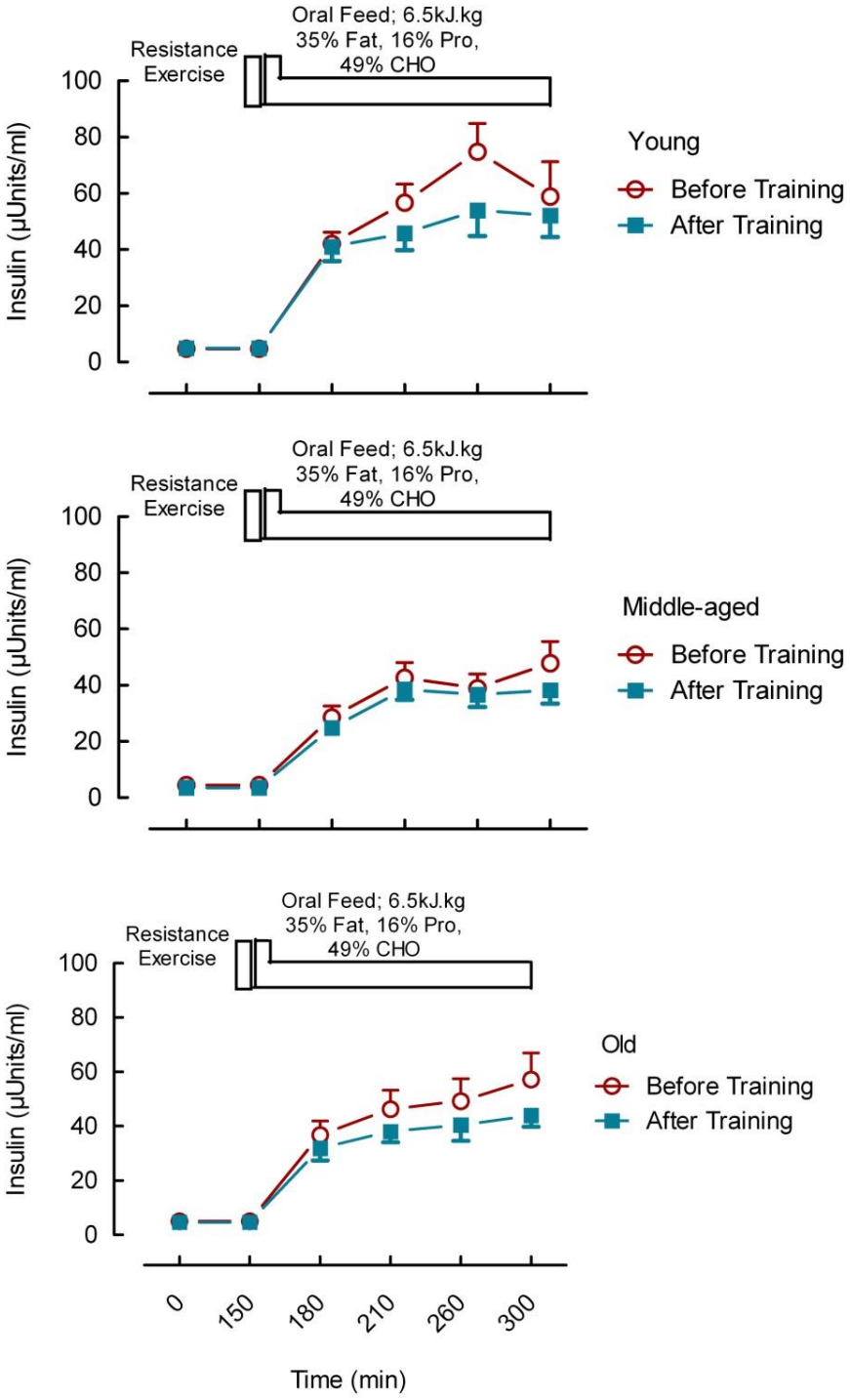


Figure 4.13 Time-course of postabsorptive and postprandial plasma insulin values in young, middle-aged and older subjects before and after RET. Values are means±SEM. Statistical analysis via ANOVA with Bonferroni post analysis on AUC values.

When all of the subjects were grouped together irrespective of age there was a significant reduction in HOMA values after RET (1.32±0.13 vs. 1.03±0.06, $P<0.01$) (Figure 4.14).

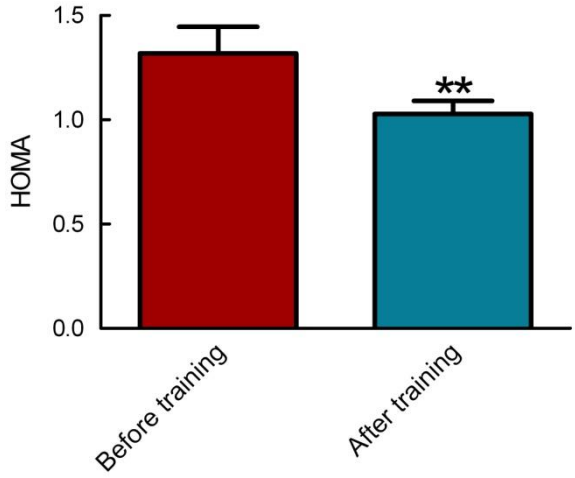


Figure 4.14 HOMA values for all subjects before and after RET. Values are means±SEM. Statistical analysis via student's t-test. **=$P<0.01$ vs. pre-training.

Fasting insulin values were not significantly different between the age-groups before or after RET (before: Y, 4.37±0.47; M, 4.41±0.52; O, 4.89±0.58; after: Y, 5.08±0.31; M, 3.55±0.37; O, 4.68±0.40 µUnits/ml). Only the middle-aged group had a difference in their fasting insulin values after RET demonstrating a reduction from 4.41±0.52 to 3.55±0.37 µUnits/ml (P<0.05) (Figure 4.15).

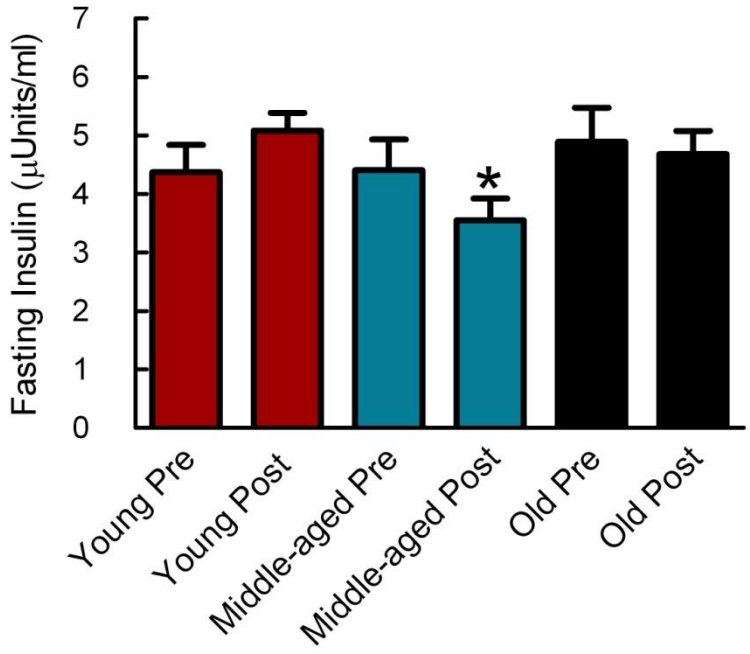


Figure 4.15 Fasting insulin values for young, middle-aged and old subjects before and after RET. Values are means±SEM. Statistical analysis via ANOVA with Bonferroni post analysis and student's t-test. *=P<0.05 vs. pre-training.

Mirroring the results seen with fasting insulin values, only the middle-aged group showed a change in their glucose/insulin ratio after RET with it increasing from 1.52±0.15 to 1.84±0.17 (P<0.05). There was no difference in the glucose/insulin ratio's between the age-groups before RET (Y, 1.28±0.15; M, 1.52±0.15; O, 1.42±0.17) but the middle-aged was higher than the young after RET (1.84±0.17 vs. 1.07±0.06, P<0.01) (Figure 4.16).

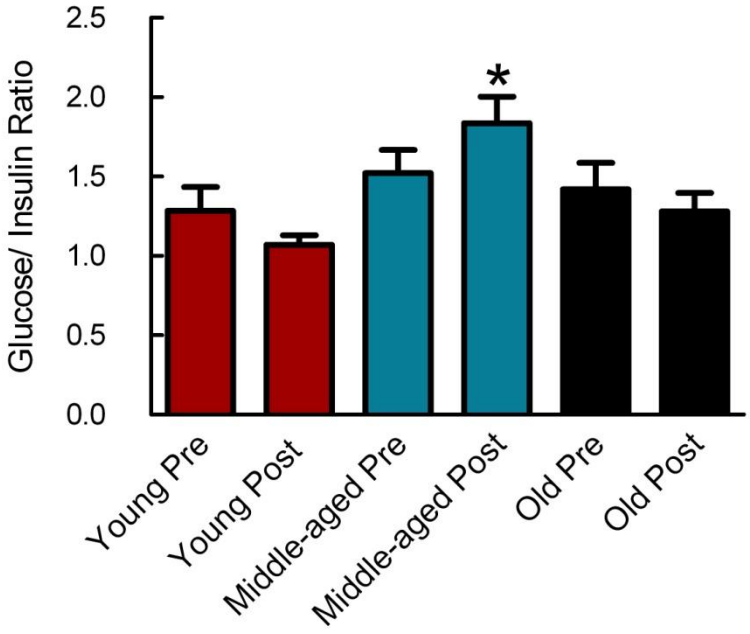


Figure 4.16 Glucose: insulin ratio's for young, middle-aged and old subjects before and after RET. Values are means±SEM. Statistical analysis via ANOVA with Bonferroni post analysis and student's t-test. *=P<0.05 vs. pre-training.

Both the middle-aged and the old groups had significantly reduced HOMA values after RET (M: 1.13±0.17 vs. 0.88±0.11 ; O: 1.70±0.28 vs. 1.11±0.12, $P<0.05$). Before RET the HOMA value of the old group was significanltly higher than the middle-aged and young groups (1.70±0.28 vs. 1.13±0.17 and 1.12±0.16, respectively, $P<0.05$), however after RET there was no difference in the HOMA values between the age-groups (Figure 4.17). QUICKI values showed an identical pattern of change.

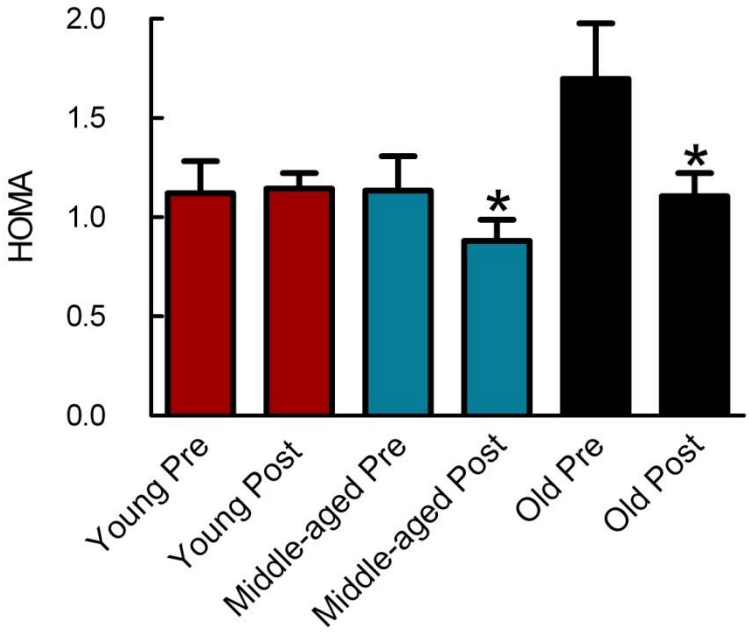


Figure 4.17 HOMA values for young, middle-aged and old subjects before and after RET. Values are means±SEM. Statistical analysis via ANOVA with Bonferroni post analysis and student's t-test. *=$P<0.05$ vs. pre-training.

Peak fed insulin was not significantly different between the age-groups either before or after RET. There was a trend for all three age-groups to reduce their peak fed insulin after RET (Y: 68.60±8.77 vs. 53.93±9.04; M: 47.63±7.40 vs. 39.25±4.61; O: 56.68±9.67 vs. 43.96±4.61, $P<0.1$) (Figure 4.18).

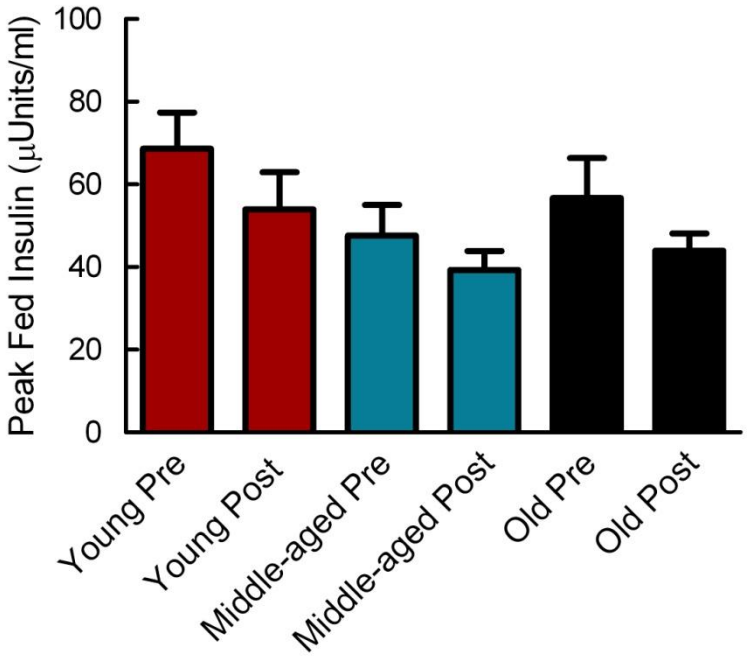


Figure 4.18 Peak insulin values in the postprandial condition for young, middle-aged and old subjects before and after RET. Values are means±SEM. Statistical analysis via ANOVA with Bonferroni post analysis and student's t-test.

