Understanding the role of physical activity, physical performance and dietary protein intake on muscle mass and insulin resistance in seniors

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1 Abstract

The decrease in physical performance (PP) with aging is in part mediated through body composition (muscle/fat) changes. With aging there is a gain and central redistribution of fat depots and a loss of lean tissue, mainly skeletal muscle. The loss of muscle mass has been implicated in the risk of developing insulin resistance. Physical activity could act as both a predictor and an outcome of PP and is a modulator as well as of insulin resistance. Age, sex, energy intake and chronic diseases can also affect PP and insulin resistance. Dietary protein intake is considered an easy and inexpensive modality to combat loss of muscle mass. Contrary to plant source of protein intake however, animal source of dietary protein may confer an increased risk of insulin resistance and diabetes. Determining insulin resistant subjects in epidemiological studies is challenging because of the lack of cut-off for scores used to assess insulin sensitivity. Analyses of effects of all interrelationships are complex and leads to challenging interpretations.

Objectives: Our first objective was to explore the complex interrelationships of body composition, physical performance, physical activity, protein intakes and insulin resistance using path analysis. Our second objective was to find out subjects who were insulin resistant subjects over a 3-year period and compare them to insulin sensitive subjects in regard to body composition measures and other baseline characteristics.

Participants: A sample of elderly men and women, non-diabetic, community-dwellers participants of the Quebec Longitudinal Study on Nutrition and Successful Aging (NuAge Study).

Methods: Tests employed to assess PP were analyzed by principal component analysis and generated two indices, one related to strength and the other to mobility. Muscle mass index (MMI; kg/height in m^2) and % body fat were derived from dual X-ray absorptiometry and bioimpedance analysis. Physical

activity was assessed by the Physical Activity Scale for the Elderly (PASE), energy intakes and protein intakes were calculated from three non-consecutive 24h-food recalls. Insulin resistance was estimated based on the Homeostasis Model Assessment (HOMA-IR) score. Proposed models associating these variables were tested for validity with the NuAge data using path analysis and employed trajectory analyses to established incidence of insulin resistance.

Results: MMI and % body fat were both negatively associated with mobility score, however, muscle mass was positively associated with strength independently of other variables. Physical activity was significantly positively and negatively associated with both MMI and % body fat respectively. Mobility was significantly positively associated with PASE score. Direct positive associations were observed for HOMA-IR score with MMI and % body fat. Physical activity was significantly positively associated with muscle mass. The direct, positive associations of animal protein intake with MMI and HOMA-IR were not significant. There was a significant, direct negative association for plant protein intake with MMI, whereas there was no association with HOMA-IR. Physical activity was also negatively associated with fat mass. Of interest, there were significant, positive indirect associations between animal protein intake and HOMA-IR score and significant negative indirect associations between plant protein intake and HOMA-IR. These indirect associations were mediated through MMI and % body fat. In the longitudinal analyses, 7 group based trajectories were identified and good posterior probabilities were obtained. A visual inspection of the curves and the posterior probabilities obtained, allowed for determination of insulin sensitive subjects and classification of insulin resistance subjects. The logistic regression with the most parsimonious model provided only 3 significant predictors of insulin resistance: higher MMI, higher body fat% and being a man.

Conclusions: Muscle mass was associated with strength but contrary to expectations, it was also positively associated with HOMA score. This relationship is counterintuitive since it suggests muscle mass with aging is

positively associated with insulin resistance. Of interest, there were significant, positive indirect associations between animal protein intake and HOMA-IR score and significant negative indirect associations between plant protein intake and HOMA-IR. These indirect associations were mediated through MMI and % body fat. Our longitudinal analyses showed that a higher muscle mass, % body fat and being a man contribute to a higher odd of insulin resistance with aging over time.

2 Résumé

La diminution de la performance physique avec l'âge est en partie affectée par la composition corporelle (masse maigre/masse adipeuse) qui s'effectue chez les personnes âgées. Le vieillissement est aussi associé à des changements de la composition corporelle se manifestant par un gain de masse grasse et une redistribution aux milieux viscéraux, ainsi qu'une perte de la masse musculaire. La perte de la masse musculaire a été impliquée dans le risque du développement de la résistance à l'insuline. L'activité physique pourrait prédire ou être le résultat de la performance physique et est aussi associée à une augmentation de la sensibilité à l'insuline. L'âge, le sexe, l'apport énergétique et les maladies chroniques pourraient aussi affecter la performance physique aussi que la résistance à l'insuline. L'apport en protéines est considéré comme étant une approche facile étant capable de combattre la perte de la masse musculaire. Il fut proposé que les protéines de source animale soient associées à un risque plus élevée de la résistance à l'insuline et du diabète, les protéines de source végétales étant moins néfaste à cet égard. D'autre part, la détermination de sujets insulino résistants est difficile dans les études épidémiologiques à cause du manque de valeurs seuils. L'analyse de tous ces facteurs est complexe et peut aboutir à des conclusions difficiles. **Objectifs**: notre premier objectif fut d'explorer les interrelations complexes de la composition corporelle, la performance physique, l'activité physique, l'apport en protéines et la résistance à l'insuline en utilisant des statistiques d'analyse de chemin. Notre second objectif fut de déterminer les sujets insulino résistants versus non insulino résistants avec le temps et comparer la différence des associations des deux groupes avec les facteurs de base. **Participants** : un échantillon de sujets non diabétiques composé d'hommes et de femmes en bonne santé, vivant en communauté, et faisant part de l'étude longitudinale sur la nutrition et le vieillissement réussi (NuAge). **Méthodes** : les tests employés pour mesurer la performance physique ont été analysés par analyses de composantes principales et ont généré deux indices : force et mobilité. L'indice de masse musculaire (MMI; kg/m^2) et le pourcentage de la masses

adipeuse ont été mesurées par DXA (Dual energy X-ray absorptiometry, technique de scan qui mesure précisément la minéralisation osseuse, la masse grasse et la masse musculaire) et par impédance bioélectrique (BIA). L'activité physique fut mesurée par un questionnaire pour les personnes âgées (PASE, Physical Activity Scale for the Elderly). Les apports en énergie et en protéines furent mesurés par trois rappels de 24 heures non consécutifs et saisis en utilisant le logiciel CANDAT. La résistance à l'insuline fut déterminée par le score HOMA-IR (Homeastatic model of assessment). Les modèles proposes ont été testes pour leur validité avec des données provenant de l'étude NuAge an utilisant des statistiques d'analyse de chemin. Des analyses de trajectoires ont été conduites afin de déterminer l'incidence de la résistance à l'insuline. **Résultats** : une association négative statistiquement significative fut observée entre l'indice de mobilité avec MMI et le pourcentage de masse adipeuse. La masse musculaire fut positivement associée avec la force. L'activité physique fut associée positivement avec le MMI et négativement avec le pourcentage en masse grasse. Des associations positives et statistiquement significatives furent observées entre HOMA-IR et MMI et HOMA-IR et %de masse grasse. L'apport protéique de source végétale fut négativement associé avec MMI (association statistiquement significative) et positivement avec le HOMA-IR (association non significative). L'association de protéines de source animale fut positive avec HOMA-IR et positive avec MMI, mais ces associations n'ont pas atteint de valeur statistiquement significatives. Les associations indirectes entre les différents apports protéiques et le HOMA-IR en passant par MMI et pourcentage de masse grasse sont dans la même direction que les associations directes et ont atteint une association statistiquement significative. Les analyses longitudinales ont identifié 7 groupes parmi lesquels ont pu identifier les participants insulino résistants versus non insulino résistants avec le temps. Les résultats de la régression logistique ont permis de montrer les prédicateurs de la résistance à l'insuline qui sont le MMI, le pourcentage de la masse grasse et le sexe. **Conclusions** : nous avons trouvé une relation positive contre-intuitive entre la masse musculaire et le

HOMA-IR. Les apports en protéines ont été associées différemment HOMA-IR en passant par MMI et pourcentage de masse grasse suggérant que les protéines de sources végétales sont associées à un HOMA-IR réduit et que le contraire fut observé avec les protéines de source animale. Les analyses longitudinales ont montré que la masse musculaire et le pourcentage de masse grasse contribuent à une chance plus élevée de développer la résistance à l'insuline avec le temps. Le sexe masculin fut associé à un risque plus élevé de développer la résistance à l'insuline.

3 Statement of support

Funding for this study was provided through a grant from the Canadian Institute for Health and Research (CIHR).

Ms. Matta was financially supported by a stipend from the Research Institute of the McGill University Health Centre provided by the Zilinksas Family for the Division of Geriatric Medicine.

4 Advance of scholarly knowledge

4.1 Original contribution to knowledge

This doctoral dissertation is the first study in Canada to examine the free-living healthy elderly, with precise and comprehensive dietary intake data, body composition and functional performance measures to address complex and relevant clinical situations regarding physical performance and insulin sensitivity with aging.

We were able to understand the complexity of physical capacity, muscle mass, protein intake and insulin resistance in healthy community-dwelling elderly. We were also able to provide two different models relating all complex measures and factors occurring with aging, one focusing on physical performance and the other focusing on body composition, protein intake and insulin resistance. We found that physical activity was highly related to mobility components in the healthy elderly. Moreover, mobility was highly associated with a decreased body fat % and increased strength component. Physical activity modulated body composition favorably. On the other hand, we found that increased muscle mass in the aged population is associated with higher insulin resistance score. It was clear that diverse protein sources were differently associated with body composition and metabolic profile components. Further, we were also able to dissociate insulin resistant versus non-insulin resistant individuals over time by conducting trajectory analyses, and were able to understand some of the baseline characteristics differentiating these individuals.

4.2 Research manuscripts in preparation for submission to refereed journals

Manuscript 1

Interrelated factors favouring physical performance and activity in older adults from the NuAge cohort study

Joane Matta, Nancy Mayo, Isabelle Dionne, Pierrette Gaudreau, Tamas Fulop, Daniel Tessier, Katherine Gray-Donald, Bryna Shatenstein, Jose A Morais.

Manuscript 2

The Homeostatic Model Assessment of insulin resistance (HOMA-IR) score is negatively associated with muscle mass index and protein intake in elderly participants of the Quebec Longitudinal Study on Nutrition and Successful Aging (NuAge)

Joane Matta, Nancy Mayo, Isabelle Dionne, Pierrette Gaudreau, Tamas Fulop, Daniel Tessier, Katherine Gray-Donald, Bryna Shatenstein, Jose A Morais.

Manuscript 3

Trajectory model of the Homeostatic Model Assessment of insulin resistance (HOMA-IR) score and its association with body composition measures in elderly participants of the Quebec Longitudinal Study on Nutrition and Aging NuAge

Joane Matta, Nancy Mayo, Isabelle Dionne, Pierrette Gaudreau, Tamas Fulop, Daniel Tessier, Katherine Gray-Donald, Bryna Shatenstein, Jose A Morais.

4.3 Abstracts and presentations

Methodological challenges in developing indices of physical capacity in healthy elderly people from the NuAge study.

Joane Matta, Carolina Moriello, Nancy Mayo, Pierrette Gaudreau, Helene Payette, Jose A. Morais and other investigators of the NuAge study.Canadian Association of Gerontology Montreal, Dec 2010 and McGill Nutrition Research Day, March 2011. (Oral)

Components of body composition affect differently physical capacity in elderly participants of the NuAge study.

Joane Matta, Carolina Moriello, Nancy Mayo, Pierrette Gaudreau, Jose A. Morais and other investigators of the NuAge study. Canadian Geriatrics Society, Vancouver April 2011. (Oral)

The Homeostatic Model Assessment of insulin resistance (HOMA-IR) score is associated with muscle mass index in elderly participants of the Quebec Longitudinal Study on Nutrition and Aging NuAge.

Joane Matta, Isabelle Dionne, Helene Payette, Pierrette Gaudreau, Nancy Mayo, Tamas Fulop, Daniel Tessier, Katherine Gray-Donald, Bryna Shatenstein, Jose A Morais. Canadian Nutrition Society Conference, Guelph June 2011. (Poster)

Insulin resistance is associated with muscle mass and sources of protein intake in elderly participants of the Quebec Longitudinal Study on Nutrition and Aging (NuAge)

Joane Matta, Nancy Mayo, Isabelle Dionne, Helene Payette, Katherine Gray-Donald, Pierrette Gaudreau, Tamas Fulop, Daniel Tessier, Bryna Shatenstein, Jose A Morais. Canadian Geriatrics Society, Quebec City April 2012. (Oral)

5 Contribution of authors to manuscripts

For manuscript one, the candidate learned factor and principal component analyses, path analysis and structural equation modeling for the purpose of analyzing her research question. The candidate developed the research question and developed a model for testing using the database available. The candidate familiarized herself with new statistical concepts and procedures to be able to answer her intended hypothesis. She verified the data and wrote the first draft of manuscript one.

For manuscript two, the candidate applied her statistical knowledge to the second part of her complex model. The candidate participated in organizing the analysis of the insulin resistance score, entered all related data. The candidate developed the research question and developed a model for testing using the database available. She verified the food groupings related to protein intake sources and worked on the best model that would answer and verify her hypotheses. The candidate verified the data and wrote the first draft of manuscript 2.

For manuscript 3, the candidate learnt epidemiological analyses and longitudinal analyses procedures. She verified the data, developed the research question and wrote the first draft of manuscript 3.

Supervisors and committee members

Dr Jose A. Morais, the candidate's supervisor, contributed to the development of the concepts and design of the 3 studies. Dr Morais provided all essential laboratory materials for the assessment of insulin resistance of the participants of the NuAge study within his laboratory. He was a key-person in selecting variables and accessing the NuAge data. Dr Morais supervised interpretation of data for all manuscripts. He also contributed in writing and editing the manuscripts presented in this thesis.

Dr. Nancy Mayo, the candidate's co-supervisor, contributed to the development of the concepts and designs of the 3 studies in this thesis. Dr. Mayo supervised statistical

analysis and interpretation of data for all manuscripts. She also assisted in writing and editing the manuscripts presented in this thesis.

Dr Katherine Gray-Donald, the candidate's committee member contributed to the development and formulation of hypotheses for the second and third manuscript. She contributed especially in the nutritional content of the thesis work. Analyses of the 24-hour food recalls and different protein sources of the participants were done under her supervision. Dr Gray Donald also helped in editing and writing the manuscripts presented in this thesis.

Drs Pierrette Gaudreau, Bryna Shatenstein, Isabelle Dionne, investigators of the NuAge study, helped in editing and writing manuscripts 1 and 2 presented in this thesis.

6 Acknowledgements

Looking back, I am so grateful for what I have learned throughout my PhD years at McGill. The experience was demanding but rewarding at the same time.