4.3.1.2.1 Molecular markers of insulin signalling

Before RET all three age-groups demonstrated increased AKT phosphorylation in response to feeding alone (1.00±0.00 vs. Y: 1.26±0.06, *P*<0.001; M: 1.14±0.04, *P*<0.01; O: 1.17±0.04, *P*<0.01) and to the combination of exercise-plus-feeding (1.00±0.00 vs. Y: 1.24±0.07; M: 1.25±0.05; O: 1.20±0.05, all *P*<0.001). After RET all three groups still responded to both feeding alone (Y: 0.97±0.08 vs. 1.19±0.06, *P*<0.01; M: 1.00±0.06 vs. 1.21±0.06, *P*<0.001; O: 0.96±0.04 vs. 1.16±0.05, *P*<0.001) and exercise-plus-feeding (Y: 0.97±0.08 vs. 1.28±0.06; M: 1.00±0.06 vs. 1.22±0.06; O: 0.96±0.04 vs. 1.29±0.05, all *P*<0.001), with the old displaying an additive effect of exercise-plus-feeding compared to feeding alone (1.16±0.05 vs. 1.29±0.05, *P*<0.05). At no time point was there any differences between the age-groups (Figure 4.19).

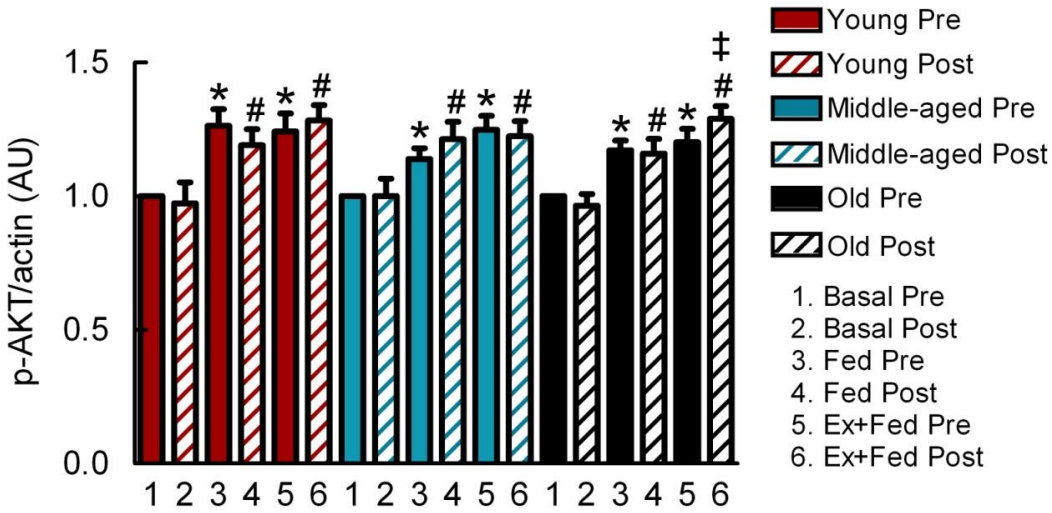


Figure 4.19 Effect of RET on AKT phosphorylation in young, middle-aged and old subjects. *= P<0.05 vs. basal pre-training; #= P<0.05 vs. basal post-training; ‡= P<0.05 vs. fed post-training. All via 2-way ANOVA with Tukey post-hoc analysis.

Before RET only the young demonstrated increased IRS-1 protein concentration in response to feeding alone (1.00±0.00 vs. 1.19±0.04, *P*<0.01) or exercise-plus-feeding (1.00±0.00 vs. 1.20±0.07, *P*<0.01). After RET all three age-groups displayed increased IRS-1 protein concentration after exercise-plus-feeding when compared to basal values (Y: 1.01±0.06 vs. 1.27±0.05, *P*<0.001; M: 0.95±0.04 vs. 1.07±0.05, *P*<0.05; O: 1.01±0.03 vs. 1.15±0.04, *P*<0.01). After RET in response to exercise-plus-feeding the young had significantly higher levels of IRS-1 than the middle-aged group (1.27±0.05 vs. 1.07±0.05, *P*<0.05) (Figure 4.20).

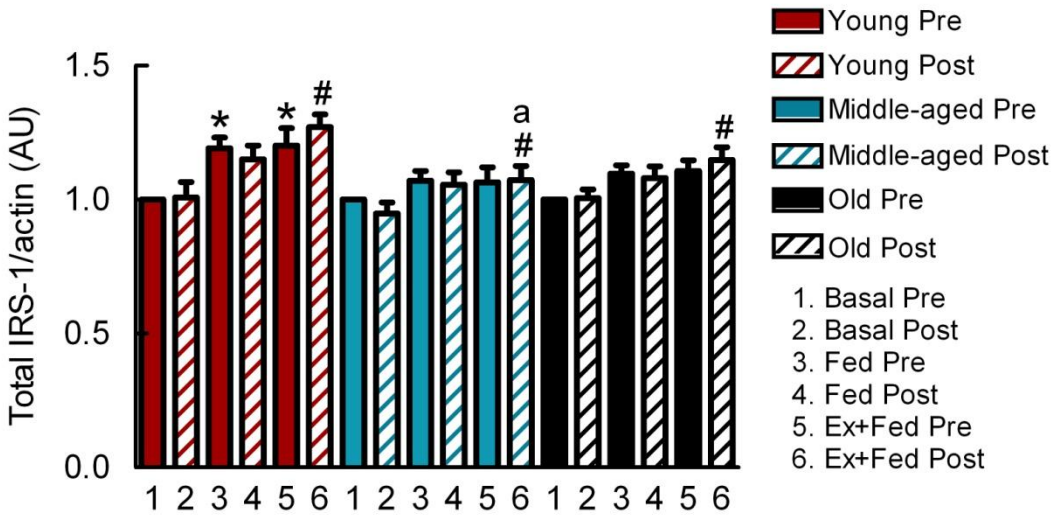


Figure 4.20 Effect of RET on Total IRS-1 concentration in young, middle-aged and old subjects. *= P<0.05 vs. basal pre-training; #= P<0.05 vs. basal post-training; a= P<0.05 vs. young in the same condition. All via 2-way ANOVA with Tukey pos-hoc analysis.

Before RET the young and the old groups showed increased PRAS 40 protein concentration after feeding alone (1.00±0.00 vs. Y: 1.19±0.04; O: 1.15±0.02, *P*<0.001) with all three age-groups showing increased PRAS 40 protein concentration after exercise-plus-feeding (1.00±0.00 vs. Y: 1.28±0.04, *P*<0.001; M: 1.10±0.03, *P*<0.05; O: 1.17±0.04, *P*<0.001) with the concentration significantly higher in the young than in the middle-aged after exercise-plus-feeding (1.28±0.04 vs. 1.10±0.03, *P*<0.01). All three age-groups maintained these increases after RET in response to both feeding alone (Y: 1.02±0.03 vs. 1.14±0.04; M: 0.92±0.04 vs. 1.06±0.05; O: 0.98±0.03 vs. 1.13±0.04) and exercise-plus-feeding (Y: 1.02±0.03 vs. 1.23±0.04; M: 0.92±0.04 vs. 1.09±0.05; O: 0.98±0.03 vs. 1.14±0.05) with no significant differences between the age-groups after RET (Figure 4.21).

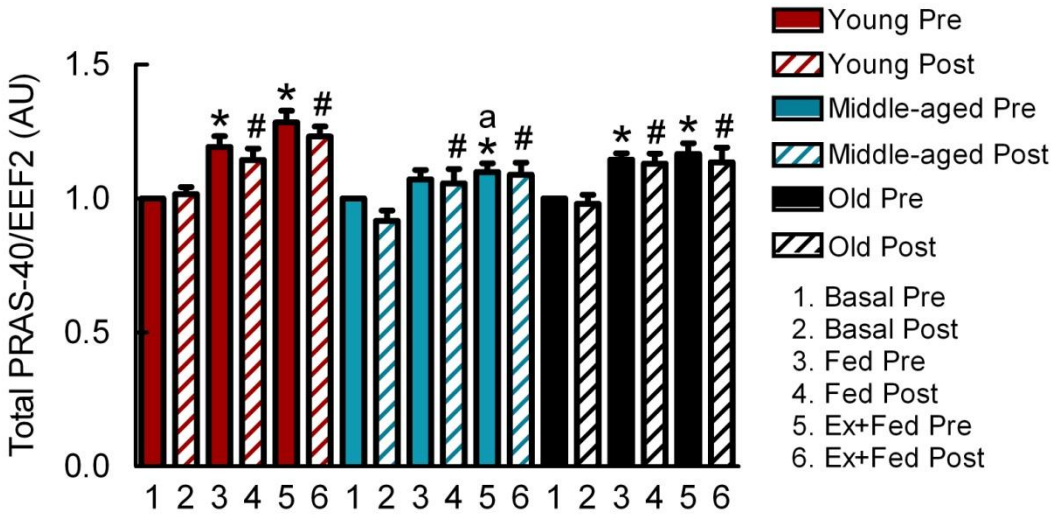


Figure 4.21 Effect of RET on Total PRAS-40 concentration in young, middle-aged and old subjects. *= P<0.05 vs. basal pre-training; #= P<0.05 vs. basal post-training; a= P<0.05 vs. young in the same condition. All via 2-way ANOVA with Tukey post-hoc analysis.

Before RET only the middle-aged demonstrated increased AS160 protein concentrations in response to feeding alone (1.00±0.00 vs. 1.14±0.04, P<0.01) while all three age-groups increased AS160 protein concentrations in response to exercise-plus-feeding both before (Y: 1.00±0.00 vs. 1.15±0.07, *P*<0.05; M: 1.00±0.00 vs. 1.23±0.07, *P*<0.001; O: 1.00±0.00 vs. 1.12±0.04) and after RET (Y: 1.03±0.02 vs. 1.21±0.05, *P*<0.01; M: 1.05±0.06 vs. 1.23±0.05, *P*<0.001; O: 0.90±0.04 vs. 1.04±0.05, *P*<0.01). Before RET the combined stimuli of exercise-plus-feeding had an additive effect compared to feeding alone in the old group (1.01±0.05 vs. 1.12±0.04, *P*<0.05) while after RET both the middle-aged and the old groups increased AS160 protein concentration in response to feeding alone (M: 1.05±0.06 vs. 1.16±0.04, *P*<0.04; O: 0.90±0.04 vs. 1.01±0.04), although in the old this may be attributable to the unexpected depression in basal values after RET. The AS160 protein concentration was higher in the middle-aged than in the old following exercise-plus-feeding after RET (1.23±0.05 vs. 1.04±0.05, *P*<0.05). The basal protein concentration of AS160 was significantly lower in the old after RET compared to before (1.00±0.00 vs. 0.90±0.04, *P*<0.05) (Figure 4.22)

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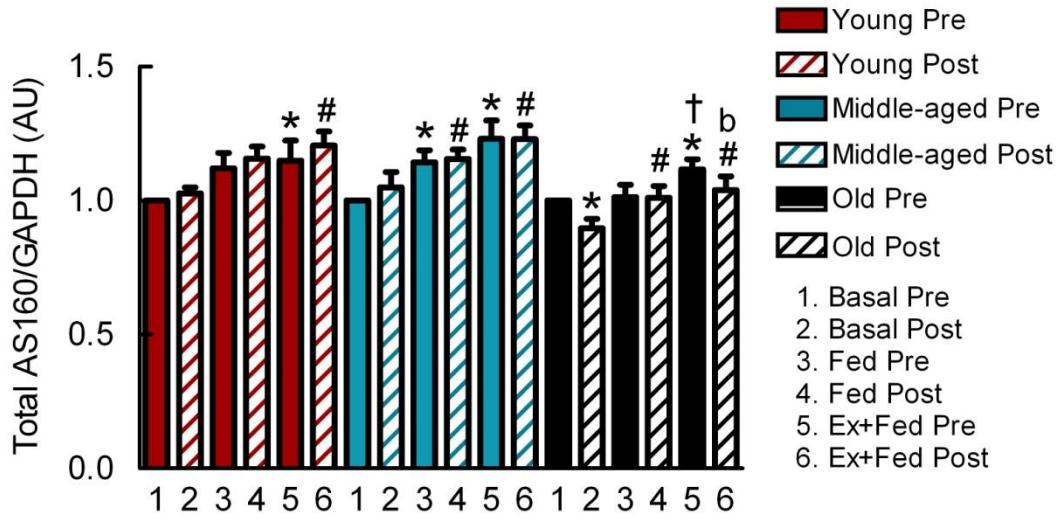

Figure 4.22 Effect of RET on Total AS160 concentration in young, middle-aged and old subjects. *= P<0.05 vs. basal pre-training; #= P<0.05 vs. basal post-training; †= P<0.05 vs. fed pre-training; b= P<0.05 vs. middle-aged in the same condition. All via 2-way ANOVA with Tukey pos-hoc analysis.

Before RET only the young showed a greater AKT substrate signature after feeding alone (1.00±0.00 vs. 1.13±0.04, *P*<0.01) while all groups demonstrated an increased signature after exercise-plus-feeding (1.00±0.00 vs. Y: 1.19±0.05, *P*<0.001; M: 1.15±0.04, *P*<0.001; O: 1.09±0.3, *P*<0.05). After RET the middle-aged and the old groups responded to feeding alone (M: 1.01±0.04 vs. 1.10±0.03, *P*<0.01; O: 0.93±0.03 vs. 1.02±0.03, *P*<0.05), again with all three groups responding to exercise-plus-feeding (Y: 1.03±0.03 vs. 1.19±0.03, *P*<0.001; M: 1.01±0.04 vs. 1.11±0.04, *P*<0.01; O: 0.93±0.03 vs. 1.03±0.03, *P*<0.01). Before RET the middle-aged group showed a greater signature after exercise-plus-feeding compared to feeding alone (1.06±0.05 vs. 1.15±0.04, *P*<0.05) and the AKT substrate signature after exercise-plus-feeding was higher in the young than in the old after RET (1.19±0.03 vs. 1.03±0.03, *P*<0.05) (Figure 4.23)

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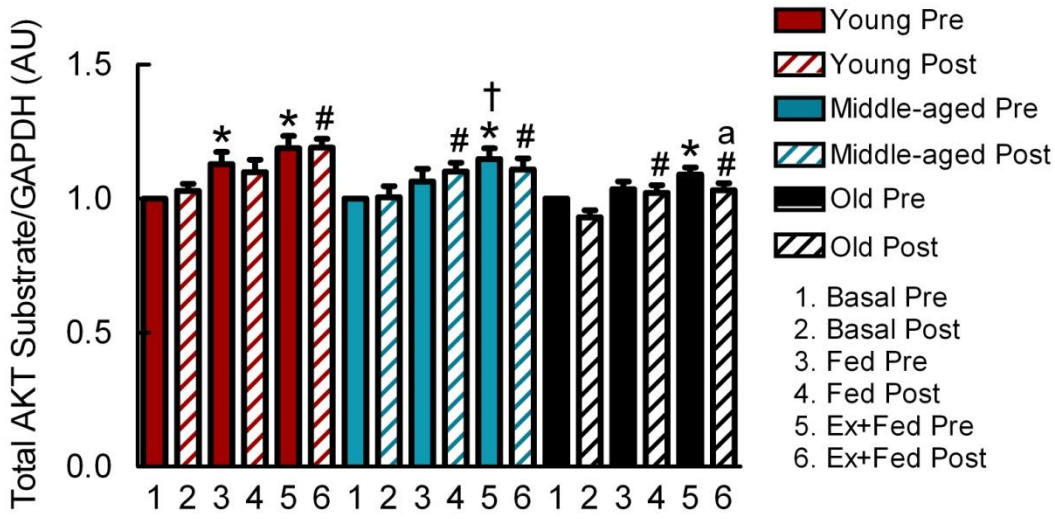

Figure 4.23 Effect of RET on Total AKT substrate concentration in young, middle-aged and old subjects. *= P<0.05 vs. basal pre-training; #= P<0.05 vs. basal post-training; †= P<0.05 vs. fed pre-training; a= P<0.05 vs. young in the same condition. All via 2-way ANOVA with Tukey post-hoc analysis.

4.3.1.3 Leg blood flow

Relationship between basal leg blood flow and age

LBF was normalized to lean leg mass with results identical to the non-normalised data presented.

Before and after RET there was no difference in basal LBF between the age groups. To determine the true relationship between basal LBF and age, we searched for a linear correlation with age (Figure 4.24). We found that both before and after RET LBF in the basal state declined with age (P<0.05).

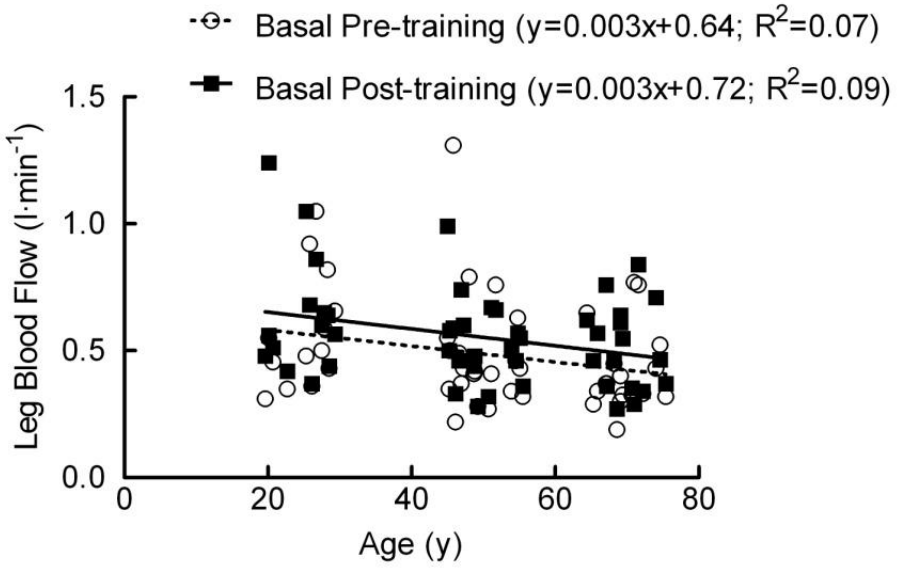

Figure 4.24 Relationship between basal femoral artery blood flow and age. Statistical analysis by Pearson's correlation.

Effects of feeding and exercise-plus-feeding on leg blood flow before and after RET

Before RET, only the young group demonstrated a significant increase in LBF in response to feeding alone (P<0.01). The post-feeding LBF in the young was significantly higher than in the middle-aged (P<0.05) and older groups (P<0.001) before RET, with no significant difference between the values in the middle-aged and older groups.

After RET the young and middle-aged groups demonstrated a significant LBF response to feeding alone (P<0.01) and fed LBF values were significantly higher in the middle-aged group than before RET (+30%, P<0.05).

Before RET the young and middle-aged groups did respond to exercise-plus-feeding (P<0.001), whereas those in the older group did not. Before RET, LBF values after exercise-plus-feeding were significantly higher in the young group compared to the older group (1.00±0.10 vs. 0.57±0.04 $l.min^{-1}$, P<0.001), with LBF values in the middle-aged group also significantly higher than in the older group (0.83±0.06 vs. 0.57±0.04 $l.min^{-1}$, P<0.05)

After RET all three age groups demonstrated a significant increase in LBF in response to exercise-plus-feeding (Y: +78.4±10.3%; M: +95.7±14.6%; O: +79.8±19.0%, all P<0.001), with LBF values significantly higher after exercise-plus-feeding than after feeding alone in all three age-groups (P<0.001). After RET, the LBF values in response to exercise-plus-feeding were significantly higher in the older group than before RET (+59.5±14%, P<0.001) such that the LBF values in the older group were no longer significantly different to those in the middle-aged group (Figure 4.25).

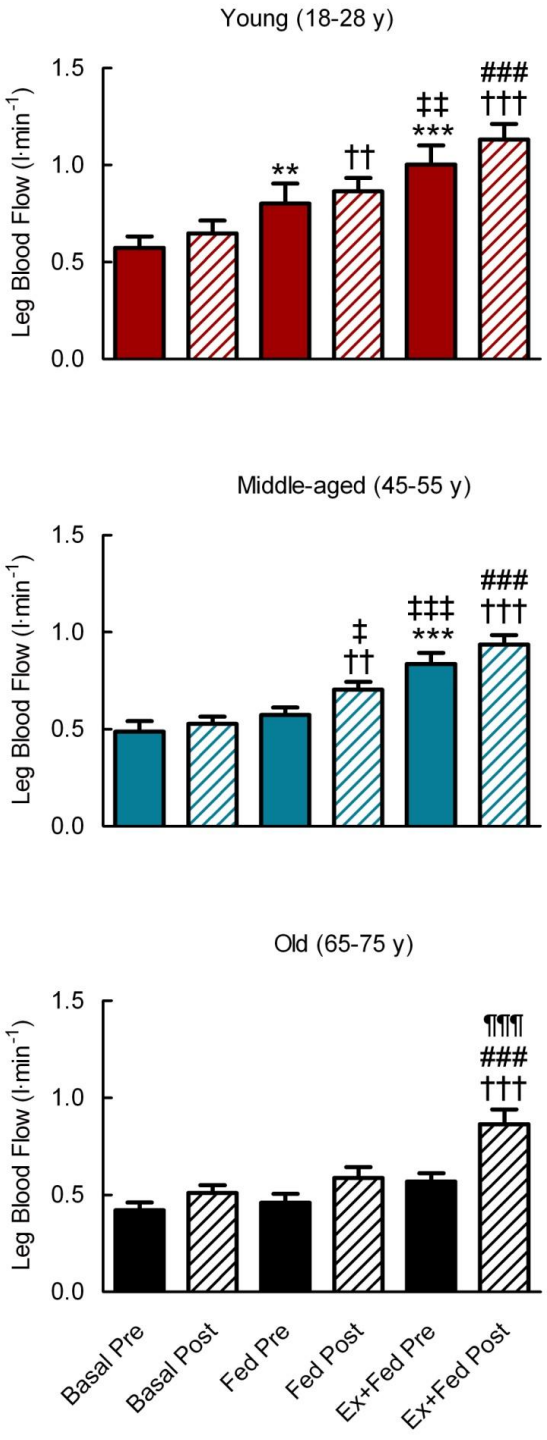

Figure 4.25 Effect of RET on femoral artery blood flow in young, middle-aged and older subjects. Values are means±SEM. Statistical analysis via 2-way ANOVA with Bonferroni post analysis. **= P<0.01 vs. basal pre-training; ***= P<0.001 vs. basal pre-training; ††= P<0.01 vs. basal post-training; †††= P<0.001 vs. basal post-training; ‡= P<0.05 vs. feeding pre-training; ‡‡= P<0.01 vs. feeding pre-training; ‡‡‡= P<0.001 vs. feeding pre-training; ### = P<0.001 vs. feeding post-training; ¶¶¶= P<0.001 vs. exercise-plus-feeding pre-training.

Relationships between leg blood flow responses to feeding/ exercise-plus-feeding and age

When we examined the data for relationships between LBF responses to feeding and to exercise-plus-feeding and age we found that before RET the responses to feeding and to exercise-plus-feeding were significantly blunted with advancing age but these age-related decreases were not apparent after RET (Figures 4.26 and 4.27).

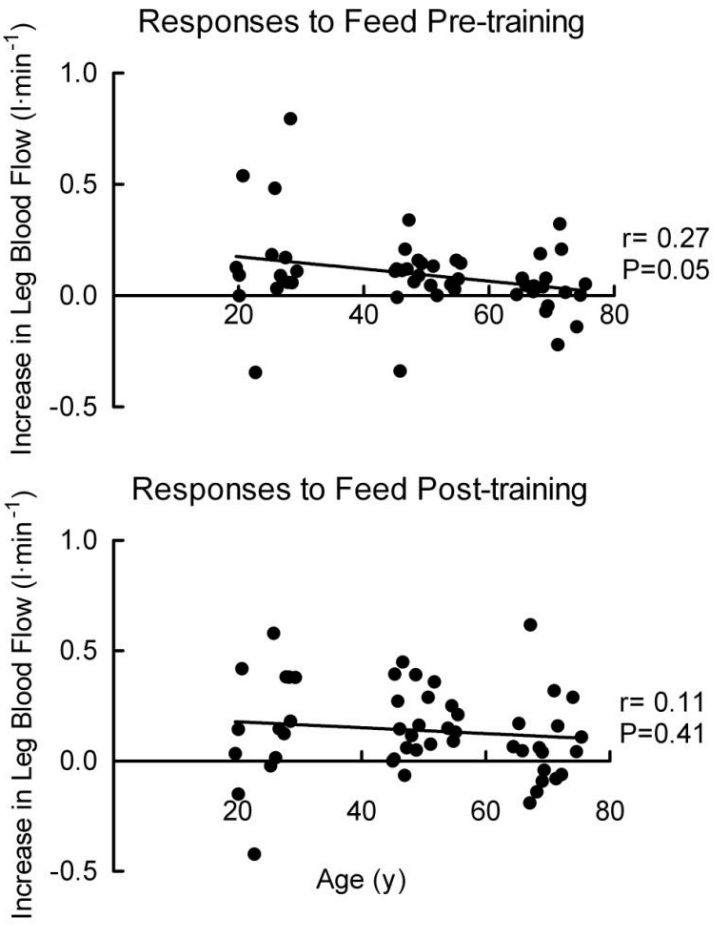

Figure 4.26 Effect of RET on the relationship between increases in femoral artery blood flow after feeding and age. Statistical analysis by Pearson's correlation.

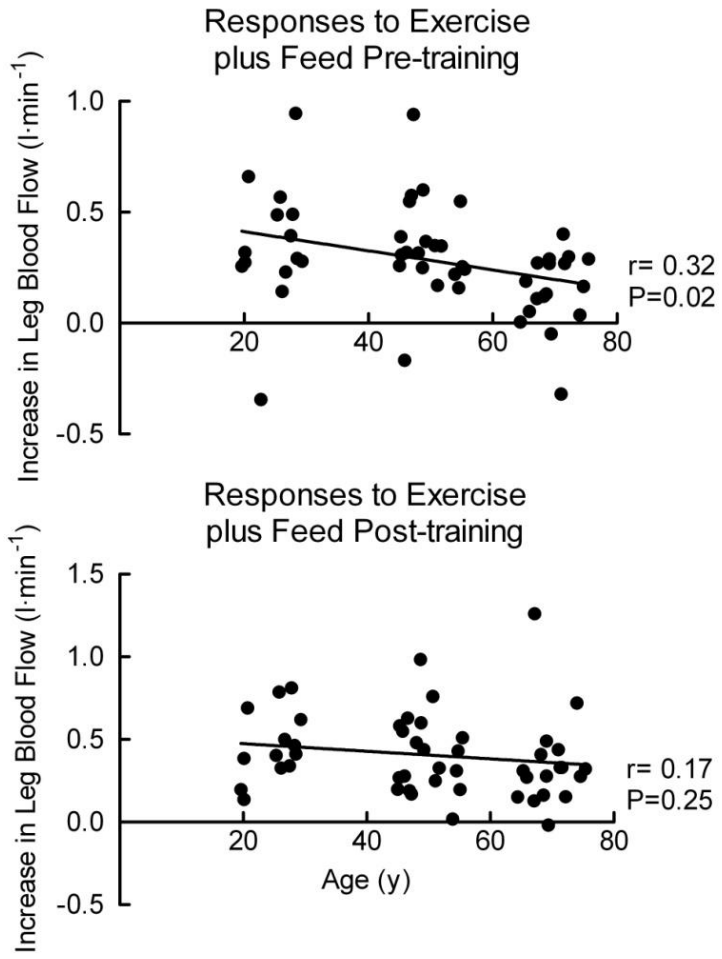

Figure 4.27 Effect of RET on the relationship between increases in femoral artery blood flow after exercise-plus-feeding and age. Statistical analysis by Pearson's correlation.

4.3.1.4 Leg Vascular Conductance

Relationships between basal limb vascular conductance and age

Basal LVC was significantly greater in the young than in the old before RET (0.56±0.06 vs. 0.36±0.03 $l \cdot min^{-1} \cdot 100mmHg^{-1}$, $P<0.01$). After RET, basal LVC in the older group was significantly higher (0.36±0.03 vs. 0.47±0.04 $l \cdot min^{-1} \cdot 100mmHg^{-1}$, $P<0.001$) and there was a trend for it to also be higher in the middle-aged group (0.43±0.05 vs. 0.50±0.04 $l \cdot min^{-1} \cdot 100mmHg^{-1}$, $P=0.095$). After RET there were no significant differences in basal LVC between the age-groups (Y: 0.63±0.06, M: 0.50±0.04, O: 0.47±0.04 $l \cdot min^{-1} \cdot 100mmHg^{-1}$).