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7 Dedication

To my mum and dad Hoda and Michel, my wonderful husband Julien and the best brother, sister and niece in the world Johnny, Jessica and Elsa

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9 List of tables

10 List of figures

11 List of abbreviations

25 (OH) D: 25-hydroxyvitamin D

3MS: Modified mini mental state examination

AA: Amino acid

ADL: Activities of daily living

ASMM: Appendicular skeletal muscle index

BIA: Bioelectrical impedance

BMI: Body mass index

CCK: Cholecystokinine

CFI: Comparative fit index

CNORM: Censored normal model

CRP: C - reactive protein

CT: Computed tomography

DXA: Dual energy x-ray absorptiometry

EAA: Essential amino acids

EPIDOS: Epidemiology of Osteoporosis

GH: Growth hormone

GLUT: Glucose transporters

HOMA: Homeostatic model of assessment

IADL: Instrumental activities of daily living

IGF-1: Insulin like growth factor-I

IL-6: Interleukine 6

IMCL: Intramyocellular lipids

IR: Insulin resistance

IRS: Insulin receptor substrates

KPa: Kilopascal

M: Men

MAPK: Ras-mitogen-activated protein kinase

MMI: Muscle mass index

MPS: Muscle protein synthesis

MRI: Magnetic resonance imaging

NFI: Normed fit index

NFκB: Nuclear factor *kappa*-light-chain-enhancer of activated *B* cells

NHANES: National Health and Nutrition Examination Survey

NPY: Neuropeptide Y

NuAge: Quebec Longitudinal Study on Nutrition and Successful Aging

OR: Odd ratio

PASE: Physical activity scale for the elderly

PCA: Principal component analysis

PI3K-AKT/protein kinase B: Phosphatidylinositol 3-kinases-protein kinase B

PP: Physical performance

PTB: Phosphotyrosine binding molecule

PTH: Parathyroid hormone values

RAMQ: Quebec Medicare Database

RDA: Recommended dietary allowances

RIA: Radioimmunoassay

RMSEA: Root mean square error of approximation

ROS: Reactive oxygen species

SD: Standard deviation

SEM: Structural equation modeling

SH2: Src Homology 2

SMI: Skeletal muscle index

SOCS: Suppressor of cytokine signalling

SRMR: Standardized root mean square residual

TBW: Total body water

TNF-α: Tumor necrosis factor

TUG: Timed up and go

VO2 max: Maximum oxygen consumption

W: Women

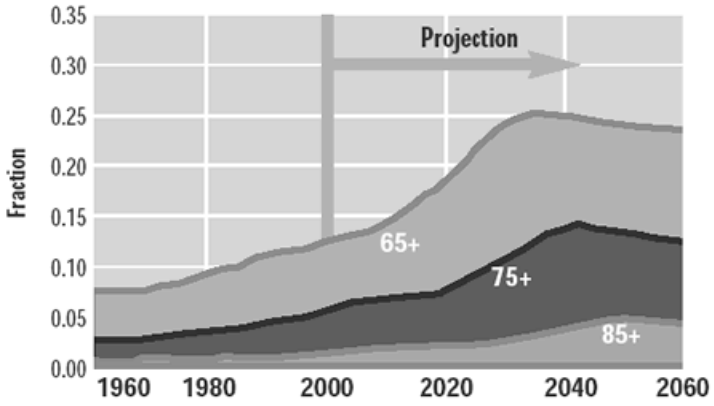
12 Chapter 1: Introduction

12.1 Background

12.1.1 Population changes

The aging of the population is increasing worldwide. In Canada, there is a trend towards a secular decline in mortality and an increase in life expectancy at birth that will continue to rise in the coming years. Since 1960, the proportion of seniors in the Canadian Society has increased from 8% to 14% in 2009. By projecting this increase to the coming years, the proportion of seniors aged 65 years and older will keep on rising to reach 23-25% in 2036 and 24-28% in 2061[1]. Figure 1 from Health Canada[2] shows the projection of the Canadian population by age fraction. In 2006, the mean age in Canada reached 38.8 years compared to 38.5 in 2005 and to 37.2 in 2001. It is estimated that in the year 2056, the mean age would reach 46.9 years[2].

Figure 1: Projection of the senior fraction of the Canadian population from 1960-2060

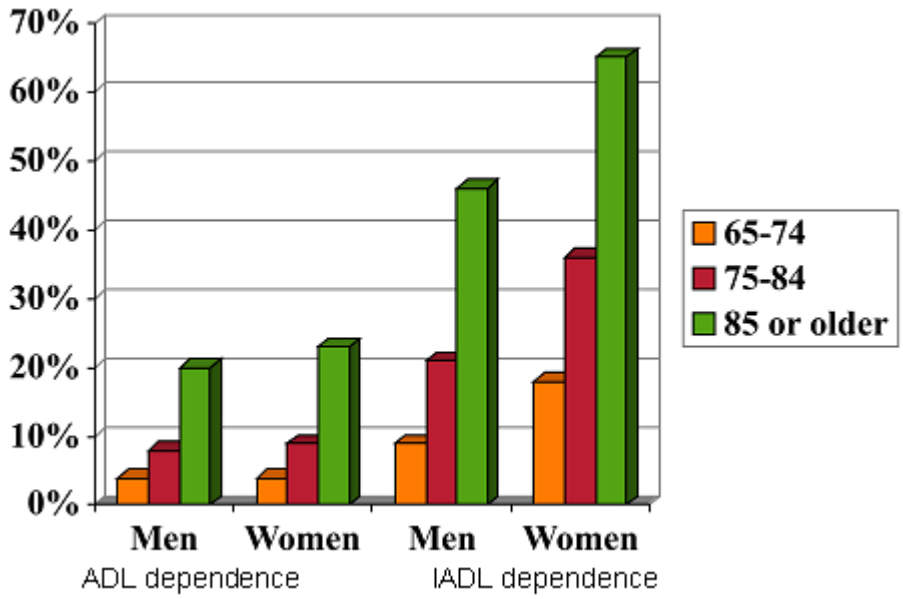


Source: Health Canada 2001[2].

The increase of the aging population adds to the public health burden. Problems of disability[3] (Figure 2) and chronic diseases[3] (Table 1) that greatly affect the senior portion of the society compared to young individuals, render the medical

care and the need for supportive and long term care very important[4]. It becomes consequently essential to address and understand factors related to problems of functionality and independence.

Figure 2: Consequences of aging to senior's functionality in Canada



Source: Adapted from Gilmour et al. [3]

Note: ADL= activities of daily living[5]; IADL=instrumental activities of daily living[6]; Frequently used indicators of physical disability

Table 1: Consequences of aging to senior's health in Canada. Prevalence of chronic conditions by age group population aged 30 or older.

Age	**30-64**	**65+**
Diabetes	4.4%	13.5%
Cancer	1.4%	5.5%
Heart disease	3.5%	19.8%
Stroke	0.6%	4.5%

Source: adapted from Gilmour et al [3].

12.1.2 Changes of body composition with aging and its relationship with insulin resistance

A change in the distribution of muscle/fat occurs with aging, with an increase in fat mass proportion and a decrease in fat-free mass mainly skeletal muscle mass; the latter referred to as sarcopenia[7] when it attains a certain threshold. Baumgartner et al. [7] defined the threshold to be a height-adjusted appendicular muscle mass of two or more standard deviations below the mean of young adults.

Data from the New Mexico Aging Process Study showed that >50% of persons over 80 years of age would be classified as being sarcopenic[7] and results from the National Health And Nutrition Examination Survey (NHANES III) showed a prevalence of 17% in the population over 60 years[8]. Data from the NHANES III showed that the likelihood of disability and functional impairment in sarcopenic individuals was twice as high as those with normal skeletal mass[9]. It has been shown that sarcopenia is associated with an increased risk of morbidity and mortality[10] and its estimated health care costs to be 18.5 billion in the year 2000 and this cost will continue to rise owing to the aging of the population[11]. Estimates from the World Health Organization (WHO) suggest that sarcopenia

affects more than 50 million people today and that it will affect more than 200 million people over the next 40 years[12].

Apart from the burden that loss of muscle mass or sarcopenia could impact on physical function and physical performance, loss of muscle mass has been hypothesized to be associated with negative metabolic outcomes[13]. The loss of muscle mass, the latter being the major organ of insulin mediated glucose disposal[14] could also have a metabolic impact on the body and could alter the function of insulin. Insulin is a hormone involved in the metabolism of glucose, lipids and proteins[15]. Insulin regulates glucose transport and utilization; a deregulation of its function, a condition termed insulin resistance (IR) could lead to type 2 diabetes[16] a disease state shown to increase with age (NHANES III prevalence of diabetes > 75 years of 13% and glucose intolerance of 14%)[17] and known to be associated with an increased risk of cardiovascular health problems, the primary cause of mortality in the elderly[18]. Insulin resistance is also linked to the metabolic syndrome[19], which predicts cardiovascular diseases[18], stroke[20] and type 2 diabetes[16], all-important causes of disability and mortality in the elderly. Sarcopenia and insulin resistance are both serious conditions that could interfere with the person's functional status and could lead to morbid outcomes, whether from sarcopenia alone, or from the direct effect of sarcopenia on the function of insulin. There has been several approaches proposed to stop the progression of sarcopenia such as hormones replacement therapy (testosterone implicated in muscle anabolism)[21], growth hormone (stimulates growth and cell reproduction)[22] and others. However, these clinical interventions have yielded different results and there are controversies surrounding the use of these techniques. A study by Blackman et al.[22] showed that administration of growth hormone for the treatment of obese elderly improved their glucose and lipoprotein profile, as well as increased their fat-free mass and decreased their fat mass. However, since growth hormone is an anti-insulinemic hormone, twice as many subjects became diabetic after 26 weeks of growth hormone treatment than in the placebo group. Another way that has been

proposed to combat sarcopenia is resistance training exercise[23, 24]. However, it appears (with some controversies)[25, 26], that resistance training alone is not sufficient to enhance muscle hypertrophy if not coupled with adequate energy or protein intake. A study by Kumar et al.[27] showed that the elderly population was able to increase muscle protein synthesis (MPS) with resistance training exercise, however this increase was still less than that of the young showing that elderly people could not get the same MPS as that of the young. Drummond et al.[28] however were able to demonstrate that an intake of 15 g of essential amino acids (EAA; amino acids that are not produced endogenously and need to be provided in the diet) coupled with resistance training showed a protein synthesis in the elderly equal to that of the young, suggesting that an intake of essential amino acids could act (but not alone) as a very important factor contributing to an increased efficiency of MPS in the elderly population. Besides, the higher prevalence of frailty in the elderly population[29] (30% after 90 years of age) renders resistance training difficult to implement and thus an optimal amount of high quality protein intake could make an option to combat sarcopenia.

It has been proposed by Rennie et al.,[30] that 80% of the effect of MPS is attributable to amino acids. Paddon Jones et al.[31] consider that "muscle deposition occurs as a complex interplay of physical activity and hormones; however the prerequisite is dietary derived amino acids". Thus, the focus on protein intake (taking into account an optimal energy intake to spare the proteins for muscles) could make a good approach to combat sarcopenia. There have been many controversies on the recommended intake of protein in elderly people [32-34]. In fact one of the most important things that have been criticized concerning the recommended dietary allowances (RDA) for protein in the elderly population is that it has been extrapolated from protein requirements of that of the young population[35]. And, with the changes of body composition that affect the aged population, the RDA of 0.8g/kg body weight of protein may not be sufficient to maintain an optimal MPS, and over the years, this slight decrease in synthesis could lead to the development of sarcopenia[36]. There is still no consensus

regarding the total protein intake for the aged population, and several authors are emphasizing as well on the quality of protein intake which could make an important factor for the maintenance of an optimal MPS in the elderly[37, 38]. Protein types are usually divided into high quality proteins or low quality protein. The quality refers to the content of essential amino acids that appear to be highly concentrated in animal protein sources and to a lesser extent in vegetable protein sources; it has been shown that the higher the EAA content in the diet the higher the MPS in men and women[39].

Pannemans et al.[37] showed that elderly women who consumed a diet high in animal protein achieved a higher net protein balance and less protein degradation than those consuming a diet high in vegetable protein. Similarly, Lord et al.,[39] in a cross sectional observational study of 38 healthy older women, showed that protein intake from animal sources was the only independent predictor of muscle mass index explaining 19% of its variance.

There also appears to be differences in the amino acids to stimulate MPS. In fact, the primary essential amino acid that has been shown to promote protein synthesis is the branched chain amino acid leucine, which appears to be highly bio available and able to escape splanchnic uptake[40] (which reduces the availability of the amino acid in the peripheral tissue and consequently muscle uptake). Large amounts (35-40 g) of an oral or intravenous amino acid (AA) mixture of leucine have been shown to stimulate muscle protein synthesis equally in elderly persons[41]. Studies in young men reported stimulation of MPS by intravenous EAA but not by non-EAA[42]. Even adding non-EAA to an oral mixture of 18 g of EAA did not further increase in muscle protein anabolism[43]. Leucine is higher in animal protein sources and meat based diet are considered to be higher in EAA content.

Supplemental intake of proteins (coupled or not with resistance exercise) has also been suggested as a possible way to delay the progress of sarcopenia, however, according to Paddon Jones et al.[31] food sources of proteins are highly available

and easily accessible, without necessarily being expensive. Besides, it appears that certain supplements could act as a meal replacement resulting in no change in total energy or protein intake. In these instances, diet could have an advantage over supplements and provide an easy and inexpensive possible way to combat sarcopenia.

On the other hand, the positive effect of protein intake on muscle mass seems to be offset by insulin resistance. Epidemiological data on long-term high protein intake support the notion that it might lead to impaired glucose metabolism, IR and type 2 diabetes[44, 45]. Many mechanisms have been proposed, including an increased glucagon secretion, gluconeogenesis and reduced glucose disposal [46]. For example, human studies of intravenous amino acids have induced lower uptake of glucose by the peripheral tissues[47]. It is still unclear however, if those negative effects of protein intake on IR could be attributed to all sources of proteins. While dietary intake of total and animal protein intake (mean intake of 1.1g/kg/day) increased the risk for type 2 diabetes after adjustments for fat intake and adiposity, plant protein intake, on the other hand, was not associated with IR[44].

We can clearly state that there is a paradigm concerning these relationships and that the optimal dietary protein recommendations for the elderly population to minimize IR or sarcopenia development remain to be determined. The recommendations need to target a minimization of the effect of sarcopenia while preventing IR development.

12.2 Rationale

Low muscle mass may be linked to a low insulin sensitivity. Because of the opposing effect that protein intake has on insulin sensitivity and muscle mass and because still, there is no consensus on what is the best level of protein intake for preventing muscle loss nor a consensus exists on what is the increased risk of dietary protein on IR, we propose to understand these complex interrelationships.

Our proposed study is even more justified as some studies have even shown a beneficial intake of amino acids on insulin sensitivity[48, 49].

To date, there is no consensus regarding these effects, and to our knowledge no study has yet determined the effect of all these variables combined together taking into consideration physical performance and physical activity. We intend to perform a path analysis of an existing prospective data set of a healthy elderly population to examine these interrelationships between the variables in a proposed model.

12.3 Statement of purpose

12.3.1 Hypotheses and objectives

The main hypotheses of the present dissertation are:

1) Muscle mass is positively associated with insulin sensitivity independently from adiposity.

2) A dietary protein intake and particularly animal protein intake is positively associated with muscle mass.

3) In contrast, animal protein intake has as negative association with insulin sensitivity independently from adiposity.

4) Personal factors (age, sex, and presence of chronic conditions) and energy intake will impact on body composition which in turn will affect physical performance which will determine the level of physical activity. Physical activity on the other hand shapes body composition.

Accordingly, the objectives of this thesis research are:

1) To understand the complex interrelationships of body composition, physical performance and physical activity.

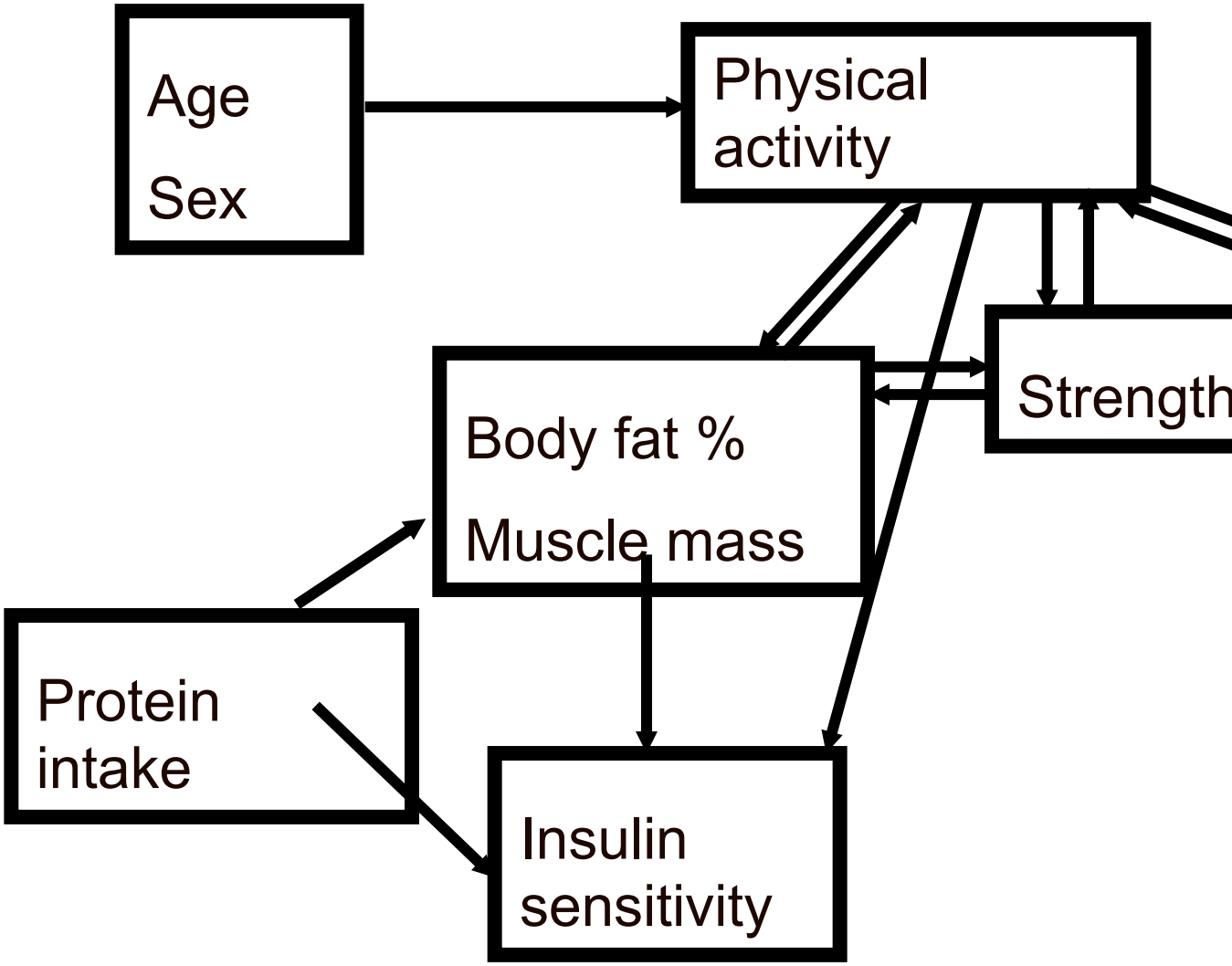
2) To understand the complex interrelationships of dietary protein intake, muscle mass and insulin resistance.

3) To understand how body composition components, protein intake from both animal and plant sources, as well as personal factors, would impact insulin sensitivity longitudinally.

12.3.2 Model to illustrate hypotheses

We propose the following model to illustrate our hypotheses:

Figure 3: Proposed model for thesis research



13 Chapter 2: Review of the literature

13.1 Definition of sarcopenia

Rosenberg[50] defined sarcopenia to be the age related decline in muscle mass among elderly individuals. The term sarcopenia is derived from the Greek word sarx (flesh) and penia (loss). Several longitudinal studies showed that muscle mass decreases by 6% per decade beginning at approximately 45 years of age[51]. According to Janssen et al.[51], even healthy adults will lose muscle mass with aging. However the difference of the impact of this muscle loss lies in the peak muscle mass attained, the age at which loss begins and the amount of muscle loss[51]. To date, there is no clear consensus on the definition of sarcopenia. It has been proposed that 2 types of sarcopenia may occur: primary and secondary. Primary sarcopenia refers to age related muscle loss that occurs in the normal process of aging, secondary sarcopenia is due to an underlying condition, disease, inactivity or bad nutrition that may contribute to the loss of muscle mass with aging[12]. However, it is hard to distinguish between primary and secondary sarcopenia as they may occur together with age.

Since loss of muscle mass may not affect similarly all older adults, several definitions for sarcopenia have been proposed. As discussed in the introduction, Baumgartner et al.[7] defined the threshold to attain sarcopenia to be a height-adjusted appendicular muscle mass of two or more standard deviations below the mean of young adults by using dual-energy x-ray absorptiometry to estimate appendicular muscle mass. Appendicular muscle mass being the skeletal muscle mass of upper and lower extremities, not taking into account organ muscles.

Table 2 lists some definitions used to estimate sarcopenia in older adults.

Table 2: Definitions of sarcopenia

Authors (year)	Methods	Criteria
Cruz Jentoft et al. 2010[52]	Bioelectrical impedance analysis	Whole body muscle mass (kg) 2 standard deviations or more below the young adult mean.
Janssen et al. 2002[8]	Bioelectrical impedance analysis (BIA)	Whole body muscle mass expressed as percentage of body weight, 2 standard deviations or more below the young adult mean.
Delmonico et al. (2007)[53]	Dual-energy X-ray absorptiometry (DXA)	Lowest sex-specific quartile of height adjusted appendicular muscle mass (kg/m^2)
Newman et al. (2003)[54]	DXA	Lowest sex-specific quartile of residuals obtained from a linear regression model predicting appendicular muscle mass from height and fat mass.
Goodpaster et al. (2006)[55], Visser et al. (2005)[56]	Computed tomography	Lowest quartile of skeletal muscle mass area in mid-thigh
Janssen et al. (2004)[11], Janssen et al. (2006)[57]	BIA	Height-adjusted whole-body muscle mass thresholds developed based on the relation between muscle mass & disability

Source: Reproduced from Janssen et al. (2011)[12].

Recently, it has been proposed that loss of muscle mass would not directly affect function. In one cross sectional study[11], it was demonstrated that a low skeletal

muscle mass in older persons is associated with physical disability. Longitudinal studies[58, 59] on the other hand, showed that weakness and indices of function carry more effect than muscle mass in predicting risk of hospitalization and disability. Health practitioners proposed that a wider definition of sarcopenia was needed to be more clinically applicable[60]. It was suggested that function must be taken into account while diagnosing and defining sarcopenia. Table 3 from Cederholm et al.[60] summarizes the recent suggested diagnostic criteria for sarcopenia.

Table 3: Recent suggested diagnostic criteria for sarcopenia

Study group	**Definition**	**Criteria**
The European Society of Parenteral and Enteral Nutrition Special Interest Groups	*Sarcopenia is a condition characterized by loss of muscle mass and muscle strength. Although sarcopenia is primarily a disease of the elderly, its development may be associated with conditions that are not exclusively seen in older persons, like disuse, malnutrition and cachexia. Like osteopenia it can also be seen in younger patients such as those with inflammatory diseases[61]*	1-Low muscle mass (>2SD below mean reference) AND 2-Walking speed <0.8m/s or reduced performance in any functional test used in geriatric assessment
The European Working Group on Sarcopenia in Older People	*Sarcopenia is a syndrome characterized by progressive and generalized loss of skeletal muscle mass and strength with a risk of adverse outcomes such as physical disability, poor quality of life and death. The condition is called primary sarcopenia when the cause is aging per se and secondary sarcopenia when disease, inactivity or malnutrition contribute[52]*	1-Low muscle mass(>2SD below mean reference) AND 2-Low muscle strength AND 3-Low physical performance (gait speed)
The International Working Group on Sarcopenia	*Sarcopenia is defined as the age-associated loss of skeletal muscle mass and function. The causes of sarcopenia are multi-factorial. While cachexia may be a component of*	1-Gait speed <1m/s AND 2-Low muscle mass (objectively measured, using

	sarcopenia, the two conditions are not the same[62]	cutoffs from literature)
The Society of Sarcopenia, Cachexia and Wasting Disorders	*Sarcopenia, reduced muscle mass with limited mobility is an important clinical entity and most older persons should be screened for this condition (personal communication between Morley et al. 2011 and Cederholm et al. 2011)*	1-Walking speed ≤1m/s or walking distance <400m during a 6-min walk AND 2-A lean appendicular muscle mass corrected for height squared of >2SDs below mean of reference population

Source: Cederholm et al. 2011[60].

It stems from the above, that many definitions for sarcopenia exist in the literature and to date; no final consensus has been reached. It has been accepted that a muscle mass below 2 standard deviations from the mean of a reference population is considered to be low. Appendicular muscle mass is sometimes used instead of total muscle mass to predict loss of muscle mass, as well as adjusting total muscle mass or appendicular muscle mass for height or body weight. Physical function is proposed to be clinically taken into consideration as muscle loss and strength may not be linearly associated. Muscle mass index (MMI), appendicular skeletal muscle index (ASMM) or skeletal muscle index (SMI) are terms used to define muscle mass or appendicular muscle mass than has been corrected for height or weight.

13.2 Etiology of sarcopenia

Many factors that occur with aging contribute to the development of primary and secondary sarcopenia. Individuals lose muscle mass as they age; this has been also seen in older athletes who did not stop being physically active, suggesting that the decline of muscle tissue with aging occurs irrespectively of the starting point of muscle mass and the peak mass achieved[63]. Primary sarcopenia or in other

terms natural loss of muscle mass that occurs with aging is a ubiquitous process that could almost affect 100% of the elderly population. The extent to which it affects health however, depends on the threshold attained. Secondary sarcopenia is due however to an underlying condition, disease or lifestyle behavior that will cause a decline in muscle mass. As noted above, it is not straight forward to differentiate between primary and secondary sarcopenia as many conditions and that could affect muscle mass could also occur with aging. Age-related changes vary substantially among persons depending on their activity levels and environmental factors[64].

The following discussion addresses the mechanisms proposed to cause both primary and/or secondary sarcopenia.

13.2.1 Lack of physical activity

It has been proposed that inactivity increases with age, especially in developed societies[65] although the underlying mechanisms and evidence are not yet clear. Some studies have shown that active elderly persons have a higher muscle strength[63] and results from bed rest studies showed that inactivity is an important contributor to the loss of muscle and strength at any given age[66]. It is not obvious yet if the decrease in physical activity leads to a loss in muscle strength or is it the decrease in muscle strength that leads to reduced activity levels. In fact, Nair[64] suggests that it is likely that the decrease in muscle mass and muscle strength that leads to reduced physical activity levels. Nair proposes that a decreased physical activity level is due to increased fatigue, muscle weakness, decreased endurance capacity and increased muscle wasting, all possible consequences of aging. Decreased levels of physical activity would in turn further increase risks of morbidity and mortality.

On the other hand however, aging per se may possibly reduce activity levels. Nair proposes that physical activity is likely regulated in two ways: spontaneous or involuntary activities being regulated by the hypothalamus and voluntary activity

being regulated by proper cognition. According to Nair, spontaneous activities may be reduced due to a decrease in muscle mitochondrial function with aging (leading to a decrease in muscle adenosine triphosphate; ATP production needed for muscle contractile activities). The down regulation of spontaneous activity may influence voluntary activities by reducing the individual's "motivation" to engage in a voluntary activity. As a result total activity decreases with age. Nair considers that although it is very common to consider that people reduce their activity levels with age, more supporting evidence is needed.

In summary inactivity adds to the lack of anabolic stimuli that may in part affect the progression of muscle loss that occurs with aging. Evidence suggests that resistance training exercise such as weight training increases muscle protein synthesis[67], muscle mass and strength[68]. Aerobic exercise such as endurance training does not increase muscle mass as much as resistance training but contributes to an increased protein synthesis[69].

13.2.2 Loss of neuro-muscular function

Evidence suggests a loss of motor units with aging. Brown[70] showed that there was a reduction in the number of motor units with aging, strength was however preserved. The loss of motor neurons with aging leads to a denervation which may be the primary mechanism leading to sarcopenia. From cross-sectional findings, decrease in alpha motor neurons occurs with age mainly affecting lower extremities. Type 2 fibers which are fast glycolytic fibers decrease whereas type I fibers (slow, oxidative) remains unchanged[71] with some studies finding that it may decrease at a much slower rate than type 2 fibers at age 80 years and above[72]. With aging, satellite cells (small cells found in matured muscle; able to fuse and augment existing muscle fibers) decrease in both fiber types but more in type 2 fibers which further contribute to the age related loss of muscle mass, strength and contractility.

13.2.3 Endocrine function

Hormonal changes may occur with age and could alter muscle mass and strength. *Growth hormone (GH) and insulin like growth factor-I (IGF-1)* decline with age which may be due to a decrease in growth hormone secretion and an increase in somatostatin (growth hormone inhibiting hormone) from the hypothalamus[73]. The decrease in both IGF-I and growth hormone has been considered as contributors to the development of sarcopenia. This hypothesis has not always been supported, however. Roubenoff et al.[65] have found that 24-hour GH secretion was highest in people with the lowest muscle mass. The authors also hypothesized that since GH is lower in obese individuals, it is most probable that it is the fat mass that is confounding the relationship between GH and sarcopenia. Roubenoff et al.[65] have in fact found that serum leptin an indicator of body fat was inversely associated with GH production.

Treatment with growth hormone has been shown to increase muscle mass, decrease fat mass, and improve muscle strength[22]. However, growth hormone is an insulin counter regulatory hormone and in the community-dwelling elderly, despite decreased fat mass and increased in fat free mass, maximum oxygen consumption (VO2 max) and muscle strength, twice as many subjects became diabetic after 26-weeks of growth hormone treatment than in the placebo group[22].

Insulin is an anti-catabolic hormone associated with glucose, lipid and protein metabolism. Aging has been associated with an increased risk of insulin resistance. Loss of muscle mass with aging, is accompanied by the loss of myocyte number affecting preferentially type 2 muscle fibers whereas type I fibers remains unchanged[71]. The latter contains most of the muscle mitochondria and aging affects skeletal mitochondrial function. In fact, one theory of aging is that it is due to the progressive accrual of reactive oxygen species (ROS) inflicted damage, including mitochondrial mutations and deletions[74]. This damage leads to mitochondrial dysfunction, which in turn has been linked to

insulin resistance[75]. Furthermore, the decrease in muscle protein in elderly persons has been linked to elevated markers of inflammation, which are implicated in insulin resistance[76].

During the life span, body weight gradually increases until the sixties [77], and thereafter it tends to decrease. Both cross-sectional and longitudinal studies of body composition have shown increases in fat mass with aging [78, 79] with a propensity for a more centrally distributed fat, especially visceral fat (omental and mesenteric fat depots)[80]. The increase in adiposity (and obesity) with aging has attracted the attention of researchers because of its relationship with IR, cardiovascular and other health problems, including type 2 diabetes[81]. Classical factors associated with obesity comprise total adiposity, especially the visceral adiposity[82, 83], reduced physical activity [84, 85] and high fat intake[86, 87]. Obesity with its visceral fat has also been associated with inflammation, as adipocytes secrete several pro-inflammatory mediators[88]. Furthermore, obesity is directly associated with the presence of intramyocellular lipids (IMCL), a putative explanatory mechanism relating fat and IR[89]. How obesity causes IR is not fully understood but once the fat mass is expanded it likely exacerbates the effect of sedentarity and impedes insulin action through mediators such as adipokines or increased availability of free-fatty acids[90]. Inflammation is involved in both IR and aging, through common inflammatory mediators (especially Il-6 (interleukine 6), TNF-α (tumor necrosis factor) and CRP (C - reactive protein), [76, 88, 91, 92]. For example, a low grade of systemic inflammation due to elevations of IL-6 and CRP was associated with late-life disability in elderly persons[93] and Il-6, TNF-α and CRP were associated with IR in non-diabetic elderly persons[94]. Cytokines are soluble peptide messengers synthesized and secreted by lymphocytes, neutrophils, macrophages and neuronal cells that modulate the inflammatory response[95]. The inflammatory cytokines (and their common hepatic protein indicator, CRP) are markers of a fundamental impairment of metabolism that sustains life, i.e. the mitochondrial respiratory chain reaction, whose dysfunction with aging triggers ROS production, a common

mediator of nuclear factor *kappa*-light-chain-enhancer of activated *B* cells (NFκB) activation, the major transcription factor of inflammation[96]. The deregulation of insulin because of the factors cited above can have a catabolic effect on muscle.

Besides, it appears that the anabolic effect of insulin may be impaired with aging[97]. Volpi et al.[97] have demonstrated that muscle protein anabolism is impaired in healthy elderly volunteers during combined hyperaminoacidemia and endogenous hyperinsulinemia after ingestion of amino acids and glucose. The authors have concluded that this might contribute to the development of sarcopenia with age. In contrast, other studies have failed to find a difference between young and healthy elderly in muscle protein anabolism after an infusion of amino acids and insulin[98]. The lack of agreement between different labs may lie in methodological differences (e.g. the timing of muscle biopsies to determine muscle protein synthesis rate), physical activity level of subjects (as physical activity contributes in muscle protein anabolism) and control group characteristics[99]. Moreover, it appears that the different doses and quality of proteins administered may affect muscle protein synthesis (MPS) differently, with those of higher leucine and essential amino acids providing more beneficial effects. An MPS response may be higher after ingestion of large[98] (30g) versus small boluses[100] (7g) of amino acids. Some studies[101] showed that the anabolic action of insulin in response to a meal is hindered in elderly people and affects consequently muscle protein synthesis (MPS). The latter may also be affected by impairment in protein digestion and/or absorption[102]. Inflammatory factors are directly linked to muscle catabolism and sarcopenia. It has been shown[103] that elevated levels of serum Il-6 is associated with a greater risk of disability in elderly individuals. It has also been found[104] that Il-6 predict sarcopenia in old men and women using data from the Framingham Heart Study.