Limb vascular conductance in response to feeding and exercise-plus-feeding before and after RET

Before RET only the young demonstrated an increased LVC in response to feeding (0.56±0.06 vs. 0.78±0.10 l·min^{-1}·100mmHg^{-1}, P<0.01), after RET the young (0.63±0.06 vs. 0.86±0.08 l·min^{-1}·100mmHg^{-1}, P<0.001) and the middle-aged groups (0.50±0.04 vs. 0.67±0.04 l·min^{-1}·100mmHg^{-1}, P<0.01) demonstrated increased LVC in response to feeding. All three age-groups responded to exercise-plus-feeding with increased LVC both before (Y: 0.56±0.06 vs. 0.98±0.09, P<0.001, M: 0.43±0.05 vs. 0.77±0.05, P<0.001, O: 0.36±0.03 vs. 0.50±0.04 l·min^{-1}·100mmHg^{-1}, P<0.05) and after RET (Y: 0.63±0.06 vs. 1.12±0.09, M: 0.50±0.04 vs. 0.89±0.05, O: 0.47±0.04 vs. 0.81±0.08 l·min^{-1}·100mmHg^{-1}, all P<0.001) with the increase in the old significantly greater after RET (49±11 vs. 80±19%, P<0.01). Before RET the young and middle-aged groups demonstrated a further increase in LVC in response to exercise-plus-feeding compared to feeding alone (Y: 0.78±0.10 vs. 0.98±0.09, P<0.01, M: 0.53±0.04 vs. 0.77±0.05 l·min^{-1}·100mmHg^{-1}, P<0.001), an increase that could be seen in all three age-groups after RET (Y: 0.86±0.08 vs. 1.12±0.09, M: 0.67±0.04 vs. 0.89±0.05, O: 0.55±0.06 vs. 0.81±0.08 l·min^{-1}·100mmHg^{-1}, all P<0.001). LVC in response to feeding was significantly higher in the middle-aged and older groups after RET (M: 0.53±0.04 vs. 0.67±0.04, O: 0.41±0.05 vs. 0.55±0.06 l·min^{-1}·100mmHg^{-1}, both P<0.05). LVC in response to exercise-plus-feeding was significantly higher in the young and the old after RET (Y: 0.98±0.09 vs. 1.12±0.09, P<0.05, O: 0.50±0.04 vs. 0.81±0.08 l·min^{-1}·100mmHg^{-1}, P<0.001) such that the LVC in the old after RET was not significantly different to that of the young before RET, effectively restoring LVC in this condition (Figure 4.28).

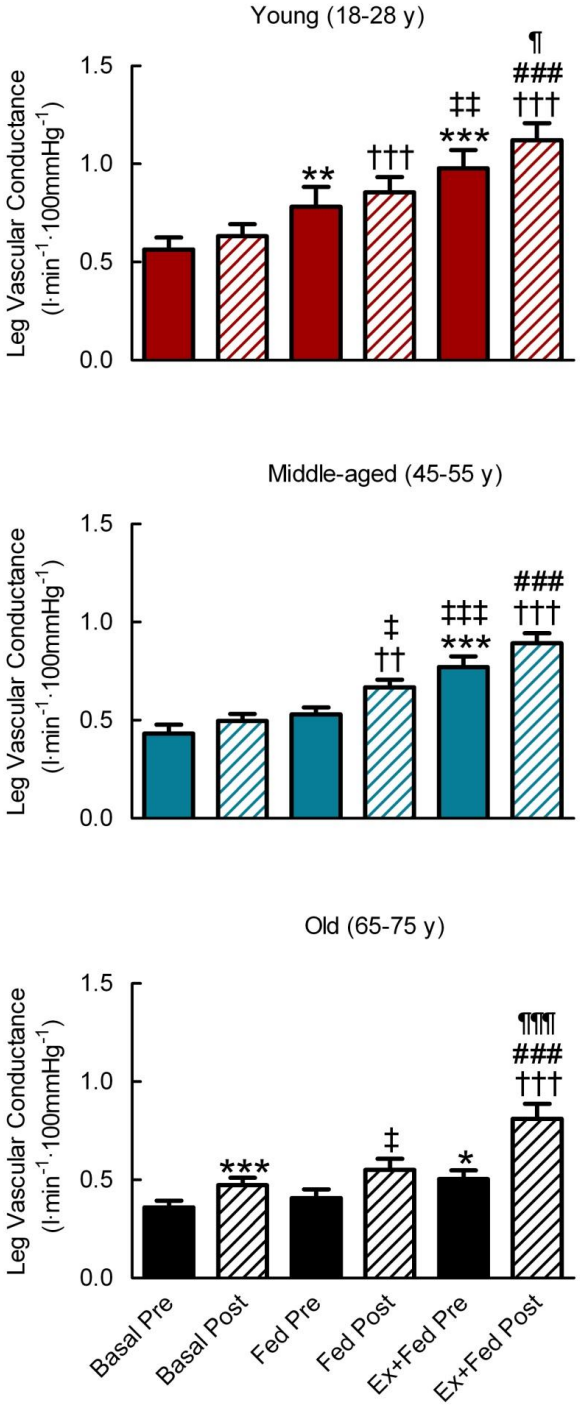

Figure 4.28 Effect of RET on leg vascular conductance in young, middle-aged and older subjects. Values are means±SEM. Statistical analysis via 2-way ANOVA with Bonferroni post analysis. *= P<0.05 vs. basal pre-training; **= P<0.01 vs. basal pre-training; ***= P<0.001 vs. basal pre-training; ††= P<0.01 vs. basal post-training; †††= P<0.001 vs. basal post-training; ‡= P<0.05 vs. feeding pre-training; ‡‡= P<0.01 vs. feeding pre-training; ‡‡‡= P<0.001 vs. feeding pre-training; ### = P<0.001 vs. feeding post-training; ¶= P<0.05 vs. exercise-plus-feeding pre-training; ¶¶¶= P<0.001 vs. exercise-plus-feeding pre-training.

4.3.1.5 Leg Peripheral Resistance

Before RET basal leg peripheral resistance (LPR) was lower in the young group that in the old (63.15±5.51 vs. 41.16±4.54, $P<0.001$) and LPR after feeding alone was lower in the young (58.28±5.63 vs. 30.93±3.83, $P<0.001$) and the middle-aged groups (58.28±5.63 vs. 41.80±3.20, $P<0.01$) compared to the old; this was also true after exercise-plus-feeding (44.55±3.98 vs. Y: 23.20±2.71 and M: 28.47±1.99, $P<0.01$). After RET there were no significant difference in LPR between the age groups in any of the three conditions, effectively restoring the LPR of the old to that of younger individuals.

Before RET, in the young, both feeding (41.16±4.54 vs. 30.93±3.83, $P<0.05$) and exercise-plus-feeding (41.16±4.54 vs. 23.20±2.71, $P<0.001$) reduced LPR. After RET only exercise-plus-feeding reduced LPR (35.43±3.23 vs. 19.72±2.05, $P<0.001$) but this may be attributable to the lowered (albeit not significantly) basal value (41.16±4.54 vs. 35.43±3.23).

As with the young, before RET the middle-aged group demonstrated reduced LPR in response to feeding and exercise-plus-feeding (55.09±4.86 vs. 41.80±3.20 and 28.47±1.99 respectively, $P<0.001$). The LPR was significantly lower after exercise-plus-feeding compared to feeding alone ($P<0.001$). After RET the middle-aged group displayed reduced basal LPR compared to before RET (55.09±4.86 vs. 44.08±3.15, $P<0.01$) and still demonstrated reduced LPR after both feeding alone and exercise-plus-feeding (44.08±3.15 vs. 31.67±1.70 and 23.94±1.52 respectively, $P<0.001$). The LPR after feeding alone was significantly lower after RET (41.80±3.20 vs. 31.67±1.70, $P<0.01$).

The old group showed reduced LPR only after exercise-plus-feeding before RET (63.15±5.51 vs. 44.55±3.98, $P<0.001$) and this was lower that the LPR after feeding alone (58.28±5.63 vs. 44.55±3.98, $P<0.001$). After RET the old group had significantly lower basal LPR than before RET (63.15±5.51 vs. 47.14±3.94, $P<0.001$) and demonstrated a further reduction after exercise-plus-feeding (47.14±3.94 vs. 28.42±2.70,

P<0.001). After RET the LPR after feeding alone (58.28±5.63 vs. 43.99±5.21, *P*<0.01) and after exercise-plus-feeding (44.55±3.98 vs. 28.42±2.70, *P*<0.001) was significantly lower compared to the same condition before RET (Figure 4.29).

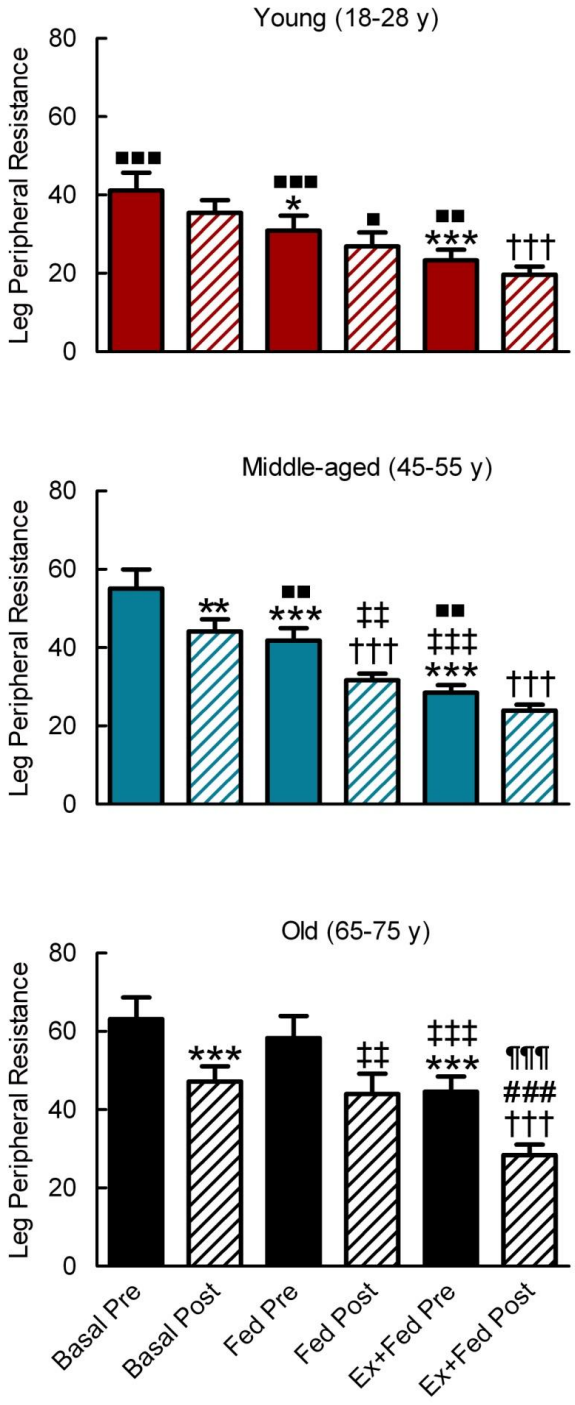

Figure 4.29 Effect of RET on leg peripheral resistance in young, middle-aged and older subjects. Values are means±SEM. Statistical analysis via 2-way ANOVA with Bonferroni post analysis. *= P<0.05 vs. basal pre-training; **= P<0.01 vs. basal pre-training; ***= P<0.001 vs. basal pre-training; †††= P<0.001 vs. basal post-training; ‡‡= P<0.01 vs. feeding pre-training; ‡‡‡= P<0.001 vs. feeding pre-training; ### = P<0.001 vs. feeding post-training; ¶¶¶= P<0.001 vs. exercise-plus-feeding pre-training; ■= P<0.05 vs. old in the same condition; ■■= P<0.01 vs. old in the same condition; ■■■= P<0.001 vs. .old in the same condition.

4.3.1.6 Molecular markers of endothelial capacity and vasodilatory action

There were no differences in basal muscle concentrations of Alpha-adrenergic receptor 1 (α-1) between the age groups before or after RET. RET had no effect on these protein concentrations (Figure 4.30).

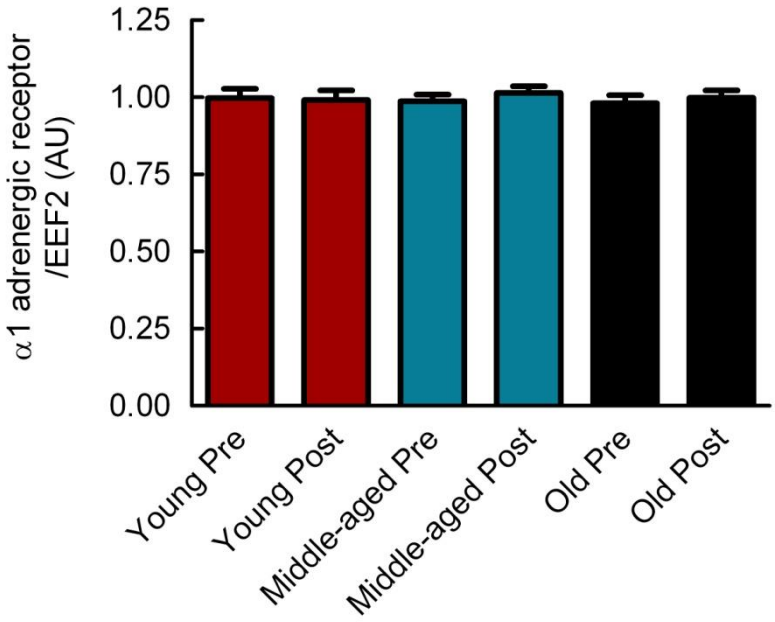

Figure 4.30 Protein expression of Alpha-adrenergic receptor 1 in young, middle-aged and older subjects before and after RET. Values are means±SEM. Statistical analysis via ANOVA with Bonferroni post analysis.

There were no differences in basal muscle concentrations of Beta-adrenergic receptor 2 (β-1) between the age groups before or after RET. RET had no effect on these protein concentrations (Figure 4.31).

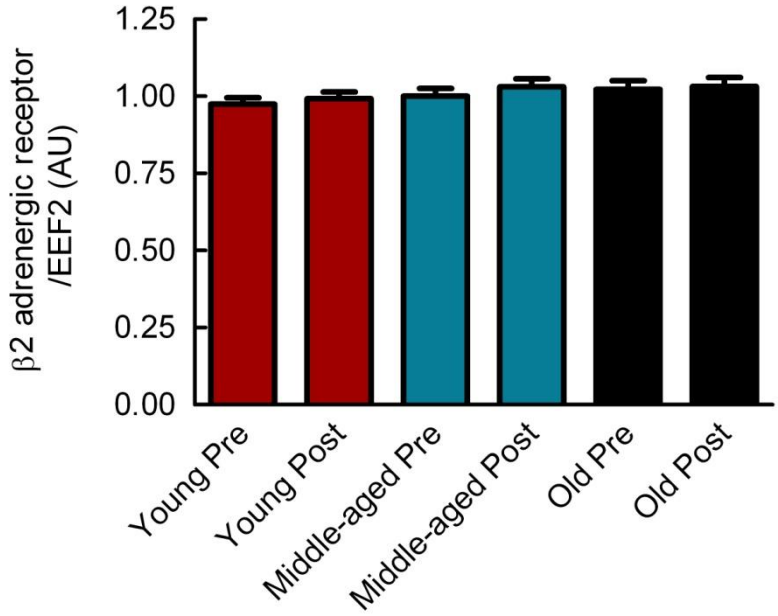

Figure 4.31 Protein expression of Beta-adrenergic receptor 2 in young, middle-aged and older subjects before and after RET. Values are means±SEM. Statistical analysis via ANOVA with Bonferroni post analysis.

There were no differences in basal muscle concentrations of Platelet endothelial cell adhesion molecule-1 (PECAM) between the age groups before or after RET. RET had no effect on these protein concentrations (Figure 4.32).

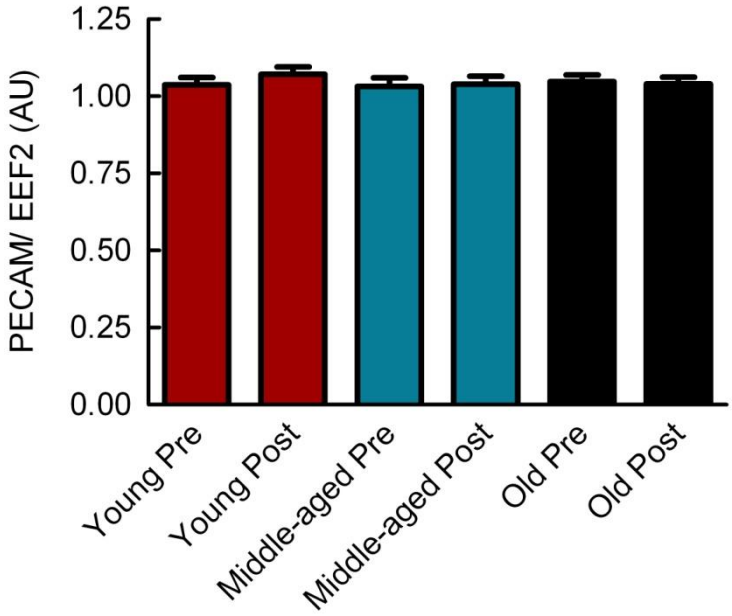

Figure 4.32 Protein expression of Platelet endothelial-cell adhesion molecule in young, middle-aged and older subjects before and after RET. Values are means±SEM. Statistical analysis via ANOVA with Bonferroni post analysis.

Endothelial nitric oxide synthase (eNOS) phosphorylation was unchanged by feeding or exercise-plus-feeding before and after RET and was not changed in any age-group by RET. Before RET eNOS phosphorylation in response to feeding alone was higher in the middle-aged group than the old (1.09±0.06 vs. 0.92±0.04, $P<0.05$) (Figure 4.33).

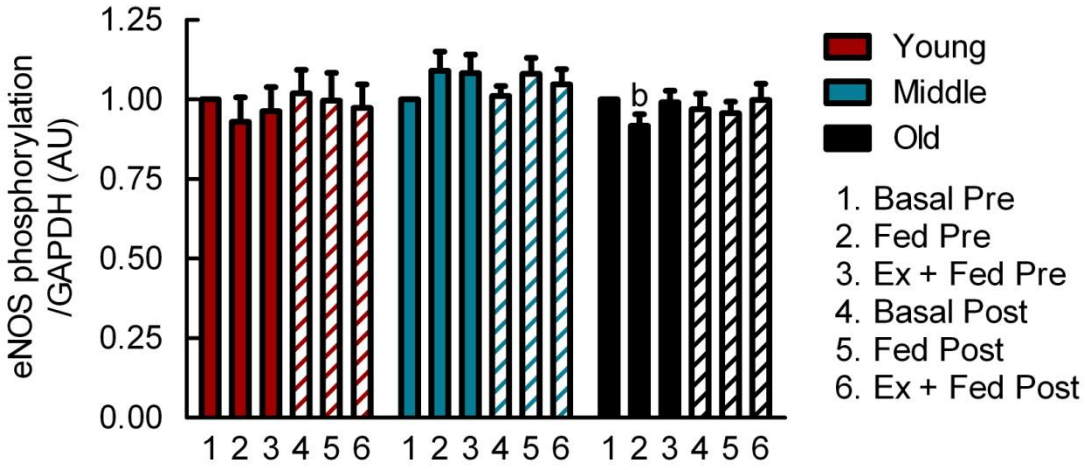

Figure 4.33 Endothelial nitric oxide synthase phosphorylation in young, middle-aged and older subjects before and after RET. Values are means±SEM. Statistical analysis via 2-way ANOVA with Bonferroni post analysis. b=P<0.05 vs. middle-aged in the same condition.

PKC-alpha (PKC-α) phosphorylation was unchanged by feeding or exercise-plus-feeding in the young and old groups before and after RET. In the middle-aged group feeding alone increased PKC-α phosphorylation before RET (1.00±0.00 vs. 1.05±0.02, *P*<0.01) and phosphorylation in response to exercise-plus-feeding was significantly higher after RET compared to before (1.08±0.02 vs. 1.10±0.03, *P*<0.05). PKC-α phosphorylation after exercise-plus-feeding was significantly higher in the middle-aged group compared to the old before (1.08±0.02 vs. 0.96±0.02, *P*<0.05) and after (1.10±0.03 vs. 0.99±0.02, *P*<0.05) RET, this was also true in the basal state after RET (1.08±0.02 vs. 0.97±0.02, *P*<0.05) (Figure 4.34).

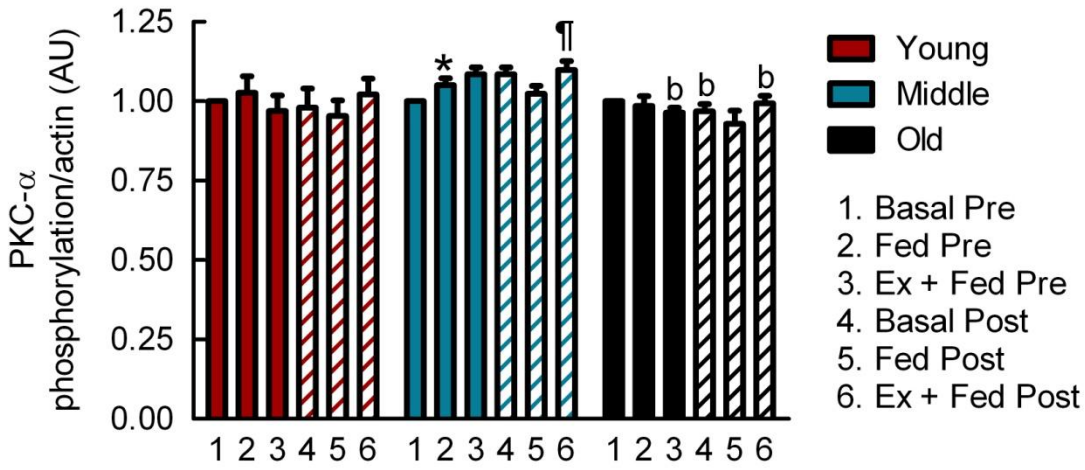

Figure 4.34PKC-alpha phosphorylation in young, middle-aged and older subjects before and after RET. Values are means±SEM. Statistical analysis via 2-way ANOVA with Bonferroni post analysis. *=P<0.05 vs. basal pre; ¶=P<0.05 vs. the same condition pre-training; b=P<0.05 vs. middle-aged in the same condition.

4.3.2 Risk factors for cardiovascular disease

4.3.2.1 Blood pressure and resting heart rate

Before RET MAP was significantly lower in the young than in the middle-aged (105±2 vs. 114±2, *P*<0.05) and older groups (105±2 vs. 120±2, *P*<0.001). The MAP in both the middle-aged (114±2 vs. 105±2, *P*<0.001) and older groups (120±2 vs. 111±2, *P*<0.001) were significantly lower

after RET; such that there was no difference between the MAP of the three groups after RET (Y, 105±2; M, 105±2; O, 111±2).

There was no difference in the RHR between the age groups either before (Y, 68±2; M, 64±2; O, 67±3) or after RET (Y, 60±2; M, 57±2; O, 65±2), although the RHR of the young and middle-aged groups were significantly lower after RET (P<0.001) (Figure 4.35).

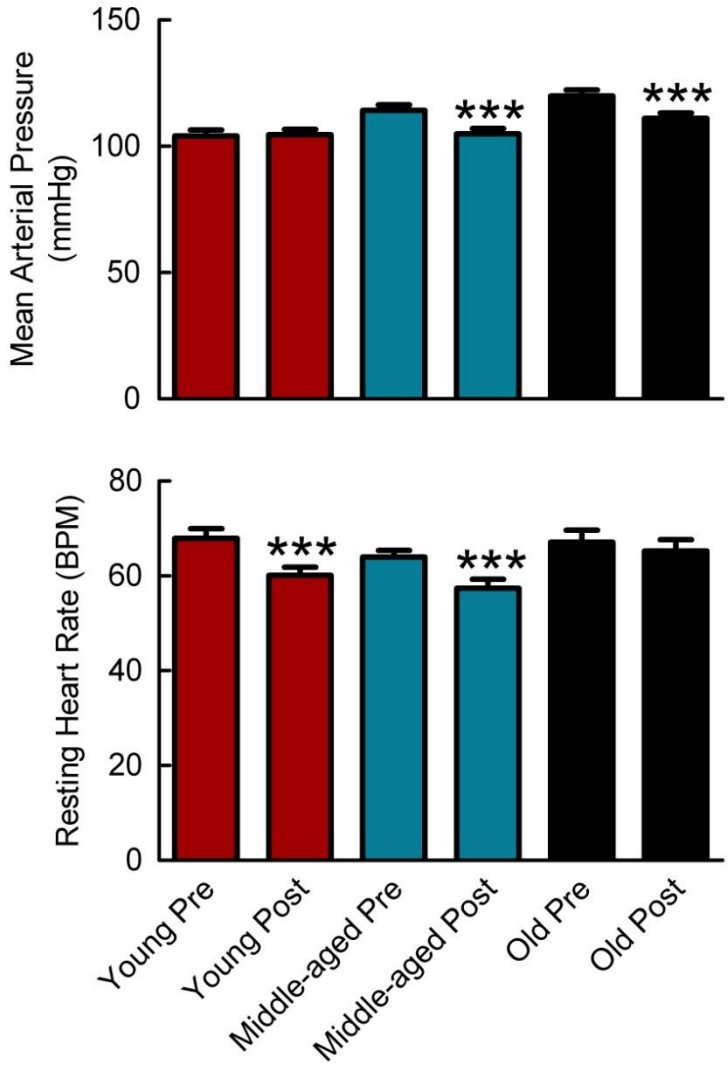

Figure 4.35 Mean arterial pressure and resting heart rate in young, middle-aged and older subjects before and after RET. Values are means±SEM. Statistical analysis via ANOVA with Bonferroni post-analysis and student's t-test. ***=*P*<0.001 vs. pre-training.

4.3.2.2 Cholesterol profiles

Total cholesterol levels were not significantly different between the age-groups either before (Y, 4.28±0.44; M, 4.78±0.27; O, 4.87±0.28) or after RET (Y, 4.4±0.48; M, 4.59±0.21; O, 5.04±0.25). RET had no effect on the total cholesterol levels in any of the age-groups.

Neither LDL nor HDL cholesterol levels were altered after RET in any of the age-groups. LDL cholesterol levels were not different between the age groups either before (Y, 2.66±0.44; M, 3.0±0.28; O, 3.11±0.23) or after RET (Y, 2.86±0.50; M, 2.86±0.20; O, 3.2±0.21). The same was true for HDL cholesterol (before: Y, 1.21±0.09; M, 1.25±0.06; O, 1.22±0.09, after: Y, 1.18±0.11; M, 1.25±0.06; O, 1.33±0.11) (Figure 4.36).