Less evidence exists on the role of *estrogen* and loss of muscle mass with aging. One study[105] found that estrone levels (estrogenic hormone) prevent the loss of

appendicular muscle mass, but predicted loss of muscle strength after adjusting for muscle mass, however. Van Geel et al.[106] have shown a positive association between lean mass and estrogen levels. On the other hand, some researchers[107] have found that estrogen levels do not predict skeletal muscle mass in elderly men and women. Besides, in the Health, Aging and Body Composition Study, estrogen replacement was associated with increased quadriceps strength but not with knee strength[108]. The protective mechanism by which estrogen can prevent loss of muscle mass is believed to be in their ability to modulate defense systems against oxidative stress. It has been suggested that low estrogen concentrations are associated with an increase in inflammatory factors as seen in vitro by Girasole et al.[106], who found that estrogens prevent the increase in Il-6. More evidence is needed on estrogen and its protective role on lean body mass. It is also worth mentioning that adipose tissue is a significant source of estrogen and high levels of the latter might be found in obese individuals. This might lead to a spurious association between estrogen and muscle mass which may disappear after controlling for body mass index (BMI). [109]

It has been found[110] that testosterone levels decrease at a level of 1% per year. Studies propose that testosterone decrease might be associated with low levels of muscle mass and strength. Baumgartner et al.[107] have found a significant positive association between muscle mass and serum testosterone in men. Additionally, some findings[111] in healthy elderly men showed that testosterone was positively related to muscle strength and muscle mass, but inversely related to fat mass suggesting that the physiological changes that occur with aging might be related to a decrease in testosterone levels with advancing age. Supplementation with testosterone has increased muscle mass and strength in men in some studies, but not in others[53]. The increase in muscle with testosterone supplementation may be due to increase in muscle fibers. A study in skeletal muscle of older men has shown that testosterone might inhibit myostatin[112]; a transforming growth factor beta protein that inhibits muscle differentiation and growth. Further studies

are needed to test the effect of testosterone replacement therapy on muscle mass in the elderly population.

Vitamin D has also been suggested to be related to loss of muscle mass. Vitamin D deficiency is common in elderly individuals[113, 114] in some studies, whereas this has not been shown in Canadian data[115] among elderly individuals and among participants of the NuAge study[116]. The difference between Canadian data and the rest of some other studies may possibly lie in the population studied (indoor stay versus community dwelling) supplement intake and food intake affecting vitamin D status. Souberbielle et al.[114, 117] measured serum 25-hydroxyvitamin D (25OHD, an indicator of vitamin D status) in 280 healthy men and women aged 60-79 years old and found a 59.6% prevalence of low vitamin D status when normal levels were defined as being > 75 nmol/L in this population of community dwelling elderly. In the latter study, the authors also reported higher parathyroid hormone values (PTH) for individuals with a 25(OH) D level of less than 30nmol/L (50 nmol/L being the minimal status to achieve) which reflects secondary hyperparathyroidism. PTH may be involved in pro inflammatory pathways leading to muscle loss and may cause sarcopenia independently of low vitamin D levels[99]. It has been stated[118] that older adults are efficient in maintaining 25(OH) D as much as young individuals with specific vitamin D intakes. However, older adults needed more vitamin D to produce higher levels of 25(OH) D for them to be able to overcome the hyperparathyroidism that occurs with age due to decreased renal function, low vitamin D status and low dietary calcium. Several reasons for low vitamin D status in the elderly are observed, among others, a low exposure to sun and a reduced skin capacity to synthesize vitamin D_3[119]. Results from the Longitudinal Aging Study Amsterdam showed that lower levels of 25(OH) D and higher PTH were associated with an increased level of sarcopenia in 1008 individual aged 65 years and above[120]. Sarcopenia in the latter study[120] was defined as a loss of grip strength greater than 40% over 3 years and a loss of 3% of appendicular skeletal muscle mass on follow-up. Potential mechanisms relating low vitamin D and risk of loss of muscle mass

include decreased muscle anabolism[100] and reduced insulin secretion as found in rats[98].

13.2.4 Nutrition

13.2.4.1 Food intake

Food intake declines with age[121]. It has been proposed that elderly individuals suffer from perturbations at the physical, sensory and regulatory levels, which can contribute in part to the anorexia of aging[122]. In general, older individuals possess reduced hunger and thirst signals. It has been proposed[122] that satiety is rapidly attained and that less frequent snacks are likely to be consumed leading to a total reduction in caloric intake. Central factors such as neurotransmitters and neuromediators may be involved in the lower energy intake consumed by elderly individuals as well as genetic (apolipoprotein E) and metabolic factors (insulin resistance, ghreline, cholecystokinine (CCK), noradrenaline, and serotonin, among others). Peripheral factors contribute also to the anorexia of aging. In fact, early satiety has been proposed to originate from the inability of the fundus to respond by relaxation in older individuals in contrast to young population. Table 4 summarizes some of the factors potentially related to the anorexia of aging.

Table 4: Potential factors related to the anorexia of aging

Decreased taste and smell
Early satiety
Increased cytokine activity (common with aging)
Gastro intestinal alterations (early satiation, decreased stomach emptying rate)
Decreased gastric emptying
CCK: increased response to fat load in elderly versus individuals
Leptin: secreted by adipose cells and may be involved in reduced food intake
Ghreline : orexigenic hormone, possible reduction with age (more evidence

needed)
NPY (Neuropeptide Y) : orexigenic neuropeptide, possible reduction with age (more evidence needed)
Appetite reduction

Source: Adapted from Chapman[122].

Social factors may be related to decreased food consumption with advancing age. According to Keller[123], conditions that frequently accompany aging, such as difficulty in transportation, meal preparation and loneliness, among others contribute to decreased food intake. If anorexia of aging leads to sarcopenia by decreasing protein intake or by decreasing other essential dietary nutrients is still unknown[124], but anorexia can predispose elderly individuals to protein energy malnutrition especially in the case of disease.

13.2.4.2 Protein intake

Protein intake is an important determinant of a healthy diet in the elderly. However, the recommended RDI of 0.8 g/kg/d may not be adequate for elderly people to maintain their nitrogen balance[125] and skeletal muscle mass[126]. Furthermore, in one study, reducing the protein intake from the usual 1.1 g/kg/d to 0.75 g/kg/d in young adults was associated with a transient reduction in erythrocyte glutathione, suggesting a reduced antioxidant capacity[127], a situation that would threat the delicate antioxidant/oxidant balance of elderly people. Rates of protein turnover are related to protein intake[128] and higher rates may confer extra benefits in the elderly, as reviewed by Morais et al[129]. Higher than usually recommended protein intakes have also been associated with increased intake of vitamin D and calcium[130]. A prospective study of dietary protein intake and risk of hip fracture in postmenopausal women has shown that the relative risk of hip fractures decreases across increasing quartiles of intake of animal protein, in a multivariate model controlling for age, body size, smoking, alcohol intake, estrogen use and physical activity[131]. Also, in the 10-year

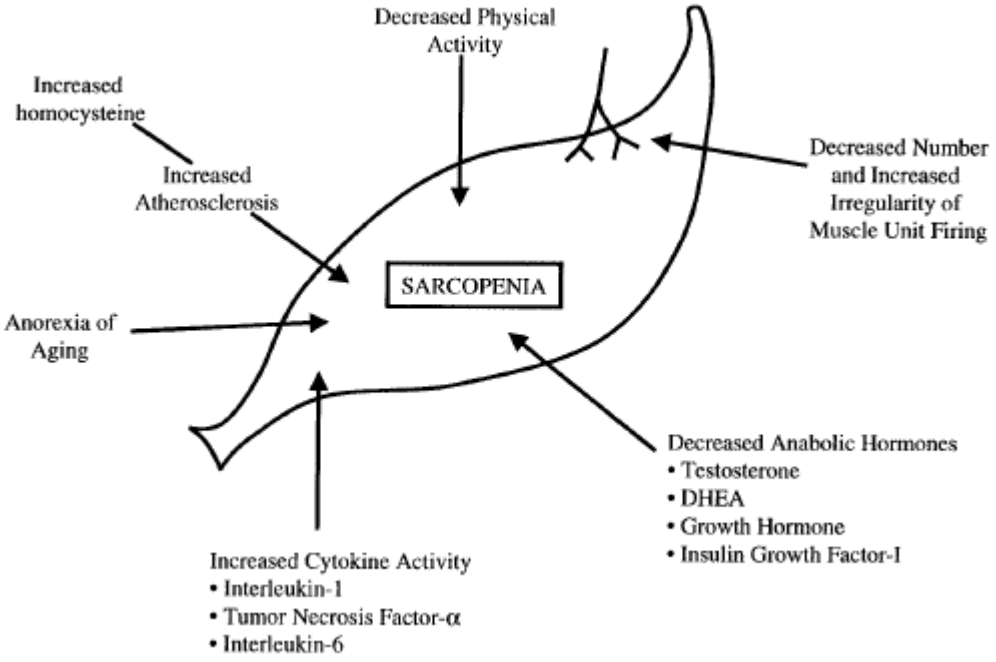
longitudinal New Mexico Aging Process Study, elderly persons consuming higher protein intakes (1.2-1.8 g/kg/d), had fewer adverse health events[132]. There also appears to be differences in amino acids to stimulate MPS. In fact, the primary essential amino acid that has been shown to promote protein synthesis is the branched chain amino acid leucine, which appears to be highly bio available and able to escape splanchnic uptake[40] (which reduces the availability of the amino acid in the peripheral tissue and consequently muscle uptake). Large amounts (35-40 g) of an oral or intravenous amino acid mixture of leucine have been shown to stimulate muscle protein synthesis equally in elderly persons[41]. Studies in young men reported stimulation of MPS by intravenous essential amino acids but not by non-essential amino acids[42]. Even adding non-EAA to an oral mixture of 18 g of EAA did not further increase in muscle protein anabolism[43]. Leucine is higher in animal protein sources and meat based diets are considered to be higher in EAA content. It has been proposed that age related impairments in muscle protein synthesis may be overcome by ingesting greater amount of high quality protein and/or free essential amino acids[99].

Homocysteine levels that may increase with age have been hypothesized to be associated with atherosclerosis and consequently muscle loss[124]. Further studies are needed to elucidate the issue.

In summary, there is a multifactorial origin of loss of muscle mass and sarcopenia. Environmental factors, mainly physical activity (especially resistance exercise) and nutritional factors may be targeted to prevent progression of muscle loss in elderly subjects. Some other approaches to combat sarcopenia (e.g. growth hormone and sex hormones replacement) seem less promising. Myostatin inhibitors, anabolic steroids as well as vitamin D supplementation are under study, and further research is required.

Figure 4 summarizes most of the factors associated with the etiology of sarcopenia.

Figure 4: Origins of muscle mass loss and sarcopenia



Source: Morley et al.[124].

13.3 Screening of muscle mass and sarcopenia

Several methods of assessing muscle mass exist[52]. Body imaging techniques are employed to estimate muscle mass or lean body mass. They include magnetic resonance imaging (MRI), computed tomography (CT scan) and dual energy X-ray absorptiometry (DXA). MRI and CT scan are considered to be the gold standard for assessing muscle mass, as they may be very accurate. DXA is another method used in clinical settings to assess fat, bone mineral and lean tissues. DXA can be used in epidemiological studies but the equipment is large and cannot be transported, making it one of its major drawbacks. DXA is considered to be an alternative source to MRI and CT scan to be used in clinical settings. DXA will provide information of appendicular muscle mass versus organ muscle mass.

Bio impedance analysis estimates fat and fat-free mass. BIA is portable and easy to use, making it efficient in clinical studies. BIA is an alternative to DXA, and has been validated against MRI, the gold standard for assessing muscle mass where good correlations results (0.907-0.952) have been obtained[133].

Brief description of the DXA method: two X-ray beams with differing energy levels are aimed at the patient's bones. When soft tissue absorption is subtracted out, the bone mineral density can be determined from the absorption of each beam by the bone. It can also be used to measure total body composition and fat content. DXA provides a good estimate for the measure of whole body composition while providing a three compartment model: bone mineral, fat, and bone-free lean masses and is thus a non invasive technique with minimal radiation exposure[134]. Brief description of BIA: determines the electrical impedance, or opposition of the flow of an electric current through body tissues which can then be used to calculate an estimate of total body water (TBW). TBW can be used to estimate fat-free body mass and, by difference with body weight, body fat. BIA was also shown to be an effective tool to assess body composition, it was validated against densitometry (considered an excellent instrument for body composition) and good correlations were obtained (0.907-0.952)[133]. Other

techniques to assess muscle mass exist such as total body potassium, urinary creatinine excretion and anthropometric measures. Total body potassium and creatinine excretion are not routinely used however. Anthropometric measures such as calculations based on arm circumference and calf circumference can sometimes be employed to assess muscle mass but are not recommended to be used to assess sarcopenia in older adults[52].

13.3.1 Measurements of strength and physical performance.

13.3.1.1 Muscle strength

Muscle strength can be assessed using several methods. Handgrip strength has been widely used to assess muscle strength because of its low cost and easy access[52]. Handgrip strength is strongly associated with leg strength[135] and has been shown to be associated with mobility outcomes as well as an association with the activities of daily living[136]. Strength of other muscle groups can also be measured using appropriate dynamometers.

13.3.1.2 Physical performance

Physical performance can be assessed using several standardized tests. The short physical performance battery is a composite measure of physical performance. It assesses balance, gait, strength and endurance[137]. Walking speed can be used in clinical and research settings to assess physical performance[138]. Participants walk at their usual pace over a 4 or more meter course. Time in seconds is usually recorded with a stop watch. Distance (m) divided by time (s) is used to calculate walking speed. Gait speed alone has been found to predict many outcomes[139]. One other test that may be used to assess physical performance is the Timed up and go (TUG)[138]; the test requires a subject to stand up, walk 3 m (10 ft), turn, walk back, and sit down. Time taken to complete the test is recorded in s. As with gait speed, TUG predicts many outcomes[140] [141]. Endurance can be assessed by different ways, including the 6-minute walk test[142]. A combination of endurance, balance and strength can be measured by the chair rising test during 5

trials and recording the time spent to perform the tests[137]. Finally, balance is captured by one-leg stand test. All of these tests are part of the short physical performance battery[137].

13.4 Prevalence and health care costs of sarcopenia

Data from the New Mexico Elder Health Survey showed that >50% of persons over 80 years of age are suffering from sarcopenia[143] when sarcopenia was defined as an appendicular skeletal muscle index (appendicular skeletal muscle mass/height in m^2) of 2 standard deviations below the mean of young adults and using an equation calibrated against DXA to estimate muscle mass. In another study, however, using DXA, prevalence using the same definition for sarcopenia ranged from 12% for those less than 70 years old to 30% for those older than 80 years old[144]. Results from NHANES III showed a prevalence of 17% of class II sarcopenia defined as a skeletal muscle index (muscle mass/weight X 100) below 2 standard deviation than the mean reference population, in the population over 60 years using BIA[145]. Other cross sectional data also presents high prevalence of sarcopenia in the elderly population: 22.6% in women and 26.8% in men using DXA. A subgroup analysis of women and men 80 years or older revealed prevalence rates of 31.0% and 52.9% respectively[122]. The latter studies reflect the prevalence of sarcopenia in American cohorts using a variety of methods and definitions. Using data from European cohorts, analyses of data from the Epidemiology of Osteoporosis (EPIDOS) study has found a prevalence of 9.5% of sarcopenia in healthy elderly women[146]. In a Chinese cohort, prevalence of sarcopenia was estimated to be 12.3% and 7.2% in men and women respectively[137]. The difference between diverse studies lie in the methods used to determine muscle mass, different definitions of sarcopenia, population studied as well as reference population used. Most of the studies show prevalence of muscle loss and sarcopenia, even though different methods were employed. Table 5 lists studies on the prevalence of sarcopenia.

It has also been shown that sarcopenia is associated with elevated health care related costs[11], which is expected to rise owing to the aging of the population. Sarcopenia is a serious condition that if left untreated could lead to an increased risk of falls, impaired thermoregulation, decreased food intake and consequently malnutrition, osteoporosis and many morbid outcomes which further exacerbate the health care spending[147]. Janssen et al.,[148] estimated the health care costs of sarcopenia to be 18.5 billion in the year 2000 and this cost will be increasing with the growth of the aging population.

Table 5: Studies on prevalence of sarcopenia

Authors	**Study**	**Methods of assessing muscle mass**	**Definition of sarcopenia**	**Prevalence of sarcopenia**
Baumgartner et al.[7]	NewMexico Elder Health Survey 883 men & women Age 65+	Equation derived from DXA	*ASMI used* 2SD below the mean of reference population	-15-25% <70 yrs ->50% above 80 years
Janssen et al.[7, 8]	NHANESIII[a] 14800men & women Age 18+	BIA	*SMI used* Type I: SMI between 1-2SD of reference population Type 2: SMI below 2SD of reference population	-17% over 60 yrs -18% over 80 yrs
Delmonico et al.[53][149]	Health ABC study[b] 2,976 men & women Age 70-79 years	DXA	*2 methods used:* 1-Appendicular skeletal muscle mass in relation to height: classified as sarcopenic if	-Method 1: 8.9% in men 7.1% in women -Method 2: 26.9% in men 36.1% in women

			fall in the lowest 20% of sex-specific index distribution 2- Appendicular skeletal muscle mass in relation to height and fat mass: residual method , 20th percentile used as cut-off for sarcopenia	
Rolland et al.[146]	EPIDOS[c] 1458 women Age 70+	DXA	ASMM < 2SD from mean reference population	9.5%
Lauretani et al.[135]	In Chianti 1030 men & women Age 20-102 yrs	CT-scan	Muscle mass <2SD from mean reference population	-In men: 20% at 65 yrs 70% at 85 yrs -In women: 5% at 65 yrs 15% at 85 yrs
Visser et al.[136]	LASA[d] 520 men & women Age 65+	DXA	>3% decline of muscle mass during 3 yr follow-up	15.7% after 3 yrs of follow up
Lau et al.[137]	Chinese cohort 527 men & women Age 70+	DXA	ASMM<2SD from reference population	-In men: 12.3% -In women: 7.2%

a: National Health and Nutrition Examination Survey.
b: Health Aging and Body Composition Study.
c: Epidemiology of Osteoporosis.
d: Longitudinal Aging Study Amsterdam.

13.5 Consequences of sarcopenia

Cross sectional studies have shown that loss of muscle and sarcopenia are associated with lower functional status and higher disability risk. Cross sectional data from the NHANES III showed that the likelihood of disability and functional impairment in sarcopenic individuals was twice as high as those with normal skeletal mass[8]. The authors of the latter study concluded that reduced skeletal muscle mass is independently associated with functional limitation and disability especially among older women. Cross sectional results from the New Mexico Elder Health Survey have found that low relative muscle mass is associated with self reported disability in men and women independently of obesity and morbidity problems. Data from the Health Aging and Body composition study have shown that elderly individuals in the lowest quintile for SMI had 80-90% risk of mobility impairment versus individuals in the highest quintile of skeletal muscle index[54]. However, the relationship between muscle mass and physical disability may be overestimated in cross sectional studies. Janssen et al.[57] have found using data from the same Cardiovascular Health Study that disability was 79% greater in those with sarcopenia at baseline, but only 27% greater during follow-up. Results by Visser et al. have shown that thigh cross sectional area was not associated with mobility limitations during 2.5 yrs of follow up in healthy elderly men and women after adjustment for muscle strength and fat infiltration[56].

It is still debated however, whether the decrease in muscle mass or the decrease in muscle strength (dynapenia) is responsible for the increased risk of falls and functional limitations in the aging population. Although it has been found cross-sectionally that low skeletal muscle mass in older persons is associated with physical disability[11], longitudinal studies[58, 59] on the other hand, demonstrated that weakness has a greater impact than muscle mass in predicting risk of hospitalization and disability. Cawthon et al.[58] found that strength and muscle mass were not associated with disability risk in elderly participants of the Health, Aging and Body Composition Study. It is also worth mentioning that

increased fat mass by itself increases the risk of disability[59, 150]. The combination of a low muscle mass with high fat mass predicts greater disability in the elderly compared with sarcopenia alone[151], although this has not been found consistently[106]. Although body composition (muscle/fat) mediates physical performance, the latter depends also on power, balance and preserved neurological and cardiovascular systems. Interventions to increase muscle mass do not always translate into increased muscle strength, however resistance training achieves improved muscle strength without necessarily affecting muscle mass [53, 152, 153]. The specific contribution of increased muscle strength caused by exercise independently of the positive effects of exercise interventions (balance, depression control among others) have not been investigated yet [56].

In summary, loss of muscle and strength may be associated with higher risk of decreased physical performance and functional limitations in the elderly population. Further research is needed to understand the complex relationships between changes in body composition, muscle strength and functional capacity.

Other than its effect on physical performance, loss of muscle mass may be associated with adverse metabolic outcomes. In this research, we will focus on the association between muscle mass and insulin resistance.

13.5.1 Loss of muscle mass and insulin resistance

There is evidence that at an advanced age, total body fat increases[154] and it has been shown that the accumulation of fat occurs more in the visceral region (omental and mesenteric fat depots)[155]. Increased visceral fat have been linked to the secretion of inflammatory markers such as adipokines and cytokines[76] and have been associated with an increased risk of insulin resistance[156]. In fact TNF-α, a cytokine secreted in inflammatory state and obesity, has been shown to act negatively on the modulation of insulin action and thus lead to its reduced function[157]. The loss of skeletal muscle mass, the latter being considered a very active metabolic tissue which is responsible for most of the resting energy

expenditure[158], will lead to an increased predisposition to increased adiposity as the excess glucose will be converted to fat. Thus, the loss of skeletal mass will lead to obesity and by the mechanism cited above; obesity will possibly lead to insulin resistance. Sarcopenia could therefore be considered to be responsible in part for insulin resistance, indirectly by causing obesity.

In fact, there are also other factors unrelated to obesity associating sarcopenia to IR. Sarcopenia is accompanied by the loss of myocyte number and the decrease in the protein content of muscle; as well defined as the loss of muscle quality[147]. There is evidence that type 2 muscle fibers size decrease whereas type I fiber size remains unchanged. But only type 2 decreases in number[147]. Type I fiber contains most the muscle mitochondria and aging affects skeletal mitochondrial function. In fact, one theory behind aging is that aging is due to the progressive accrual of ROS inflicted damage, including mitochondrial mutations and deletions[74]. This damage leads to mitochondrial dysfunction. It has been shown that these ROS species (e.g. OH^-, H_2O_2, O^-) which are by products of the mitochondrial oxidative metabolism not concealed by our antioxidant defense mechanisms, interfere with the modulation of insulin action, and thus lead to its resistance[75]. In a study on rats, oxidative stress caused by buthionine sulfoxamine an inhibitor of glutathione synthase (implicated in the modulation of insulin action) impaired glucose uptake by insulin through phosphorylation blockade of insulin receptor substrate and activated the NFκB pathway. Furthermore, the decrease in muscle protein in elderly persons has been linked to elevated markers of inflammation, which are implicated in insulin resistance[76].

The mitochondrial dysfunction in the elderly can also lead to a decreased fat oxidation in muscles[159]; as a consequence, there is an accumulation of intramyocellular lipid (IMCL) which has been implicated in insulin resistance development[160]. Some studies have linked obesity to IMCL and to insulin resistance[161]. However, accumulation of IMCL in the muscles of the aging population is independent of overall adiposity as IMCL has been linked to a

decreased muscle fat oxidation from a cellular dysfunction shown in the elderly[159]. In addition, a study by Petersen et al.[160] has demonstrated that mitochondrial dysfunction has been associated with reduced insulin mediated glucose disposal and increased IMCL in elderly compared to young persons and this finding was independent of overall adiposity. Furthermore, in elderly persons, the decrease in muscle protein synthesis has been linked with markers of inflammation, such as CRP, IL-6 and tumor necrosis factor-α (TNF- α) receptor II[162]. These mediators of inflammation are implicated in the development of IR[88, 91], as well as in other deleterious effects of aging[92].

Another fact relating sarcopenia to IR independently from adiposity comes from the study conducted at the McGill Nutrition and Food Science Centre which has demonstrated that net protein anabolism, a marker of insulin sensitivity, was correlated with fat free mass and thus its loss (mainly muscle) should be associated with IR[163].

We can state that there are many factors linking sarcopenia to IR whether from sarcopenia leading to obesity and consequently IR or independently from adiposity from the mitochondrial dysfunction of the aging remaining muscle.

The first part of this literature review discussed in depth loss of muscle mass and sarcopenia that may occur with aging. The next part will focus on insulin resistance.

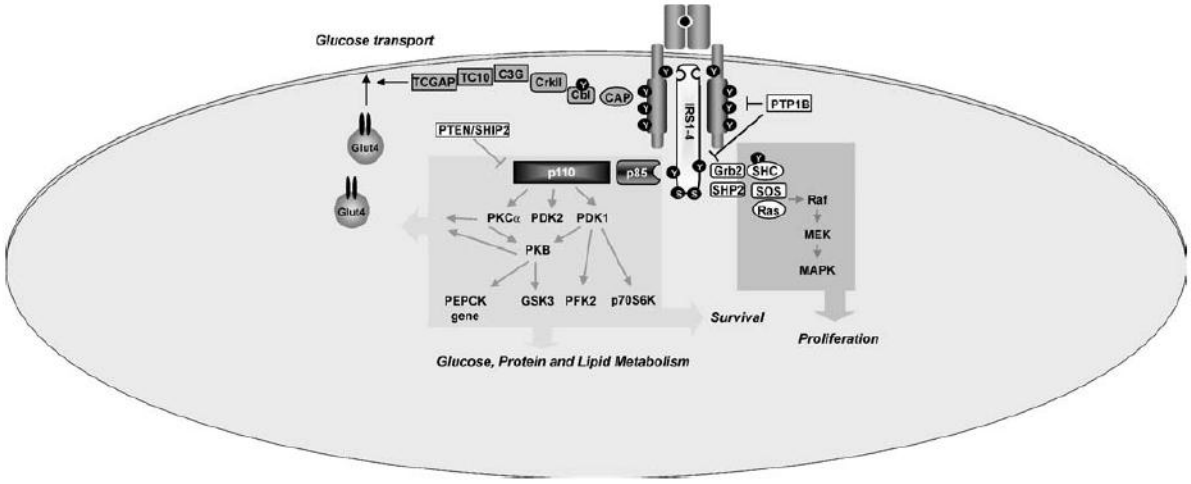
13.6 Insulin function

Insulin is a key hormone in regulating metabolism and in the maintenance of normoglycemia and normolipidemia. Insulin stimulates glucose uptake into adipose and muscle tissues to promote its storage as intracellular triglycerides as well as glycogen in muscle. Insulin also inhibits liver glucose production and release (gluconeogenesis and glycogenolysis). In a healthy person, this would lead to maintenance of plasma glucose at normal levels[164]. Insulin is also involved in the metabolism of other nutrients. When insulin binds on its receptor, it will

signal a cascade of events all responsible for the numerous insulin mediated physiological responses[15]. Through different signalling pathways, insulin will play a role in glucose, protein and lipid metabolism[15]. Insulin signalling is mediated by several complex processes. Insulin binds to its receptor the latter being a heterotetrameric membrane protein consisting of α and β subunits. Upon binding on the receptor's α subunit, insulin will result in an activation of the intrinsic kinase activity of the β subunit. The consequent result would be an autophosphorylation reaction where one β subunit tyrosine phosphorylates the adjacent β subunit. The insulin receptor substrates (IRS) are a family of proteins that interact with the phosphorylated insulin receptor through a phosphotyrosine binding molecule (PTB) to facilitate phosphorylation of IRS on a number of tyrosine residues [144]. The insulin receptor substrate proteins (IRS proteins) are linked to the activation of three main signalling pathways: Phosphatidylinositol 3-kinases-protein kinase B (PI3K-AKT/protein kinase B) pathway mostly involved with insulin action; the CAP/Cbl/Tc10 [15] also mainly involved in insulin action and the Ras-mitogen-activated protein kinase (MAPK) pathway which is more involved in the regulation of gene expression and cell proliferation[165]. Upon activation of the insulin receptor, IRS1 and IRS2 are phosphorylated themselves which will provide docking sites for Src Homology 2 (SH2) domain-containing proteins. These events will result in the activation of the three pathways discussed above and ultimately result in the physiological effects on insulin[15]. Figure 5 shows the principal components of the insulin signaling pathway.

Glucose transport is mediated through carriers referred to as the glucose transporters (GLUT) family. The GLUT-4 protein is restricted to fat and muscle and responsible for insulin stimulated glucose uptake[164]. The activation of the PI3K pathway will result in GLUT 4 translocation, glycogen synthesis and lipogenesis and a general control of gene expression patterns. The other pathway that has been also shown to be involved with GLUT-4 translocation is the CAP/Cbl/Tc10 pathway that occurs when insulin binds on its receptor[164].

Figure 5: Insulin signaling pathway



Source: Pirola et al. [15].

13.7 Insulin resistance

Insulin resistance is characterized by a diminished response to the action of insulin at its target organs. The combined inability of muscle and adipose to facilitate glucose uptake and of the liver to suppress glucose output in response to increasing insulin are referred to as insulin resistance[164]. Decreased insulin action has been associated with a defect in PI3K activity and an alteration of glucose transporters but many other mechanisms have been proposed.

13.7.1 Causes of insulin resistance

13.7.1.1 Hyperinsulinemia and hyperglycemia

In an insulin resistant state, plasma glucose is minimally elevated and remains at normal levels. At first, the pancreas compensates by secreting more insulin, and the presence of normal to high insulin levels is noticed[164]. Both hyperinsulinemia and hyperglycemia will further exacerbate the insulin resistant state[15].

With some controversies[166], hyperinsulinemia has been shown to impair tyrosine kinase activity[167]. Hyperinsulinemia has been shown to affect insulin

function on several sites along the insulin signaling pathway [15]. In vitro studies using adipocytes exposed to insulin have found that the rate of degradation of insulin receptor substrate 1 is ten times faster in insulin treated cells versus basal cells[168]. IRS 2 is an important IRS protein and mediates peripheral insulin action; exposure of 3T3-L1 adipocytes to insulin induced a degradation of IRS2 proteins, further showing the negative effect of hyperinsulinemia on the insulin signalling pathway [169]. Additionally, in vivo studies have shown that GLUT-4 expression is lower in healthy individuals with insulin resistant profiles[170]. Moreover, a study using a model of obese, insulin resistant and type 2 diabetic mice, has found a decreased PKB activation in adipose tissue and skeletal muscle suggesting that a high exposure to insulin might hinder normal phosphorylation[171].

Hyperglycemia may also affect insulin signaling. In Zucker diabetic fatty rat liver, exposure to hyposinulinemic and hyperglycemic phase enhanced phosphorylation on IRS-1 and IRS-2 but phosphorylation of Akt/PKB were severely suppressed. Restoration to normoglycemia normalized Akt/PKB activation. The authors suggest that hyperglycemia may reduce the efficiency of Akt/PKB kinase and it may underlie the hyperglycemia-induced insulin resistance in the liver[171].

13.7.1.2 Fatty acids and obesity

Circulating fatty acids can lead to insulin resistance by decreasing insulin induced PI3K activation[15]. One study showed that high fatty acids exposure lead to an impairment in IRS-1 and IRS-2 phosphorylation[172]. Another in vitro study showed that free-fatty acids play a role in the failure of pancreatic beta cell mass expansion to compensate for peripheral insulin resistance. The negative effects of fatty acids cannot be attributed to all types of fatty acids[173]. A rat study showed that animals fed a high polyunsaturated fatty acid diet were able to maintain insulin sensitivity in the muscle but not in the liver[174].

Obesity has been associated with insulin resistance. One mechanism behind obesity and insulin resistance might account for the increased expression of several protein tyrosine phosphatases which dephosphorylate and terminate signaling[175]. Additionally, a major factor contributing to impaired glucose transport in adipocytes is the down regulation of GLUT4. It appears that even though GLUT4 expression is normal in obese individuals; its translocation however might be affected [175]. Obesity carries complex relationship with insulin resistance development, it is now widely recognized that the adipose organ functions as endocrine glands and releases a variety of molecules such as hormones, cytokines and free fatty acids which allows it to play a role in glucose homeostasis[175].

13.7.1.3 Cytokines and glycated proteins

High levels of cytokines have been associated with a risk of developing insulin resistance and diabetes. TNF-α was recognized as an agent causing insulin resistance and is increased in obese state. TNF-α impairs phosphorylation of IRS molecules via phosphorylation of IRS-1 S307 and induction of SOCS proteins[15]. Another mechanism by which TNF-α may lead to insulin resistance is through induction of serine/threonine phosphorylation on IRS proteins. Interleukine 6 (IL-6) is an inflammatory factor also implicated in insulin resistance. It appears that it also induces SOCS proteins[15].

Glucose can react with amino groups forming glycation products. Glycation can occur on any protein, when insulin itself gets glycated, this result in impaired biological activity[15, 176].

13.7.1.4 Dietary protein intake on insulin resistance

A number of studies have suggested that a diet high in protein and mainly animal protein is involved with an increased risk of IR development[177, 178]. In fact, protein intake has increased in Western countries, and possibly to an extent of 1.5-2 times the recommended dietary allowances (RDA)[179]. It is still unclear

whether the aging population consumes a high intake of protein but recent data in French adults suggested that 50% of the aged population consumed less than 1.14g/kg (above RDA recommendation) and 25% consumed less than RDA recommendation[180]. It is worth mentioning however, that the data concerning the optimal protein intake for the aged population is still highly controversial with some considering the RDA recommendation to be insufficient to maintain an optimal MPS[35]. It is therefore difficult to conclude what could be considered a high intake of proteins in the elderly and it is still a matter of debate.