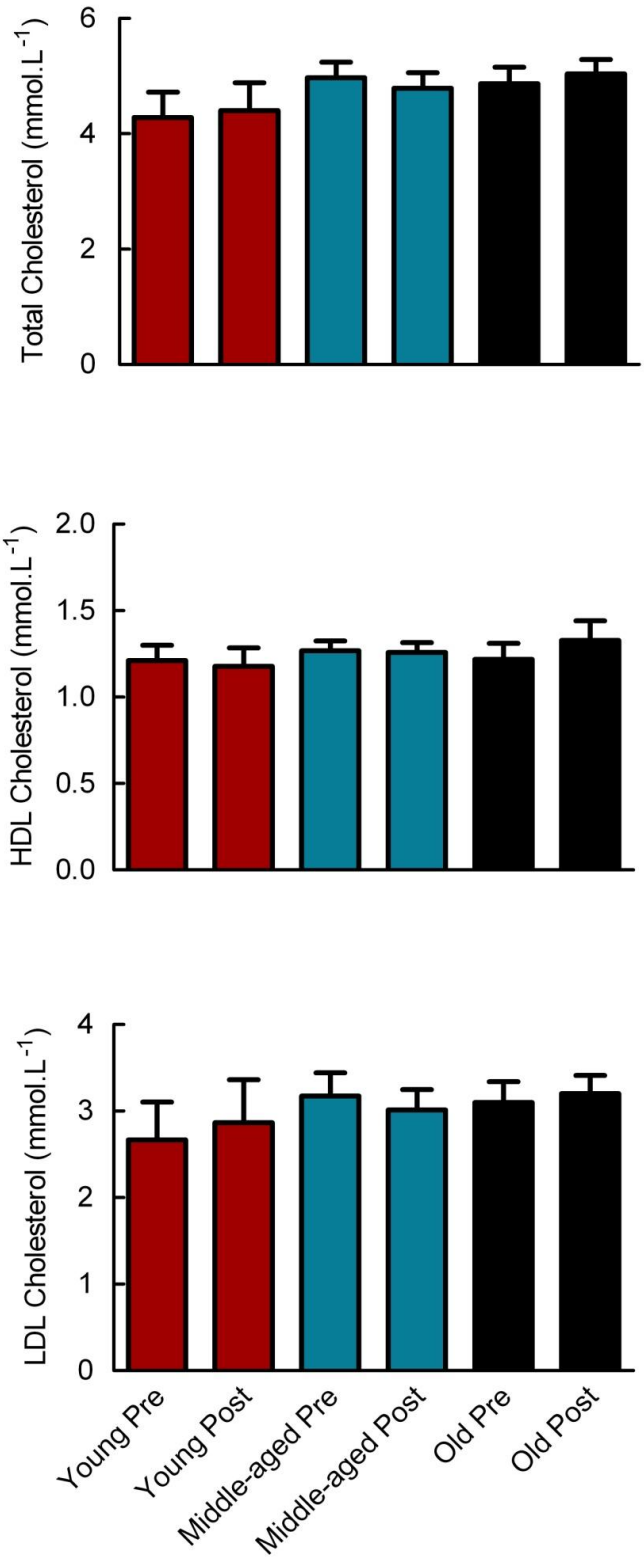

Figure 4.36 Total cholesterol, high density lipoprotein cholesterol and low density lipoprotein cholesterol in young, middle-aged and older subjects before and after RET. Values are means±SEM. Statistical analysis via ANOVA with Bonferroni post analysis and students t-test.

Neither total cholesterol: HDL (TC: HDL) ratios nor LDL: HDL cholesterol ratios were altered after RET in any of the age-groups. TC: HDL ratios were not different between the age groups either before (Y, 3.70±0.47; M, 3.96±0.28; O, 4.18±0.34) or after RET (Y, 3.47±0.42; M, 3.79±0.23; O, 4.01±0.33). The same was true for LDL: HDL cholesterol ratios (before: Y, 2.35±0.51; M, 2.66±0.25; O, 2.67±0.27, after: Y, 2.09±0.46; M, 2.52±0.21; O, 2.58±0.28).

Triglyceride levels were significantly lower in the young than the middle-aged and old both before and after RET (before: 0.88±0.07 vs. 1.14±0.11 and 1.18±0.14, respectively, after: 0.79±0.09 vs. 1.05±0.08 and 1.12±0.09, respectively). Triglyceride levels were not significantly different between the middle-aged and old either before or after RET. RET had no effect on the triglyceride levels in any of the age-groups (Figure 4.37).

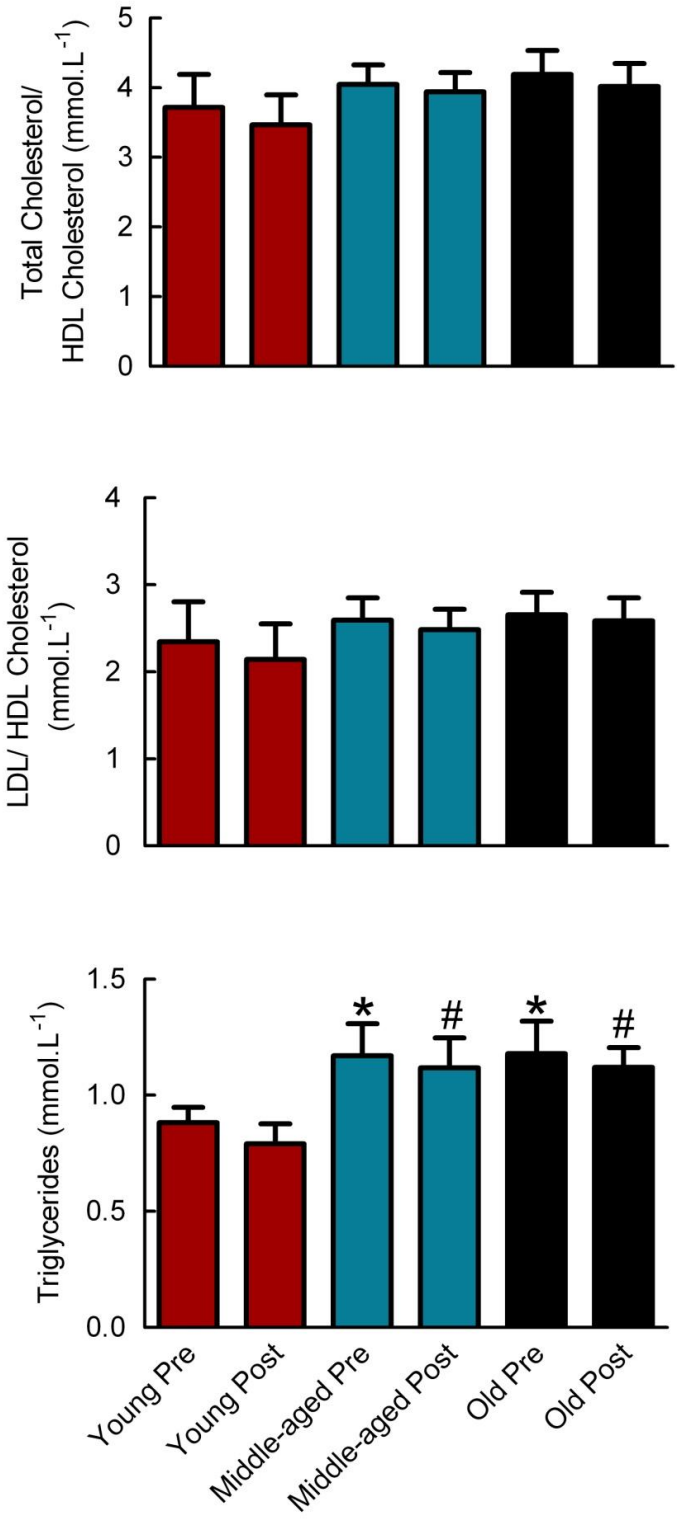

Figure 4.37 Total cholesterol: high density lipoprotein cholesterol, low density lipoprotein cholesterol: high density lipoprotein cholesterol and triglycerides in young, middle-aged and older subjects before and after RET. Values are means±SEM. Statistical analysis via ANOVA with Bonferroni post analysis and students t-test. *=P<0.05 vs. young pre, #=P<0.05 vs. young post.

4.3.2.3 Cytokines

There were no differences in basal plasma concentrations of the classic inflammatory markers TNF-α or IL-6 between the age groups before or after RET and RET had no effect on these cytokine concentrations. There were no significant differences in IL-10 concentrations between the age-groups before or after RET, although the young did demonstrate significantly higher IL-10 after RET ($P<0.05$) (Figure 4.38).

There were also no differences in basal plasma levels of interleukin-8 (IL-8), IL-1α, transforming growth factor beta (TGF-β), interferon-gamma (IFN-γ), monocyte chemoattractant protein-1 (MCP-1), MCP-2, stromal cell-derived factor-1 beta (SDF-1β), monokine induced by IFN-gamma (MIG) or granulocyte colony-stimulating factor (GCSF) between the age-groups before or after RET and levels of these cytokines were unchanged by RET (Table 4.6).

In addition there were no differences in basal plasma concentrations of IL-1β, IL-16, IL-4, IL-2, IL-12, IL-15, granulocyte-macrophage colony-stimulating factor (GM-CSF), regulated upon activation, normal T-cell expressed and secreted (RANTES), IFN-β, macrophage inflammatory protein-1 alpha (MIP-1α) or MIP-1β between the age-groups before or after RET although at least one of the age-groups did have significantly altered levels of these cytokines after RET. GM-CSF was the only cytokine increased in all three age-groups following RET (Y, $P<0.01$; M, $P<0.01$; O, $P<0.05$). IL-1β, IL-16 and IL-2 were significantly higher in the young following RET ($P<0.01$). RANTES ($P<0.01$), IFN-β ($P<0.001$), IL-15 ($P<0.05$) and MIP-1β ($P<0.05$) were increased only in the old group after RET. IL-4 was increased in the middle-aged and old group following RET ($P<0.001$ and <0.05, respectively) while IL-12 and MIP-1α were higher in both the middle-aged (IL 12, $P<0.05$; MIP-1α, $P<0.01$) and old group (IL 12, $P<0.001$; MIP-1α, $P<0.01$) after RET (Table 4.6).

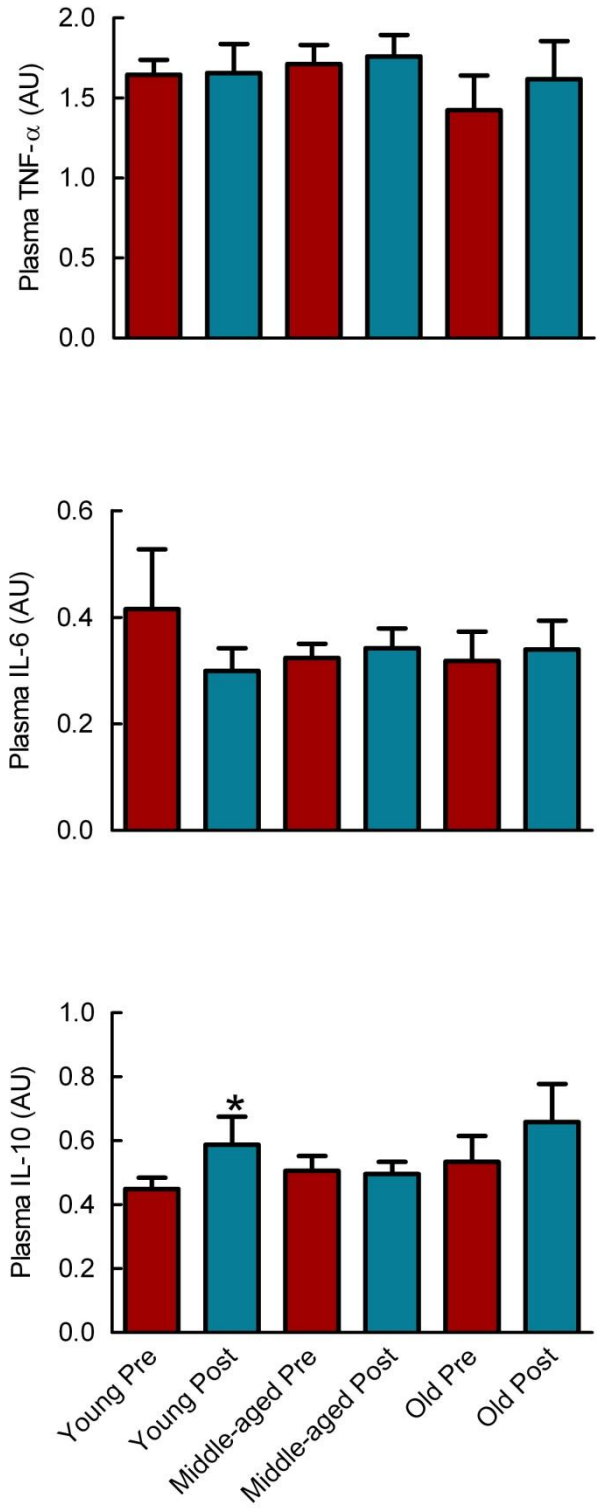

Figure 4.38 Plasma concentrations of TNF-α, IL-6 and IL-10 in young, middle-aged and older subjects before and after RET. Values are means±SEM. Statistical analysis via ANOVA with Bonferroni post analysis and students t-test. *=P<0.05 vs. young pre.

Table 4.6 Cytokine concentrations before and after RET

Cytokine	*Young*		*Middle-aged*		*Old*	
	pre	***post***	***pre***	***post***	***Pre***	***post***
TNF-α	1.6500± 0.0900	1.7600± 0.1100	1.8200± 0.0600	1.8400± 0.0800	1.7100± 0.1200	1.9400± 0.1100
IL-6	0.4200± 0.1100	0.3000± 0.0400	0.3400± 0.0200	0.3600± 0.0300	0.3600± 0.0500	0.3700± 0.0500
IL-10	0.4500± 0.0300	0.5600± 0.0900*	0.5100± 0.0500	0.5000± 0.0400	0.5300± 0.0800	0.6600± 0.1200
IL-8	0.0017± 0.0002	0.0019± 0.0002	0.0022± 0.0002	0.0021± 0.0002	0.0020± 0.0002	0.0030± 0.0006
IL-1α	0.0051± 0.0003	0.0055± 0.0005	0.0055± 0.0002	0.0058± 0.0002	0.0055± 0.0004	0.0066± 0.0006
IL-1β	0.0057± 0.0003	0.0073± 0.0006**	0.0056± 0.0004	0.0062± 0.0003	0.0060± 0.0007	0.0064± 0.0008
IL-16	0.3988± 0.0365	0.4694± 0.0505**	0.3894± 0.0411	0.3713± 0.0373	0.3734± 0.0677	0.3993± 0.0449
IL-4	0.2001± 0.0130	0.2808± 0.0214***	0.2223± 0.0121	0.2403± 0.0100*	0.2347± 0.0184	0.2682± 0.0153*
IL-2	0.0916± 0.0046	0.1060± 0.0053**	0.1074± 0.0122	0.1029± 0.0045	0.1109± 0.0202	0.1217± 0.0059
IL-12	0.0380± 0.0043	0.0418± 0.0056	0.0377± 0.0033	0.0450± 0.0029*	0.0384± 0.0042	0.0528± 0.0047***
IL-15	0.0146± 0.0010	0.0165± 0.0015	0.0166± 0.0008	0.0165± 0.0007	0.0155± 0.0009	0.0179± 0.0018*
GCSF	0.0668± 0.0028	0.0644± 0.0036	0.0664± 0.0026	0.0735± 0.0057	0.0681± 0.0028	0.0728± 0.0032
MCP-1	0.0450± 0.0069	0.0512± 0.0105	0.0537± 0.0066	0.0693± 0.0153	0.0595± 0.0103	0.1025± 0.0224
MCP-2	0.0038± 0.0003	0.0037± 0.0002	0.0042± 0.0003	0.0039± 0.0003	0.0033± 0.0002	0.0040± 0.0003
GM-CSF	0.0173± 0.0008	0.0202± 0.0012**	0.0153± 0.0009	0.0173± 0.0006**	0.0151± 0.0012	0.0181± 0.0012*
TGF-β	1.3996± 0.0638	1.3922± 0.0659	1.4644± 0.0349	1.5197± 0.0581	1.4501± 0.0706	1.5209± 0.0730
RANTES	0.9984± 0.2514	1.1678± 0.2897	1.1480± 0.2609	1.4827± 0.2471	0.8901± 0.2681	1.9001± 0.3295**
IFN-γ	1.4214± 0.1078	1.5560± 0.1728	1.5736± 0.1094	1.6419± 0.1043	1.6038± 0.1311	1.8504± 0.2325
IFN-β	0.9588± 0.1165	0.9354± 0.1198	0.9803± 0.0697	1.0161± 0.0554	0.7238± 0.0898	1.1425± 0.1063***
SDF-1β	1.7129 0.1273	1.8255 0.1636	1.8988 0.0876	1.8691 0.1051	1.9568 0.1256	2.0942 0.1925
MIG	0.1552 0.0087	0.1664 0.0112	0.1644 0.0049	0.1706 0.0053	0.1740 0.0096	0.2077 0.0314
MIP-1α	0.0757± 0.0057	0.0780± 0.0069	0.0805± 0.0045	0.0888± 0.0049**	0.0792± 0.0063	0.0975± 0.0060**
MIP-1β	0.0876± 0.0072	0.0905± 0.0087	0.0879± 0.0031	0.0880± 0.0076	0.0912± 0.0053	0.1267± 0.0196*