It has long been believed that a high intake of protein might be related to weight loss through an early satiety effect and consequently to a better metabolic profile and probably better effect on insulin sensitivity (insulin action)[181]. However, epidemiological data concerning long-term high protein intake shows that this might lead to an impaired glucose metabolism and possibly to IR development and type 2 diabetes[177, 178]. Many mechanisms have been proposed. It has been suggested that a high intake of protein might cause “weakness of the pancreas”, and possibly a low level of insulin secretion[182]. A study by Linn et al.[46] showed that a high intake of protein (1.87±0.26 g/kg) in healthy individuals for a duration of 6 months was associated with an increased glucagon secretion (counter regulatory hormone), and increased gluconeogenesis (liver glucose production, known as endogenous glucose production) usually suppressed by an optimal insulin action. Furthermore, overall glucose disposal was reduced, which shows that the consumption of a very high protein diet increases the risk of insulin resistance. Studies performed under controlled conditions, showed that an intravenous administration of amino acids raised plasma amino acids and induced a lower uptake of glucose by the peripheral tissues[47]. Besides, in vitro studies showed that amino acids could negatively affect glucose disposal by disturbing the modulation of insulin action and negatively influencing essential mechanisms involved in the cascade of events for insulin action (inhibition of phosphorylation of insulin receptor substrate1 and impaired activation of PI3 kinase) in skeletal

muscle[183]. It is still unclear however, if those negative effects of protein intake on IR could be attributed to all sources of proteins. In fact, Lavigne et al.[184] on a study performed on rats fed a high fat diet showed that cod protein intake adjusted for omega-3 intake in fish oil, decreased the risk of IR development in contrast to casein (major milk protein) and soy protein (vegetable source). Those beneficial effects of cod protein intake were found without any weight loss in the population, suggesting that the effect of protein per se independently from adiposity is an important determinant of insulin action. A prospective study by Song et al.[178] in the Women's Health study context showed that red meat consumption, animal protein intake and heme iron (iron from animal source) increased the risk of type 2 diabetes development in elderly women after adjustment for total fat intake. It was suggested by the authors, that red meat consumption is highly linked with preservatives and additives intake, as well as with chemical compounds and advanced glycated end products (due to the meat processing) that could be linked to an increased risk of IR development. Besides, meat is high in heme iron and in a study by Forouhi et al.[185], it was suggested that a high level of iron increases diabetes risk. A recent prospective study by Sluijs et al.[177] showed that a dietary intake of total and animal protein intake (mean intake of total protein was 75.7g/day approximately 1.1g/kg/day) increased the risk for type 2 diabetes development. These findings were adjusted for confounders, particularly for fat intake and adiposity, which further suggests that the effect of animal protein per se could be responsible for IR development. Vegetable protein intake mainly from fruits, vegetables, bread and potatoes were not associated with IR development. No analysis concerning fish intake alone was done to test its role on insulin sensitivity. It could be that, with these results showing negative effects of animal protein intake on insulin function independently from fat intake, certain amino acids be implicated in those adverse effect. In fact, the amino acid leucine, which is high in animal protein and has been associated with a positive effect on muscle mass, was suggested to be independently related to glucose uptake impairment in rats, even with a high

insulin level[186]. *It appears that total protein intake and mainly animal protein intake are associated with an increased risk of IR and diabetes type 2 developments. We propose to test the putative role of protein intake on IR development independently from adiposity, particularly animal protein intake in the healthy elderly population that took part in the NuAge study, described below.*

13.7.2 Consequences of insulin resistance

Insulin resistance is responsible for increases in blood glucose and lipid concentrations, hyperinsulinemia and eventually overt type 2 diabetes[175]. IR is also a strong risk factor for the metabolic syndrome, a cluster of metabolic and cardiovascular disorders, which according to NIH 3th Report of the National Cholesterol Education Program Expert Panel 2001 criteria[187] comprises excess abdominal fat, impaired fasting glucose, dyslipidemia and hypertension, and affects 40% of those > 60y[188]. IR is believed to be the factor that could explain the clustering of the diagnostic criteria of the metabolic syndrome[189]. In prospective cohort studies, this syndrome predicts cardiovascular disease, the leading cause of death in the elderly[18], stroke[20] and type 2 diabetes[16], all-important causes of disability, as well as mortality.

13.7.3 Prevalence of insulin resistance in the elderly

IR increases with age as data from NHANES III showed a prevalence of type 2 diabetes in the elderly > 75 y of 13% with another 14% showing impaired glucose tolerance, that is twice that of the younger population[190]. With the increase in the aging population, the number of elderly persons continues to rise with a parallel rise in type 2 diabetes[117]. Data from the In Chianti study showed that insulin resistance increases with aging using a representative sample of 968 participants of 22-104 years of age[191]. In the latter study, sex stratified analyses adjusting for multiple confounders found that IR was significantly associated with poor muscle strength in women but not in men[191].

13.7.4 Assessing insulin resistance

The hyperinsulinemic, euglcycemic clamp technique is the gold standard method to determine insulin resistance [185]. The clamp method consists of maintaining plasma glucose concentration at basal levels with a glucose infusion and adjusting rates according to frequent plasma glucose monitoring while infusing also insulin to elevate concentrations to postprandial levels (typically ~500 ρmol/L). Because plasma glucose is maintained constant by a continuous glucose infusion (euglycemia), the glucose infusion rate equals glucose uptake by all the tissues and can therefore be considered a measure of tissue sensitivity to exogenous insulin[192]. The glucose clamp technique measures IR directly and this is why it is considered as the gold standard method for assessing insulin resistance.

Other non-direct, but also less invasive measures exist for assessing insulin resistance. These methods are based on the principle that as insulin resistance progresses, there is a compensatory increase of insulin secretion from the pancreas that maintains plasma glucose in normal range[193]. However, as IR develops, higher insulin concentration may not overcome this resistance or not be sustained and glucose begins to rise to the impaired fasting values of 5.6-6.9 mmol/L, before attaining levels consistent with diabetes of ≥6.9 mmol/L [194]. Thus, measuring fasting glucose and insulin captures the state of IR. IR can be assessed by the Homeostatic Model Assessment (HOMA-IR), an index of resistance that has been compared with the gold-standard, euglycemic hyperinsulinemic clamp in different age groups where good correlations (r=0.69-0.83) were obtained[195]. Despite some limitations [196] the HOMA-IR has been used in numerous large population samples to estimate IR. This index requires only determination of fasting plasma glucose and insulin and the HOMA-IR score is given by the formula: [insulin (μU/mL) x glucose (mmol/L) / 22.5] [197].

We suggest that total protein intake particularly animal source could have a beneficial effect on muscle mass. We propose to perform a secondary analysis of an existing observational database of healthy elderly men and women to answer our question and test the association of dietary protein intake on muscle mass.

The previous sections described the relationship between each two variables in our model: sarcopenia on IR, dietary protein effect on sarcopenia and dietary protein on IR risk. We can clearly state that there is a paradigm concerning these relationships and that the optimal dietary protein recommendations for the elderly population to minimize IR or sarcopenia development remain to be determined. The recommendations need to target a minimization of the effect of sarcopenia while preventing IR development. Because of the opposing effect that protein intake has on these two variables and because still, there is no consensus on what protein intake is best for preventing sarcopenia nor a consensus exists on what is the increased risk of dietary protein on IR, our approach is well justified. Our proposed study is even more justified as some studies have shown even a beneficial intake of amino acids on insulin sensitivity[48, 49]. To date, there is no consensus regarding these effects, and to our knowledge no study has yet determined the effect of all these variables combined together. We propose to do a path analysis of an existing data set of a healthy elderly population to examine these interrelationships between the variables in our model and to determine, if any, the type of dietary protein intake, particularly sources of protein intake, could be implicated in reducing the risk be it of IR or loss of muscle mass.

13.8 Path analysis:

We will use path analysis to analyze our proposed models. Path analysis combines factor analysis, correlation and regression but all variables are observed. This is in contrast to structural equation modeling (SEM) which is based on latent variables to express constructs that are not directly measurable[198]. All our variables in the models were continuous and observed variables. Path analysis is used to test a confirmatory versus an exploratory hypothesis or theory with the main purpose to

find out if the model fits with the analyzed dataset. The benefit of path analysis would be to test the direct and indirect effects that we have in our model independently of all other associations[199].

The path analysis involves several steps: (i)-specification of the model based on theories and hypotheses; (ii) identification of the model to check if the values could be used to be analysed; (iii)- Estimation technique, the one used in this article was the maximum likelihood estimation; (iv)- Evaluation of the fit statistics and the results obtained if they fit or not with data; (v)- Modification of the model based on rationale and hypotheses, by adding paths that allow for better fit of the data[199]. We will use the goodness of fit indices to evaluate our models.

13.9 The NuAge Study

We propose to do a secondary analysis of an existing prospective observational data set collected in healthy elderly men and women in 3 age strata (68-72, 73-77, and 78-82). The dataset that we aim to analyze is part of the NuAge dataset that was collected between the years 2005 and 2008 with repeated measures at 4 time sets: T1, T2, T3, and T4[200]. NuAge is a Canadian Institute of Health and Research (CIHR) funded longitudinal study that collects a wealth of data with the goal of establishing the temporal sequence of events and the directions of causal relationships. We consider that the longitudinal design of the NuAge study will provide as much as possible information to answer our questions and the opportunity to test the interrelationships among the factors in the models we wish to study. A random sample stratified for age and sex of elderly persons living in Montreal and Sherbrooke areas were recruited using the Quebec Medical Bureau Database (Régie de l’assurance maladie du Québec). The Régie's databanks are grouped into two categories: databanks owned by the Régie and databanks administered by the Régie. Databanks owned by the Régie: the Régie carries out all data management functions for these databanks, ranging from obtaining data from health professionals or healthcare facilities to providing information

products for various clienteles, such as persons doing research in the field of health[200]. The content of these databanks is specific to the various laws and programs administered by the Régie.

Study population: The participants were selected in two phases: a telephone survey and a clinical examination. The purpose was to generate 600 people per sex-age stratum equally divided between sexes. A total of 1793 subjects were included in the first year (T1) that ended in March 2005. This number declined to 1699 at T2 giving an attrition rate of approximately 5% annually (deaths 1%, dropouts: 3%, moved away: 1%). The study ended in March 2008 (T4). The recruitment of the population took place in Montreal and Sherbrooke areas. Subjects were contacted using the Quebec Medical Bureau Database (36,183 men and women). The recruitment and follow up interviews took place at Geriatric Research Centers of either Montreal or Sherbrooke areas (Quebec, Canada) [200].

The face-to-face interviews took place annually (if necessary at home), and telephone follow-ups were made every six months to document health events, including falls. Subjects were provided with an illustrated notebook to facilitate recall and to note health events. The community dwelling men and women needed to speak French or English and needed to commit for a five-year period. They also had to be free of disabilities in activities of daily living, without cognitive impairment, walk without help, able to walk 300 meters (one block) or to climb 10 stairs (one floor) without rest, able to sign an informed consent. Exclusion criteria consisted of people suffering from ≥class II heart failure, symptomatic chronic obstructive pulmonary disease, inflammatory digestive diseases or cancer the last five years (except basal cell of the skin) because these diseases could potentially affect and bias the study purposes[200].

The fraction of the population that we propose to study here is the population not suffering from diabetes at the start (confounding factor for insulin resistance). We propose to study an existing dataset to answer the questions that would usually

require a very expensive and time consuming study. Besides, the elderly population recruited for NuAge study is the most relevant population for the questions we need to address and subjects were a representative sample of the Quebec elderly population. In addition, the data collected contains results on body composition and dietary intake, all relevant to answer our questions. Thus, the secondary analysis of an existing dataset could be the best option for not putting such a large number of elderly persons under investigation since these results have already been collected. It is also worth mentioning that the exclusion and inclusion criteria are good for our study because they control for potential biases that could affect the results.

14 Bridge 1

Loss of muscle mass is associated with many adverse physical and metabolic outcomes.

An understanding of all the factors that occur together with aging with regard to physical performance and muscle mass, taking into consideration body fat, energy intake and other factors into a comprehensive model is very helpful.

Most of the studies that associated muscle mass with disability analyzed only a few variables at time, a model combining all variables of interest will help in better understanding the changes that occur with aging.

If one intends to develop programs to maintain physical capacity with aging, it is unclear which of the predictors of disability (e.g., age, sex, strength, smoking, physical activity, number of chronic diseases), alone or in conjunction with the others, are the most important to address. Physical activity is often proposed as a mean of maintaining capacity[201] but if one attempts to develop a model that integrates physical activity one has to recognize that since it affects both muscle mass and strength it could act as an outcome as well as a predictor of physical capacity. All of the previous variables are also affected by the burden of chronic disease that occurs with aging.

Recommending physical activity is often challenging among older population especially in the vulnerable and frail population[202]. We suggest that before recommending physical activity we must have a close look at the physical capacity of the individual even though the population in NuAge is all composed of community dwelling elderly in good health. These complex interrelationships are not well understood in the literature with a non-linear relationship between muscle mass and physical capacity[202].

15 Manuscript 1

15.1 Title: Interrelated factors favouring physical performance and activity in older adults from the NuAge cohort study

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15.2 Abstract

Background: The decrease in physical performance (PP) with aging mediated, at least in part, through changes in body composition (muscle/fat). Age, sex, energy intake and chronic diseases can also affect PP. In contrast, physical activity (PA) could both as a predictor and an outcome of PP. The effects of these interrelationships are complex and their interpretation is a challenge.

Hypotheses: We hypothesize a model including predictors such as energy intake, age, sex and chronic diseases. The latter predictors would in turn influence body composition components. Body composition components would influence PP measures. PA could act as both a predictor and an outcome of PP. **Objectives**: To explore the complex interrelationships of body composition, PP and PA to assist in indicating ways to promote healthy aging.

Participants: A sub sample of 847 non diabetic men and women community dwellers of the Quebec Longitudinal Study on Nutrition and Successful Aging (NuAge Study).

Methods: Data obtained from tests employed to assess PP were analyzed by principal component analysis and generated two indices, one related to strength and the other to mobility. Muscle mass index (MMI; kg/height in m^2) and % body fat were derived from dual X-ray absorptiometry and bio impedance analysis. PA was assessed by the Physical Activity Scale for the Elderly (PASE) and energy intakes were calculated from three non-consecutive 24h-food recalls. Baseline data was used from the NuAge dataset to test relationships. We evaluated if the proposed model fitted with the dataset of NuAge by path analysis using fit statistical indices.

Results: MMI and % body fat were both negatively associated with mobility score (beta coefficients -0.11 and -0.02 respectively), however MMI was positively associated with strength (β=0.6). Physical activity was significantly positively associated with MMI (β=0.02) and negatively with % body fat (β=-

0.16). Mobility was significantly positively associated with physical activity (β=0.65). Our hypothesized initial model did not have a good fit with the data, however with some paths according to hypotheses, the final model fits with our data: Chi-Square= 4.6385, RMSEA Estimate=0.0000.

Conclusions: We have specified the directionality of our model based on rationale and hypotheses and obtained good fit indices of the model that we proposed. The cross sectional nature of path analysis do not allow drawing conclusions of directionality, it just shows that the proposed model with these directions fits well in the population analyzed. In our dataset, PA was significantly positively associated with mobility components. Mobility was in turn positively associated with strength and negatively with % body fat. Based on the directionality of our model, mobility components (balance and walking speed) influence PA. Strength influences positively mobility whereas fat influences negatively mobility. % body fat is in turn influenced by PA. The findings of our model can shed some light on the complexity of relationships between body composition, lifestyle habits and PP. Our model has to be tested in different samples to validate our findings.

15.3 Introduction

The maintenance of physical performance with aging poses significant challenges as many factors contribute interdependently to deterioration[203]. Aging is associated with body composition changes[149] that undoubtedly affect performance. Studies have shown that, as one ages, the body undergoes a gain in fat mass and a loss in fat-free mass[7], especially involving skeletal muscle mass, the latter referred to as sarcopenia[7] when it attains a certain threshold. Data from the New Mexico Elder Health Survey showed that >50% of persons over 80 years of age would be classified as being sarcopenic[7] and results from the National Health and Nutrition Examination Survey (NHANES) III showed a prevalence of 17% in the population over 60 years[8]. The likelihood of disability and functional impairment in sarcopenic individuals is twice as high as those with normal skeletal muscle mass[9]. It has also been shown that sarcopenia is associated with an increased risk of morbidity and mortality[10] and with elevated health care related costs[11], which are expected to rise owing to the aging of the population. Although it has been found cross-sectionally that low skeletal muscle mass in older persons is associated with physical disability[11], longitudinal studies[58, 59] on the other hand, demonstrated that low strength has a greater impact than muscle mass in predicting risk of hospitalization and disability. It is also worth mentioning that increased fat mass by itself increases the risk of disability[59, 150]. The combination of a low muscle mass with high fat mass predicts greater disability in the elderly compared with sarcopenia alone[151], although this has not been found consistently in another study[106]. Although body composition (muscle/fat) mediates PP, the latter depends also on power, balance and preserved neurological and cardiovascular systems. Interventions to increase muscle mass do not always translate into increased muscle strength, however resistance training achieves improved muscle strength without necessarily affecting muscle mass in older adults [53, 152, 153].

Thus, if one intends to develop programs to promote healthy aging, it is unclear which of the predictors of performance discussed so far, alone or in conjunction

with the others, are the most important to address. PA is often proposed as a mean of maintaining performance[201] but if one attempts to develop a model that integrates PA one has to recognise that since it affects body composition and strength/mobility, it could act both as an outcome and as a predictor of PP.

All of the previous variables are also affected by the burden of chronic disease that occurs with aging[3].

Recommending physical activity is often challenging among older population especially in those with more limited mobility[202]. We suggest that before recommending physical activity we must have a close look at the physical performance of the individual. These complex interrelationships are not well understood in the literature with a non-linear relationship between the losses of muscle mass and their impact on physical capacity[202, 204].

Regression type models with one outcome, one variable under study and all other variables acting as confounders or prognostic variables do not permit the untangling of the interdependence of the variables contributing to loss of function in the elderly. The effect of one variable is adjusted for the effects of the other variables which can lead to a misinterpretation of interdependent effects.

The objective of this study is to understand the complex interrelationships of body composition, PP and PA in an elderly population with the understanding that PA is a desired behaviour for this population as it will offset to a degree the negative effects of aging. We proposed a model (Figure 1) containing variables of interest using path analysis and tested if the model fits with an analysed dataset from the Quebec Longitudinal Study on Nutrition and Successful Aging "NuAge". Path analysis can determine the direct and indirect effects that we have in our model independently of all other associations[199].

15.4 Methods

Study population

Baseline data from the Quebec Longitudinal Study on Nutrition and Successful Aging "NuAge" were used in the analysis. NuAge is a five-year observational study of 1,793 men and women aged 68–82 years in good general health at recruitment, described in details elsewhere[200]. A random sample, stratified by age and sex, from a population-wide health insurance list (Quebec Medicare database RAMQ), was used to identify participants. Community-dwelling men and women, living in the regions of Greater Montreal and Sherbrooke in Quebec, Canada, were included if they spoke French or English, were free of disabilities in activities of daily living, had no cognitive impairment (Modified Mini-Mental State Examination [3MS] score, >79[118];), were able to walk one block or to climb one flight of stairs without rest, and were willing to commit to a five-year study period. Those who had heart failure greater than or equal to Class 2, chronic obstructive pulmonary disease requiring oxygen therapy or oral steroids, inflammatory digestive diseases, or cancer treated by radiation therapy, chemotherapy, or surgery in the past five years were excluded. Participation rate among eligible participants was 65.3%. The number of participants recruited in each age strata are as follows: 70 ± 2 years: 337 women (W), 329 men (M); 75 ± 2 years: 305 W, 289 M; 80 ± 2 years: 298 W, 235 M. Participants were followed annually and underwent a series of nutritional, functional, medical, biological, and social measurements in four annual follow-ups. Computer-assisted interviews were carried out by trained research dietitians and nurses following rigorous standardized procedures. All participants signed an informed consent document approved by the Ethics Committees of Montreal and Sherbrooke Geriatrics University Institutes.For the purpose of this analysis, participants with reported diabetes mellitus or taking anti-diabetic medication, or disclosing fasting glucose > 6.9 mmol/L were excluded as it has been shown that diabetes has an effect on muscle mass and physical capacity[205]. Therefore, only 1062 non diabetic

subjects were included, and from those, a total of 847 had a complete dataset. The present study's protocol was reviewed by the Research Ethics Board of the McGill University Health Centre.

The proposed model (Figure 15.1)

We propose a model leading to physical activity as a desirable goal for health maintenance, hypothesizing that personal factors (age, sex, and presence of chronic conditions) and energy intake will impact on body composition, which in turn will affect physical performance (strength and mobility), the latter determining the level of physical activity. Physical activity on the other hand shapes body composition[202] and strength and mobility as well.

The model considers personal factors and energy intake as independent variables, called in path analysis, exogenous variables, not receiving arrows. Dependent variables are body composition measures, physical performance measures and physical activity, the latter acting both as a predictor and an outcome. The proposed model (black arrows) was tested for fitting with the NuAge Study dataset that after adding new paths led to the revised model (includes both black and grey arrows) in Figure 1.

Measurement Strategy

Physical activity was assessed using the Physical Activity Scale for the Elderly (PASE)[206], which is validated in older populations. Daily activity was then recorded as a global score according to the intensity and time of reported activities. Reported activities include leisure time activities, housework, gardening, and home repair among others. Any work that does not involve mostly sitting activity is recorded as well.

Personal factors: Age, sex and comorbidity were available.Diseases were reported using a modified version of the Older American Resources and Services questionnaire[207]. Participants were asked to answer ‘‘yes’’ or ‘‘no’’ if they had

been diagnosed by a physician for 25 common conditions (e.g. osteoporosis, high blood pressure). Participants also had the possibility of adding other conditions at the end of the questionnaire. The total number of conditions was used as an indicator for comorbidity.

Total energy intake: Dietary intake was measured by three non-consecutive 24-hour dietary recalls, one face-to-face and two telephone interviews conducted by trained dietitians. The 24-hour dietary recall has been shown to be an effective tool to measure dietary intake in such large populations, providing good estimates of intake in healthy elderly people[208] The 24-hour recalls were analyzed using CANDAT program (©Godin, London ON), which uses the 2007b Canadian Nutrient File, Health Canada.

Assessment of body composition: Body composition was assessed by dual energy X-ray absorptiometry (DXA; GE Lunar Prodigy; Madison, WI) for subjects in Sherbrooke area and by bio-impedance spectrum analyzer (BIA; model 4000B, Xitron Technologies, San Diego, CA) for subjects in Montreal area. The muscle mass (kg) of the subjects undergoing BIA was estimated by entering values of reactance with a resistance at 50khz and a 800 mA current in the formula proposed by Janssen et al.[145] This formula was validated against magnetic resonance imaging (MRI), the gold standard for body composition assessment. This muscle mass value was divided by the height squared to derive the muscle mass index [MMI= muscle mass (kg) / height $(m)^2$]. Results of appendicular muscle mass obtained by DXA were extrapolated to total muscle mass using the equation of Kim et al[209], which was also validated against MRI. MMI was then calculated and entered into our model; thus the whole population was rendered similar in regard to this variable since MMI assessed by each instrument was defined against the same gold-standard of MRI. It must be noted however, that the use of either BIA or DXA is a limit to our study and that BIA must be validated against DXA in the same population, which was not the case in our subsample.

Body fat mass was estimated directly by DXA, while for that from BIA, we subtracted the fat-free mass from the weight. We then calculated percent body fat, which was entered into our model.

The tests used for assessment of physical performance were chosen for their validity and accessibility and were supervised by trained research assistants[150].

Walking speed (normal pace)[138]**.** Participants walked twice at their usual pace over a 4-meter course. Time in seconds was recorded between the 2nd and the 4th meter with a stop watch. The best trial was used for data analysis. Distance (m) divided by time (s) was used to calculate walking speed.

Balance (one leg stand)[210]**.** Participants stood without shoes, 1 meter from the wall with one foot raised about 6 inches off the ground. During the procedure, they kept their hands on their hips and stood on one leg; touching the wall or the floor or standing for 60 s would end the test. The mean performance on each side after two attempts was recorded, and the mean score for both legs was used in the analysis.

Timed up-and-go[138]: The test requires a subject to stand up, walk 3 m(10 ft), turn, walk back, and sit down. Time taken to complete the test was recorded in s.

Strength measures[211]. Quadriceps and biceps strength were recorded in kg of force using a dynamometer (Microfet 2 ^{TM}) whereas handgrip strength was measured in KPa using a Martin Vigorimeter. For analyses we used maximum scores obtained from three trials, from right hand side.

Statistical analyses

All the direct and indirect effects between all these variables were modeled by path analysis to be able to estimate all the interrelationships amongst them. Basic descriptive statistics and bi-variate relationships were used to describe our sample and test relationships between variables. As the sample sizes were very large, only correlations greater than 0.5 were considered statistically significant.

Our objective was to explore relationships among the variables of our model, we used path analysis to estimate if our theoretical model fits with the data. As the measurement of physical performance, a key construct in our theoretical model requires a large number of tests, principal component analysis (PCA) with varimax rotation was used to create indices of physical performance to be used in the path model. Path analysis combines factor analysis, correlation and regression but all variables are observed. This is in contrast to structural equation modeling (SEM) which is based on latent variables to express constructs that are not directly measurable[198]. All our variables in the model were continuous and observed variables. Path analysis is used to test a confirmatory versus an exploratory hypothesis or theory with the main purpose to find out if the model fits with the analysed dataset. The benefit of path analysis would be to test the direct and indirect effects that we have in our model independently of all other associations[199].

The path analysis involves several steps: (i)-specification of the model based on theories and hypotheses; (ii)-identification of the model to check if the values could be used to be analysed; (iii)-estimation technique, the one used in this article was the maximum likelihood estimation; (iv)- evaluation of the fit statistics and the results obtained if they fit or not with data; (v)- modification of the model based on rationale and hypotheses, by adding paths that allow for better fit of the data[199].

We used the goodness of fit indices to evaluate our model. Chi square test was used to verify the null hypothesis that the model does fit with the data. The statistical indices used included: Normed fit index(NFI); Root mean square error of approximation (RMSEA); Comparative fit index (CFI), Standardized root mean square residual(SRMR). The NFI may range in value from 0 to 1, where 0 represents the goodness of fit associated with a ‘null’ model. Values on the NFI and the CFI >0.9 indicate an acceptable fit between the model and the data. The SRMR is the average of all residuals and should not exceed 0.1. RMSEA is

favorable at a value of less than 0.05[212]. For model identification purposes, we rescaled PASE score, strength and mobility to 1/10 and energy intake to 1/100. Estimates presented are from rescaled variables entered into the model. Significance is set at $p<0.05$. Analyses conducted with SAS PROC CALIS procedure for path analysis and SPSS, version 18 for PCA.

15.5 Results

Characteristics of the study population according to sex are presented in Table 15.1. The average age of women and men and their BMI were similar. The expected differences (body fat % and muscle mass) between men and women were observed for the other variables considered in the model. The principal component analysis applied to the physical performance tests identified 2 factors with eigenvalues greater than 1 that were retained (eigenvalue= 2.942 for first factor labelled "strength" and 1.161 for second factor labelled "mobility"). The first factor explained 49.0% of the variance in physical performance and the second 19.3%. We then calculated a value for each subject on the strength and mobility indices based on the factor loadings presented in Table 15.2. These two indices were used into our model as continuous variables. We report the bivariate analyses of strength and mobility with the other variables in Table 3. The only strong positive correlation (r >0.8) was between the indices of strength and mobility. Moderate positive correlations (0.5 to 0.8) were observed for MMI and mobility and MMI and strength. A moderate negative correlation (-0.5) was seen between fat and strength. Weak correlations were observed for the other variables and are not presented (Table 15.3).

Goodness of fit indices for the initial and revised models are presented in Table 15.4. Chi square, testing the exact fit hypothesis, was significant on the initial model. Values of CFI and NFI did not exceed 0.1; RMSEA was 0.3 and SRMR 0.1. These values suggested that our model could be improved. We thus added paths consistent with theories and hypotheses[213]. Physical activity received paths from age, chronic diseases and sex, based on the fact that these variables would affect physical activity. Age, sex, body fat %, muscle mass and chronic diseases could all impact on mobility. Strength received paths from sex, age and chronic diseases. The fit statistics for the revised model include a non-significant Chi square test, indicating that we failed to reject the null hypothesis and our model had thus a good fit in our population. All other fit statistics were favourable and indicated that our model fits with the data (Table 15.4).

All paths for our revised model were significant with the exception of age on % body fat, PA on MMI, chronic diseases on MMI and % body fat on strength (data not shown). We kept the non-significant paths since they are based on our hypotheses.

As shown in Table 15.5 and Figure 15.1, several significant associations were observed between the variables in our model. Mobility and strength were significantly positively associated, where each one unit of increase in strength, was associated with a 0.37 unit increase in mobility independently from other variables in the model and this applies for all associations between variables stated throughout this article. Both % body fat and MMI were significantly negatively associated with mobility. A one unit increase in % body fat was associated to a 0.2 unit decrease in mobility. On the other hand, a 1.1 unit decrease in mobility score was associated with a one unit increase in MMI.

However, only MMI was significantly associated with strength (for each one unit increase in MMI, there was a 6 unit increase in strength). Physical activity was significantly positively and negatively associated with both MMI and % body fat respectively. Mobility, associated to both % body fat and MMI, was in turn significantly positively associated with PA.

Age was significantly negatively associated with strength, MMI, PA and mobility. Each unit increase in age is associated to a 2.3 unit decrease in strength and each unit increase in age is associated to a 0.05 unit decrease in MMI.

A counterintuitive result was found between energy intake and % body fat independently of all other variables in the model, as one unit increase in energy intake was associated significantly with a 0.0015 decrease in % body fat. This may be due do underreporting of energy intake with increased BMI and % body fat. This has also been seen in other studies where the main predictors of underreporting were old age and adiposity[214].

15.6 Discussion

Our main objective was to understand the complex interrelationships of body composition, physical performance and physical activity, taking into consideration energy intake, sex, age and diseases to promote healthy aging. To our knowledge, this is one of the first studies to investigate the associations between these different interrelated variables in older adults in one model by path analysis. We conduct principal component analysis (Table 15.2) to separate the many tests assessing physical performance, enabling one to relate specific components of performance to each body composition compartment. The richness of the data makes our model valuable in defining the independent relationships found, contributing to the pertinence of the information derived from these analyses.

The initial model did not get a good fit in our population; this led us to add paths from exogenous variables directly to physical performance measures (mobility and strength) as well as on physical activity. But all the paths added were based on hypotheses that age, sex; smoking and chronic diseases would affect directly physical performance and PA. We believe that the final model show the importance of the direct effect of exogenous variables on the endogenous variables. We discuss below the final model while acknowledging that its external validity may be limited as it only fits with the data in our population; it must be tested in different samples for the model to be generalized.

In the model, exogenous variables comprising age, sex, energy intake and chronic diseases were all related to either aspects of physical performance (strength and mobility) in our cohort. Regarding body composition aspects, parts of our results are consistent with previous analyses conducted within the framework of the NuAge study. Bouchard et al.[150] reported that fat mass but not lean body mass is related to reduced physical capacity in the elderly participants, when physical capacity was measured by one leg balance and walking speed. More recently, it was suggested that waist circumference, a proxy measure of abdominal fat accumulation could be used in older individuals to assess risk of mobility

impairment[215]. In the present analyses, which included also participants from Montreal area while excluding those with diabetes, we have as well found a significant negative association between % body fat and mobility such that for each unit increase of % body fat, there was a 0.2 unit decrease in mobility score. As with other studies [110-112, 150] % body fat was negatively associated with mobility. Body fat % in our study on the other hand was not associated with strength score.

We obtained significant positive associations between muscle mass and strength such that for each unit increase in MMI there was a 6 unit increase in strength. This association is not surprising since muscle is the substratum needed to develop strength. However, this relationship is not linear as the quality of the muscle based on strength/muscle measurement is an important component[216]. On the other hand, we have found significant negative associations between MMI and mobility controlled for sex and all other associations in the model. In the reported study of NuAge by Bouchard et al.[150], there was however a non-significant negative correlation between appendicular skeletal mass and mobility measures of 0.09 and 0.05, in men and women, respectively. Other than having excluded diabetic subjects in our analyses who are more prone to balance problems not related to muscle mass, we took also into consideration the effects of strength in our model. These might have affected the relationship between MMI and mobility, explaining at least in part the discrepancy between the previous results of NuAge[150] with the present analysis.