Values are means±SEM. Statistical analysis via ANOVA and students t-test. *=P<0.05 vs. pre RET in the same age-group, **=P<0.01 vs. pre RET in the same age-group, ***=P<0.001 vs. pre RET in the same age-group.

4.4 Discussion

Effect of RET on LBF

In summary, we report that age-related dysregulation in LBF in response to feeding and exercise-plus-feeding (but not basal) is ameliorated by RET which is concomitant with a restoration of LVC. Although we were unable to seek a mechanism for our findings in this study, it is evident that RET may in some cases reverse age-related declines in LBF/LVC which are indicative of pathological changes in vessel structure and/ or vascular tone.

Age-related reductions in basal limb blood flow and vascular conductance that occur with advancing age can be associated with the metabolic syndrome, functional impairments, osteoporosis and other clinical conditions such as CVD and atherosclerosis (Anton *et al.*, 2006). We have confirmed previous observations (Miyachi *et al.*, 2005; Dinenno *et al.*, 2001; Moreau *et al.*, 2003) that there is an age-related decline in basal LBF and also in associated LVC. Although we did not observe significant differences in LBF between our groups in the basal state, using a correlation analysis of all 51 subjects, therefore giving greater statistical power, we revealed a relationship that was obscured by comparison of the smaller data sets, demonstrating an inverse relationship between advancing age and basal LBF.

One of the normal symptoms of ageing is that of impaired glucose tolerance, especially insulin mediated glucose uptake (IMGU) which occurs primarily in skeletal muscle (Meneilly *et al.*, 1995). This impairment may be partly explained by decreased limb blood flow experienced by the elderly (Meneilly *et al.*, 1995) causing a decrease in the delivery of insulin and glucose to the muscle tissue. Oxygen, hormone and nutrient delivery may also be impaired by reduction in LBF and this may contribute to decreasing muscle mass maintenance as well as whole–body glucose intolerance and hyperinsulinaemia (Lind & Lithell, 1993).

Arterioles have high compliance, which allows them to respond to stretch stimuli and regulate blood flow without damage. Sympathetic fibres of the autonomic nervous system innervate the smooth muscle of blood vessels, typically causing the smooth muscle to contract, narrowing the vessel lumen. When sympathetic stimulation decreases or in the presence of certain receptors (i.e. β-2 adrenoreceptor) the smooth muscle fibres of the vessel relax and lumen diameter increases facilitating increases in blood flow through the vessel. In healthy young adults the ingestion of food induces vasodilation, a response mediated by the vasodilatory actions of insulin (Skilton *et al.*, 2005). This postprandial response is significantly impaired with increasing age, even when blood flow data is adjusted for reduced baseline flow values in the elderly (Skilton *et al.*, 2005).

Although there is little information available concerning acute responses of LBF to feeding Fugmann and colleagues (using venous capacitance plethysmography) showed a long lasting (<2 h) response to feeding a mixed-meal (Fugmann *et al.*, 2003). This was also shown by Hernandez et al. who demonstrated a 16-25% increase in LBF in young males (Hernandez & Jensen, 1995), although the method used (indocyanine green dye dilution) is inherently more variable than either plethysmography or Doppler techniques. Combining Doppler ultrasound and contrast enhanced ultrasound (CEUS) Vincent and colleagues found a 33% increase in bulk brachial artery blood flow 60 min after a mixed meal and also a 50% increase in forearm microvascular blood volume (Vincent *et al.*, 2006).

In line with these findings we found that before RET LBF increased in response to feeding in our younger subjects (+39.8%) but this increase was not apparent in our middle-aged or older subjects. Further age × LBF correlation analysis confirmed age-related declines in LBF responses to feeding before RET.

The reduced vasodilatory response of decreased postprandial blood flow shown with age both by us in this study and by others (Skilton *et al.*,

2005), may serve to act as a preclinical marker of vascular health. Ageing is a major risk factor for CVD (Lakatta & Yin, 1982) and the reduced vasodilatory response in the periphery experienced with age may be indicative of an impaired response of the coronary arteries to endothelium dependent vasodilators (Gerhard *et al.*, 1996). Due to the large amount of time most humans spend in the postprandial state negative changes in responses to feeding may have large implications for health.

RET improved the age-related diminutions in LBF responses to feeding such that fed LBF responses significantly increased in the middle-aged and older groups (when compared to their pre-RET values) and the negative relationship between advancing age and fed LBF was ameliorated, presumably through enhancement of fed LVC. This restoration of fed LBF responses and improved LVC after RET may positively impact age-related declines in glucose disposal and amino-acid deposition (Fugmann *et al.*, 2003; Lind & Lithell, 1993) and may be related to a re-sensitization to insulin and its effects on total flow and/ or capillary recruitment- clearly this conjecture warrants further investigation.

In this study we elected to measure LBF during the recovery period from exercise which reflects the remodeling period (when muscle protein turnover is heightened (Kumar *et al.*, 2009)). We also chose to study the combined effects of exercise and feeding (rather than exercise hyperaemia *per se*) to maximize practical ramifications of the work. For instance, most people would (or would be recommended to) combine resistance exercise with feeding to maximize muscle anabolic responses (Moore *et al.*, 2009).

Exercise hyperaemia is evident in active muscles both during, and for sustained periods afterwards and is important for performance and ensuing recovery and remodeling alike (Sjoberg *et al.*, 2011). Importantly such hyperaemic responses also seem to be diminished in age (Donato *et al.*, 2006; Poole *et al.*, 2003), which may contribute to reduced performance and maladaptation to exercise training in older age (Greig *et al.*, 2011).

In this study show we evidence of previously undescribed LBF responses to the combination of exercise-plus-feeding where LBF in older individuals appears to be completely unresponsive to feeding and barely responsive to exercise-plus-feeding but is restored after RET; rejuvenating these responses in older individuals, even surpassing pre-training values seen in middle-aged individuals.

The mechanism for capillary recruitment, and therefore increased blood flow, by insulin is early (Vincent *et al.*, 2002), nitric oxide (NO) dependent (Clark *et al.*, 2003) and likely to be mediated at the endothelial cells of the sphincters. One possible suggestion towards a mechanism for age-related reduction and RET induced improvement in LBF surrounds pro-inflammatory cytokines. Pro-inflammatory cytokines such as TNF-α have been shown to inhibit the insulin stimulated increase in femoral blood flow, and supra-physiological levels of insulin were required to recover femoral blood flow values (Zhang *et al.*, 2003). We however, observed no age-related increases in systemic TNF-α, IL-6 or any other pro-inflammatory cytokine nor did we observe a decrease as a result of RET; leading to the conclusion that this proposition cannot explain the age-related reduction in basal femoral blood flow nor the improved blood flow responses to feeding and exercise that occurred in this study.

The mechanisms for the age-related declines in LBF and the changes that are seen with RET are probably complex and both a decrease in muscle mass (Dinenno *et al.*, 2001) and chronic vasodilation (Thijssen *et al.*, 2007; Dinenno *et al.*, 2001) have been suggested as possible contributors. Although we cannot rule it out we propose that the age-related declines in LBF responses to feeding and exercise-plus-feeding are unlikely to be related to declines in muscle mass *per se*, at least in our healthy older participants, as using DEXA we did not detect significant differences in lean mass between our age-groups and other mechanisms such as chronic vasodilation due to increased α-adrenergic tone and/ or lower O_2 demand are more likely lower candidates than lower muscle mass *per se*. Our findings are consistent with work from other groups showing no significant

differences in measures of lean mass between age-groups using DEXA and who suggested that reductions in the LBF in the elderly were due to reductions in specific oxygen demand independent of total muscle mass (Dinenno *et al.*, 1999). Computed tomography (CT) or magnetic resonance imaging (MRI) cadaver validation studies have shown these methods to have much higher accuracy in quantifying muscle mass than DEXA (Mitsiopoulos *et al.*, 1998) and reports describing sarcopenia tend to use such methods (Pahor *et al.*, 2009). One explanation for this discrepancy in measures may be that CT or MRI can quantify a hallmark of ageing, myosteatosis using the Hounsfield unit of lean tissue (Lang *et al.*, 2009) but DEXA cannot and this may have lead to an over-estimation of functional muscle mass in our older subjects when measured by DEXA. Further support for the prior notion of dissociation between muscle mass and LBF *per se* exits as in this study no group demonstrated increases in basal LBF despite displaying hypertrophy.

To try and ascertain a greater understanding of the mechanisms related to the changes in LBF with age and / or RET we looked at protein expression of PECAM-1, α-1 adrenoreceptor and β-2 adrenoreceptor in the muscle to investigate the possibility of alterations in muscle endothelium. It was surprising that we saw no differences in the protein concentration of these targets either with age or RET given that chronic α-adrenergic tone is known to be elevated in older individuals (Dinenno *et al.*, 2001; Smith *et al.*, 2007a) and that RET has been reported to increase peak vasodilatory limb capacity (Rakobowchuk *et al.*, 2005). There were also no differences either before or after RET in phosphorylation of eNOS or PKC-α despite their putative role in hyperaemia (Partovian *et al.*, 2005).

RET has been shown to cause muscle hypertrophy (Schoenfeld, 2010) and although we did not observe any gains in whole-body lean mass in our older group after RET we did see increases in upper lean leg mass which may be more pertinent when considering changes in femoral artery blood flow. Other studies have demonstrated that older people retain the ability to gain muscle mass with RET (Peterson *et al.*, 2011) which suggests that as

all of our participants underwent the same training regime it may the use of DEXA is a limitation in this study (see above). As well as promoting hypertrophy RET has also been shown to increase basal LBF (Anton *et al.*, 2006; Tanimoto *et al.*, 2009), somewhat surprisingly given the common view of such vascular adaptations being specific to endurance training (Thijssen *et al.*, 2007). Tanimoto and colleagues reported that 13 weeks of high (85-90% 1-RM) or low-intensity (50-60% 1-RM) RET were equally effective in increasing basal LBF (Tanimoto *et al.*, 2009) and it has also been shown that basal LBF in resistance trained individuals is better maintained with ageing (Miyachi *et al.*, 2005). It was therefore unexpected that we saw no increases in basal LBF in any group after RET and the age-related reduction in postabsorptive LBF was still apparent after RET even when data was expressed relative to leg lean muscle mass. This was reflected by the fact that increases in basal LVC in our older subjects after RET were principally due to decreases in MAP (rather than increases in LBF) which may have been due to an amelioration of increased sympathetic nerve activity directed to the vascular beds that has been reported in ageing (Ng *et al.*, 1993).

Although we have investigated healthy subjects, it may be proposed that increased LBF and LVC in the postprandial and post-exercise states after RET may benefit individuals with CV or metabolic conditions (Baron *et al.*, 1990) and may go some way to alleviate or reverse the anabolic blunting sometimes observed in older individuals (Cuthbertson *et al.*, 2005). The mechanism for these improvements were not discovered during this study but were not related to endothelial expansion (indexed by endothelial cell markers or androgen receptors in muscle biopsies) or to phosphorylation of eNOS (Fleming & Busse, 1999) or PKC-α (Partovian *et al.*, 2005) which are supposed components of the vasodilatory responses in the microvascular beds.

Mechanisms to explain the observed positive changes in LBF after RET need further exploration as we recognize the limitations of using only these markers in muscle biopsies. Decreased α-adrenergic tone (known to be

elevated in older individuals (Dinenno *et al.*, 2001; Smith *et al.*, 2007a) and perhaps redistribution of LBF to working muscles (i.e. improved functional sympatholysis) are attractive possibilities that may contribute to improvements in LBF and corresponding normalization of LVC in response to feeding and exercise. Also, to what extent the LBF responses to exercise and to exercise-plus-feeding correspond to increases in microvascular flow, as observed by Vincent (Vincent *et al.*, 2006) also merits further investigation with one assumption that 75% of total femoral blood flow goes to the muscle (Newman *et al.*, 2007). If this assumption were to be correct the increased leg blood flow shown after RET could have very positive implications for an ageing population with regard to metabolic and anabolic status (Phillips *et al.*, 2012).

Effect of RET on mean arterial pressure and resting heart rate

High blood pressure is one of the nine leading risk factors influencing cardiovascular disease (Anand *et al.*, 2008) and is estimated to lead to over 7 million deaths each year; about 13% of total worldwide deaths (Chobanian *et al.*, 2003), with a strong independent relationship between BP and cardiovascular morbidity and mortality down to 115/75 mmHg (Lewington *et al.*, 2002). Physical activity guidelines for hypertension traditionally centre aerobic exercise (Mancia, 2007), although as RET increases in popularity due to research showing the benefits on bone, muscle and metabolism, those interested in CV health are also beginning to promote RET as an integral part of an exercise program (Braith & Stewart, 2006).

Contrary to some negative reports on the effects of RET on BP, we found that MAP, which was lower in the young group before RET, was reduced in the middle-aged and old groups after RET to the extent that there were no differences between the age groups - effectively restoring the MAP of the older groups to that of the young. These findings are similar to those reported in meta-analyses (Cornelissen *et al.*, 2011; Kelley & Kelley, 2000) and independent studies (Moraes *et al.*, 2012), showing that both moderate intensity progressive, dynamic RET and low intensity isometric

RET (Cornelissen *et al.*, 2011) may cause a reduction in systolic and diastolic BP (which would equal a reduction in MAP). In one meta-analysis dynamic RET, as was performed in this study, was also found to have other favorable effects on CV risk factors such as an increase in VO_2 max and a reduction in body fat and plasma triglycerides (Cornelissen *et al.*, 2011), which complements our findings of lower RHR in our young and middle-aged groups after RET, with a trend for this reduction in our older group.

Although the reduction in MAP exhibited by our middle-aged and older groups after RET may seem numerically small they are significant both statistically and clinically. Using information taken from large intervention studies (Stamler *et al.*, 1991) it can be suggested that BP reductions as small as 3 mmHg can reduce coronary heart disease risk by 5%, stoke by 8% and all cause mortality by 4% (Whelton *et al.*, 2002). With this information our MAP reductions of 9 mmHg in both our middle-aged and older group strongly suggest cardiovascular benefits of RET.

While the evidence for the CV benefits of RET are still not as uniform and striking as for traditional aerobic exercise, it is gaining favor in the literature and it is more widely accepted that RET does not raise resting BP (Kelley & Kelley, 2000; Rossi *et al.*, 2012; Fahs *et al.*, 2011). While the proposed potential mechanisms for RET increasing BP are multifaceted and include 1. concentric hypertrophy due to marked repetitive increases in BP during RET, which increased left ventricular wall thickness with unchanged left ventricular chamber size 2. larger conduit arteries 3. stiffer central conduit arteries (where the mechanoreceptors are located) (Sugawara *et al.*, 2012), the major proposed mechanism for RET reducing BP is that of reduced peripheral resistance (Kelley & Kelley, 2000), aligning perfectly with the results of this study regarding LPF, MAP and LVC.

Effect of RET on insulin and glucose handling

It is not only increased LBF and the positive role that it may have in ageing with regard to muscle mass maintenance, CVD, atherosclerosis and diabetes that has been demonstrated to be improved by RET in this study as we have also demonstrated changes in both insulin and glucose handling. Glucose tolerance and utilization decline with age because of an increase in obesity (central and total) and a decline in physical activity/ muscle mass (Coon *et al.*, 1992; Kohrt *et al.*, 1992), although intriguingly the most apparent changes in plasma glucose as an index of metabolic health were observed in our young group.

Our young group demonstrated reduced postprandial plasma glucose AUC in addition to reduced peak plasma glucose and also a trend for reduced postprandial plasma insulin AUC. There was a trend in all three groups for lower peak plasma insulin after RET and when all subjects were grouped HOMA was significantly lower after RET. The older group showed a reduction in peak postprandial plasma glucose but not AUC, although importantly they, along with the middle-aged group did demonstrate reduced HOMA values following RET, restoring their value to a level not different to that of the young. HOMA is the abbreviation for homeostasis model assessment and together with android obesity, hypertension, elevated plasma triglycerides and high HDL cholesterol, can be used to diagnose the metabolic syndrome (2001).

Our findings of improvements in HOMA following RET are in keeping with those of others who found that in healthy adults (Miller *et al.*, 1994; Ryan *et al.*, 1996) and in type 2 diabetics (Kadoglou *et al.*, 2012; Misra *et al.*, 2008; Sigal *et al.*, 2007; Ibanez *et al.*, 2005; Castaneda *et al.*, 2002), RET improved glycemic control, alleviated insulin resistance (marked by HOMA) and reduced HbA1c levels and ApoB: ApoA-I ratio. Increased lean mass following RET is commonly cited as one of the key reasons for improvements in insulin sensitivity and/ or glucose clearance after RET (Ryan *et al.*, 2001), although this cannot explain all of the changes as some

groups have found improvements independent of changes in lean mass (Holten *et al.*, 2004).

An alternative potential mechanism for these improvements was suggested by Holten *et al* (Holten *et al.*, 2004) when they found that in healthy controls and type 2 diabetics, RET enhances insulin action in skeletal muscle. Alongside these findings they also found that RET increased the protein contents of GLUT4 insulin receptor, protein kinase B, glycogen synthase and also glycogen synthase activity (Holten *et al.*, 2004). These signalling changes with RET are not entirely aligned with the changes we see after RET as we see no change in basal IRS-1 in any of our age groups after RET, although we do see a signalling response after exercise-plus-feeding in our middle-aged and old groups that was not apparent before RET.

Although our results for insulin and glucose handling and the associated signalling are not as striking as those found in some studies mentioned our volunteers were all healthy individuals, able to perform tasks of everyday living and Ryan *et al* found that in older individuals more insulin resistant individuals derived the greatest benefits from RET (Ryan *et al.*, 2001). In addition to the beneficial effects on metabolic health that can be achieved through RET alone, the combination of aerobic and RET has been shown to be superior (Sigal *et al.*, 2007) and therefore combining the approached used in this study with regular aerobic training may further enhance the health benefits that RET can achieve.

CHAPTER 5- DISCUSSION AND OVERVIEW

Ageing is one of the most important social and financial problems facing western society and the UK, along with the majority of other countries in the western world have an ageing population; in which the rate of ageing does not appear to be slowing. With social, clinical and economic implications of this ageing population, many to do with the associated decline in independence maintenance for these ageing individuals, any method which may prevent this decline in health and well-being that allows individuals to sustain normal daily activity and functional independence must be developed, advocated and promoted.

Although hereditary and other pre-disposing factors to chronic diseases cannot be altered, lifestyle choices can be influential in minimizing risk factors for chronic disease, especially in the elderly. Because the effects of lifestyle choices will be cumulative throughout life it is particularly important for older people to adopt diet and lifestyle practices that will improve their chances of healthy, active independent ageing.

Sarcopenia, the age-associated loss of muscle mass is a widespread syndrome that can cause frailty, decrease mobility, increase the likelihood of falls and even have the effect on BC of increasing % body fat, which itself is associated with negative health outcomes. RET may be one vehicle by which to alleviate progressive sarcopenia and may even go some way to reversing the muscle mass losses experienced by a condition that has been questioned to be due to “the physiology of disuse.”

Something which is often associated with sarcopenia but can also be an independent feature of ageing, and may negatively contribute to risk factors for chronic diseases is the redistribution of body fat with ageing. Ageing is not only associated with the loss of FFM and an increase in FM it is also associated with the preferential deposition of body fat in the trunk region which may potentially lead to android obesity; associated with heightened CVD and metabolic syndrome risk. Ageing is also associated

with a greater proportional deposition of visceral fat which increases the risk of all diseases linked to a centralized pattern of fat distribution.

Reduced BMC and BMD which are also associated with ageing may increase the risk of bone-related disorders such as osteoporosis and osteoarthritis.

Another age associated change is the change in limb blood flow and its responses to anabolic stimuli, experienced with age. Although the mechanisms behind this are not completely understood it is likely that the muscle mass loss experienced with sarcopenia cannot be the only explanation as lower limb blood flow is experienced in the elderly even when values are expressed relative to lean leg mass.

For the elderly, it is believed that maintenance of muscle mass and strength as we age leads to much improved functional capacity and quality of life (Tipton, 2001). With exercise seeming more effective at preventing muscle loss than restoring lost muscle mass (Wolfe, 2006), the strength gains that have been shown in our subjects only serve to show that if these strength gains can be made in 20 weeks, then the advocating and promotion of habitual RET, especially for those advancing in age may go a long way to helping prevent some of the associated muscle loss and associated strength and function losses that are experienced by many elderly people.

Sarcopenia can gravely affect quality of life and causes considerable morbidity. As skeletal muscle is the primary site for numerous metabolic functions, sarcopenia may contribute to negative conditions of peripheral insulin resistance, dyslipidaemia and increased adiposity. Any condition detrimental to the health of elderly individuals increases their risk of hospitalization, need for care and/ or disease impacting on the independence maintenance of the elderly individuals in question.

It is not only muscle mass, strength and endurance, but also power output that declines with ageing and associated sarcopenia and this may be

important in the incidence of falls and the inability to perform weight bearing tasks. These tasks may be as simple as raising from a chair or bed, something which the majority of healthy people would take for granted, yet are crucial for independent living.

For elderly people fear of falling and the potential outcomes of these falls are very real, with many elderly people needing hospitalization and/ or home-help following a fall. Whipple and colleagues found that the strength of elderly people who suffered falls was significantly lower than in elderly people who did not fall (Whipple *et al.*, 1987); another reason why the promotion of RET in the elderly may have both clinic and social implications for an ever growing section of society.

Although some of the risk factors for sarcopenia cannot be altered, such as advancing age or female sex, physical inactivity can be and the results of this study illustrate the benefits of RET in helping to alleviate sarcopenia in the important weight bearing lower limbs. Any method by which sarcopenia may be reduced should be embraced and promoted.

RET has been well documented to have health benefits based on improvements in muscular strength and positive changes in BMD, both of which can off-set the development of age-related conditions such as sarcopenia, osteoporosis and the frailty syndrome.

Our results have shown that a 20 week RET program elicits strength gains in all of the major muscle groups trained; a determinant of muscle function, bringing the whole-body strength of the old after RET to a level not different to the young before RET. As strength has been shown to be negatively correlated with premature mortality these strength gains serve as an indicator of positive changes in health status following RET.

Decreased trunk fat brought about by a 20 week RET program may lower the risk of CVD and associated conditions such as type 2 diabetes and the metabolic syndrome. These benefits may be further enhanced by the

reduction in whole body % body fat that has been shown to occur with the 20 week RET program in our completed subjects.

The change in basal leg blood flow and the increased responses to feeding and to exercise with feeding shown by our completed subjects suggests that a 20 week RET program is associated with positive effects on leg blood flow *per se* and also improves the leg blood flow responses to feeding alone and to a single bout of resistance exercise combined with feeding. A response to feeding can have significant implications as for the average person a relatively large proportion of the waking day is spent in the postprandial state. The clinical implications that may be associated with increased leg blood flow include: an improved clearance of lipoproteins and alleviation of age-associated reductions in muscle glucose uptake and insulin sensitivity, which are key features of the metabolic syndrome, type 2 diabetes and a factor that potentially contributes to CV disease. Delivery of oxygen and nutrients to the muscle are also likely increased with improved leg blood flow and this may play a role in overcoming the anabolic blunting observed with age.

The results from this study show that 20 weeks RET can lower MAP, a risk factor for CVD if elevated and improved fasting metabolic status and glucose handling in older individuals.

Significant, novel findings of this study are the positive effects of RET on limb blood flow in response to exercise and the combination of exercise-plus-feeding. The possible advantages that this may have are already discussed and knowledge of the distribution of this blood flow i.e. nutritive vs. non-nutritive blood flow, would allow us greater insight into the possible health benefits of this improved blood flow caused by RET.

There is evidence to support the idea of two vascular routes either within, or closely related to skeletal muscle. One route has intimate contact with muscle cells and is known as ‘nutritive’, the other ‘non-nutritive’, acts as a vascular shunt and is thought to be, at least in part, the vessels in closely

associated connective tissue that nourishes fat cells attached to the muscle (Clark *et al.*, 2000). The presence of these two vascular routes in skeletal muscle has two main consequences; 1. In relation to energy balance, the proportion of flow between the nutritive and non-nutritive routes sets the BMR as an increase in the proportion of total flow that is nutritive increases basal energy consumption. 2. The presence of two vascular routes effects fuel partitioning within the muscle. As the non-nutritive route supplies connective tissue and associated adipocytes and the nutritive route the muscle cells, a high nutritive: non-nutritive flow ratio favors delivery of nutrients and hormones to the muscle cells. However if a high non-nutritive flow remains nutrient delivery reaches the connective tissues and fat cells. Although these cells are much less metabolically active than muscle cells, nutrients such as glucose, insulin and triglycerides will still be utilized enabling fat cell growth (Clark *et al.*, 2000). A study measuring leg *muscle* blood flow using a contrast-enhanced ultra sound (CEUS) would allow us to examine the blood flow into the muscle (nutritive flow) and if the magnitude of change following RET was the same as for whole leg blood flow this may further advocate clinical benefits of RET as improved nutritive leg blood flow may be associated with; an improved clearance of lipoproteins, alleviation of some of the age-associated reductions in muscle glucose uptake and insulin sensitivity and improve nutrient and oxygen delivery to the muscle which may be key for MPS.

Any positive changes in health-related markers measured in this study can only serve to act as evidence for the benefits of physical activity, specifically RET. The economic, clinical and social implications of physical inactivity are well known and positive results of physical activity based research can only act to drive forward the concept of physical activity for health.

Based on the results of this study there is scope for future work to alter the length and/ or the intensity of the RET intervention, to establish what is the minimum time-frame or intensity needed to obtain the positive outcomes that we observed. Although advocating habitual RET would be optimal, to

try and maximize reduction in risk factors for chronic disease and the maintenance of independence in the elderly there may be clinical situations (i.e. pre-operative or post-fall) in which it would be beneficial to have knowledge of the minimum time-scale or work intensity needed to elicit these gains in the elderly.

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Awards

2012 Young Investigator Award (Oral Presentation) - European College of Sport Science; Annual Congress.

2012 International Early-Career Physiologist Travel Award - The American Physiological Society; Experimental Biology Meeting.

2010 International Early-Career Physiologist Travel Award - The American Physiological Society; Experimental Biology Meeting.

2009 The Physiological Society Blue Riband Presentation Prize - The Physiological Society; Annual Meeting.

2008 1st Prize Presentation Award - University of Nottingham; Integrated Systems Biology and Medicine Meeting.