The counterintuitive negative association between MMI and mobility score independent of all other variables in our model could be explained by the concept of muscle quality with aging. Muscle quality is defined as the strength divided by muscle mass. In our study, we did not measure muscle density and fat infiltration into the muscle tissue, which have been linked with decreased mobility, even after correction for % body fat[208]. As stated previously, mobility requires more than muscle mass alone and the usefulness of the latter rests in the development of strength, a marker of its quality. The association between the concept of muscle

quality and muscle density[58] may explain some of the apparent discrepancy found between muscle mass and mobility. This concept of muscle quality extends also to metabolic aspects as it has been shown that insulin resistance was exacerbated with increased lean body mass in postmenopausal women[119]. Exercise increases strength but does not always translate into increased muscle mass. Although the latter findings may not be related to the association we report between mobility and muscle mass, they further illustrate the concept of worsening muscle quality in aging.

Additionally, we found a significant positive association between strength and mobility in our model showing for each unit increase in strength there was a 0.37 unit increase in mobility. Within the context of the NuAge study, individuals with a low relative strength measured by handgrip and quadriceps strength were more likely to have lower mobility scores[108]. By including subjects of both sites, we are thus confirming the solidity of previous findings and this by including all aspects at once.

Physical activity in our model was negatively associated with % body fat but positively so with MMI (physical activity being a predictor). These associations suggest that physical activity is the right approach to improve physical performance since it was associated with mobility, independently from the effect on % body fat and it is envisioned that this is mediated through better quality of muscle mass with exercise[152] since strength impacts on mobility. Based on our model, one can infer that for older persons to engage in physical activity the prerequisite is to have sufficient mobility and strength. This indicates that these aspects would need to be addressed for older persons to be more involved in exercise activities. It is well known that one of the barriers to perform exercise lies in limited mobility[217]. Likewise low muscle strength makes exercise so demanding that any program of exercise for older adults needs to be tailored to their low performance[218].

Due to the originality of our model, one can infer relationships with practical application to everyday life. Thus, our model suggests that greater muscle mass

may not be the best target to enhance mobility, and coupled with the fact that % body fat is also negatively associated with mobility, weight loss would be desirable in this population to achieve mobility purposes. In fact, a BMI threshold of 30.5 kg/m^2 above which women is expected to have greater risk of impaired mobility has been suggested with no threshold being identified for men[106]. We are cognizant that weight loss might not always be advantageous considering the impact on other aspects of function and health in older adults[219]. However, based on our results, weight loss should be directed exclusively towards fat mass and not muscle mass since the latter has a significant positive association with strength, which if lost, would affect the functionality of this population. Some intervention studies in mildly obese elderly persons have shown that this is feasible through a negative energy balance combined with physical activity[109].

We have found a counterintuitive association between % body fat and energy intake in our model this may be due to overweight people underreporting and underweight people over reporting.

Our study has several limitations including the path analysis statistics for our cross sectional analysis of associations, which cannot assume causality since this is a design issue and not an analysis one. We are also cognizant that our model could include other relationships providing good fit with other or different paths added. However, we conducted thorough analysis and we consider that both the initial and the revised model provided at the very least, a minimally acceptable fit to the data. The revised model provided the best fit with a good model chi-square value. In addition, the revised model demonstrated very high values in excess of 0.99 on the NFI, NNFI and CFI. We added paths in our revised model, however according to MacCallum et al.[213] changes must only be made when they are theoretically meaningful. In our view, the paths we added are clearly interpretable within theories. Still, the revised model is itself of admittedly questionable validity, since it results from data-driven modifications made to a rejected initial model, and it is based on a single sample. It is therefore possible that the model will not generalize to other samples from a different population. Future path

analytic studies should be conducted to test the validity of this model, preferably by comparing the two models investigated in this study as a priori models, to determine which provides best fit to the data in new samples.

Of note, we included men and women together in our sample, despite known differences in body composition. However, we also tested them separately to fit our model by gender and the results did not change; the fit statistics were the same and as good as those provided in the present analyses. This adds to the validity of our results. In this study, we have merged body composition measurements of participants from the two recruitment sites of the NuAge Study despite the different techniques being employed. For this latter aspect, we took great care of assembling the data on muscle mass by adjusting quantities based on the same gold standard, i.e. magnetic resonance imaging. Finally, our analyses were limited to a healthy population without disability at baseline and our findings may not be applicable to other elderly populations such as the frail elderly. However we detected associations in a healthy sample which may be even stronger in disabled or diseased participants. Associating these variables and significant paths to diseases and outcomes requires longitudinal analyses but our model, using cross sectional data at this stage, can assist in better defining ways of counteracting the expected decrease in physical performance associated with aging.

We must also be cognizant that our model shows a circular pattern. With a lack of exercise, people lose strength and muscle; on the other hand, they reduce physical activity which in turn affects body composition and physical performance. How to break this circular pattern needs further elucidation by using longitudinal type of analyses. This model could explain in part the complex interrelationships that occur with aging and the potential factors that could be targeted to assist older individuals in better aging.

In conclusion, our inclusive model of path analysis which fitted with our data indicated that mobility components influence PA. Mobility would in turn be influenced positively by strength and negatively by % body fat. Strength is influenced by MMI. PA influences % body fat. We proposed and confirmed a

model taking into consideration PA, PP and body composition components among a national sample of Canadian community dwelling older men and women. This model suggests that many aspects must be taken into consideration when assessing factors association with PP and PA in older adults.

Table 15.1: Characteristics of study population

Participants	Women	Men	P value
N	470	377	
Age (y)	73.9 ± 4.1	73.6 ± 4.0	0.26
Chronic diseases (median±SEM)	3.0±0.09	2.0 ± 0.09	<0.0001
BMI (kg/m^2)	27.2± 4.4	27.5 ± 3.9	0.29
Energy intake (kcal/d)	1687 ± 399	2105 ± 497	<0.0001
Fat mass (%)	39.0 ± 6.9	27.1 ± 7.1	<0.0001
Muscle mass index (MMI; kg/m^2)	7.4 ± 1.0	9.9± 1.0	<0.0001
Walking speed (m/s)	1.1± 0.2	1.2 ± 0.2	<0.0001
One leg balance (s)	13.8 ± 16.6	21.0 ± 21.4	<0.0001
Timed up and go (s)	10.4 ± 2.1	10.0 ± 1.8	0.0002
Handgrip strength (KPa)	59.1 ± 16.1	75.4 ± 17.2	<0.0001
Biceps strength (kg)	29.4 ± 7.2	55.1± 12.8	<0.0001
Quadriceps strength (kg)	43.4 ± 13.8	72.2 ± 20.6	<0.0001
Physical activity (score)	93.5 ± 46.7	119.0 ± 58.4	<0.0001

Results are mean±sd

p value significant at <0.05

Table 15.2: Principal component analysis for physical capacity measures:

Physical function	Index 1 Strength	Index 2 Mobility
TUG	0.13	**0.87**
One leg balance	0.28	**0.53**
Walking speed (m/s)	0.16	**0.86**
Quadriceps strength	**0.82**	0.23
Biceps strength	**0.90**	0.14
Handgrip strength	**0.75**	0.21

We calculated each subject's strength and mobility indices by the following equations:

***Strength (Index 1)** = (0.16 X walking speed) + (0.28 X balance) + (0.82 X quad right) + (0.90 X biceps right) + (0.75 X handgrip) + 0.13(TUG)*

***Mobility (Index 2)** = (0.86 X walking speed) + (0.53 X balance) + (0.23 X quad right) + (0.14 X biceps right) + (0.21 X handgrip) + 0.87(TUG)*

Table 15.3: Significant Pearson correlations of variables with strength and mobility indices

	Fat (%)	MMI	BMI	Strength	Mobility
Fat (%)	1				
MMI	-0.50*(-0.54;-0.45)	1			
BMI	0.55*(0.51;0.59)		1		
Strength	-0.50*(0.55;-0.46)	0.68* (0.65;0.71)		1	
Mobility				0.863* (0.849; 0.876)	1

Results are r (95%CI)

**. Correlation is significant at the 0.01 level (2-tailed) and r > 0.5*

Table 15.4: Goodness of fit indices for initial and revised models

Model	Chi-square	Df	Pr	NFI	RMSEA	95% CI RMSEA	CFI	SRMR
Initial model	1226.44	15	<0.0001	0.67	0.30	0.29-0.32	0.67	0.10
Revised model	4.24	5	0.51	0.99	0.000	0-0.044	1.00	0.0073

Note: N= 847. NFI= Normed fit index; RMSEA= Root mean square error of approximation; CFI= Comparative fit index, SRMR= Standardized root mean square residual

The NFI may range in value from 0 to 1, where 0 represents the goodness of fit associated with a 'null' model. Values on the NFI and the CFI >0.9 indicate an acceptable fit between the model and the data.

The SRMR is the average of all residuals and should not exceed 0.1

RMSEA: favorable at a value less than 0.05

Table 15.5: Estimates and standard errors of the path analysis of the revised model

Physical activity on:	Estimate	Standard error	t-test[1]
Mobility	0.65	0.13	4.88*
Age	-0.17	0.04	-3.72*
Sex	1.077	0.41	2.57*
Chronic diseases	-0.28	0.088	-3.17*
Mobility on:			
Strength	0.37	0.01	35.91*
Fat mass	-0.02	0.003	-6.04*
Muscle mass index	-0.11	0.029	-3.9*
Age	-0.04	0.007	-5.4*
Sex	-0.58	0.11	-5.3*
Chronic diseases	-0.07	0.014	-4.94*
Strength on:	Estimate	Standard error	t-test
Fat mass	-0.004	0.01	-0.3
Muscle mass index	0.6	0.09	6.4*
Age	-0.231	0.02	-10.19*
Sex	4.44	0.33	13.40*
Chronic diseases	-0.12	0.04	-2.7*
Fat mass on:			
Physical activity	-0.16	0.04	-3.40*
Age	0.03	0.05	0.53
Sex	-10.65	0.54	-19.61*
Energy intake	-0.15	0.053	-2.95*
Chronic diseases	0.25	0.12	2.11*
Muscle mass index on:			
Physical activity	0.02	0.006	3.07*
Age	-0.05	0.008	-6.3*
Sex	2.36	0.075	31.26*
Energy intake	0.0225	0.0074	3.03*
Chronic diseases	0.0339	0.017	1.98

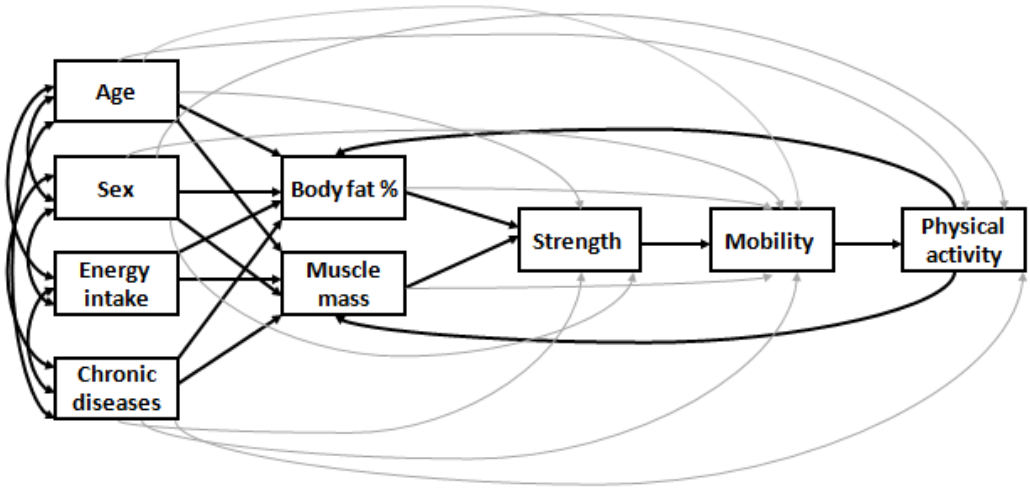
[1]: statistical test to determine the independent association between each two variables

**: significant at t in absolute value>2*

Value for men entered as 1, women 0

Results are presented as Y on X

Figure 15.1: Proposed and Revised model



Note:

Endogenous variable: variable receiving an effect from a predictor and acting as an outcome

Exogenous: predictor not receiving arrows

Double headed arrow: covariance between exogenous variables

Single headed arrow: path from an exogenous to an endogenous variable

Muscle mass representing muscle mass index

____: proposed model

____: added paths which complement the proposed model to attain the revised model.

Please refer to methodology for description of variables in the model.

16 Bridge 2

The purpose of the first manuscript was to understand cross sectional relationships between muscle mass, physical performance measures and physical activity.

As physical activity modulates body composition favorably, it also has a role in preventing insulin resistance. However, the association between muscle mass and insulin resistance is not fully understood, neither the role of different sources of dietary protein intakes on muscle mass and insulin sensitivity. Our second objective is to thus understand cross sectional associations among body composition, insulin resistance and dietary protein intake taking into consideration other factors that occur with aging.

Aside from the association of muscle mass with performance, skeletal muscle has also been associated with metabolic outcomes. Srikanthan et al. showed however that higher muscle mass was associated with better insulin sensitivity using data from the third National Health and Nutrition Examination Survey data III[220]. We hypothesize that muscle mass is negatively associated with insulin resistance, and that skeletal muscle could be positively associated with higher insulin sensitivity.

Additionally, protein intake particularly animal sources might prevent further muscle loss and hence prevent insulin resistance, however high protein intakes and particularly animal protein intakes might be associated with increased risk of insulin resistance and diabetes development.

To untangle the issue of those paradoxes, we propose to study a proposed model combining all factors cited above (body composition components, dietary protein intakes, physical activity and insulin resistance, as well as other factors that occur with aging).

17 Manuscript 2

17.1 Title : The Homeostatic Model Assessment of insulin resistance (HOMA-IR) score is negatively associated with muscle mass index and protein intake in elderly participants of the Quebec Longitudinal Study on Nutrition and Successful Aging (NuAge)

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17.2 Abstract

Background: Aging is associated with a gain and central redistribution of fat depots and a loss of lean tissue, mainly skeletal muscle, predisposing to insulin resistance. Ensuring adequate dietary protein intake is considered an easy and inexpensive way to combat loss of muscle mass. However, unlike plant sources of protein, animal source proteins may confer an increased risk of insulin resistance and diabetes.

Hypotheses: Muscle mass is positively associated with insulin sensitivity. Animal protein intake is positively associated with muscle mass and negatively with insulin sensitivity. Plant protein intake is negatively associated with MMI but positively with insulin sensitivity. **Objective**: To test if a proposed model taking into consideration different sources of protein intake, body composition components and insulin sensitivity fits with our data.

Participants: A total of 441 non-diabetic participants of the Quebec Longitudinal Study on Nutrition and Successful Aging (NuAge) from the complete dataset with all available measures.

Methods: Muscle mass index (MMI; kg/height in m^2) and % body fat were derived from DXA or bio impedance analysis. Insulin resistance was estimated based on the HOMA-IR score. Physical activity was assessed by the PASE questionnaire. Protein intakes and sources were obtained from three non-consecutive 24h-diet recalls. We propose a model hypothesizing that dietary intake from different protein sources and fat intake, all corrected for energy intake would be associated with body composition. Body composition measures would in turn be associated with HOMA-IR score. Path analysis of a proposed model was used to determine whether our theoretical causal pathway fit the data. We used several fit statistical indices to evaluate and improve our path analysis model. Path analysis provides associations between each two variables correcting for all other variables in the model.

Results: In the final model, direct positive associations were observed between HOMA-IR score and MMI and % body fat. There were no associations between animal protein intake and MMI neither between animal protein intake and HOMA-IR. There was a significant direct negative association between plant protein intake and MMI, whereas it was not related to HOMA-IR. Of interest, there were significant, positive indirect associations between animal protein intake and HOMA-IR score and significant negative indirect associations between plant protein intake and HOMA-IR. These indirect associations were mediated through MMI and % body fat. As expected, physical activity in our model favorably modulated body composition. All the associations between each two variables were independent of all other variables in the model.

Our hypothesized initial model did not get a good fit with the data without the effect modifiers, the final model taking into consideration age, sex, smoking, number of chronic diseases and physical activity fits with our data: Chi-Square= 4.83, RMSEA Estimate=0.0000.

Conclusions: Our final model indicated that contrary to expectations, MMI and HOMA score were significantly positively associated. This relationship is counterintuitive since it suggests that higher muscle mass with aging is associated with insulin resistance. Protein intake sources were related to HOMA-IR score differently through MMI and % body fat, respectively, suggesting that plant protein intake was associated negatively with insulin resistance and negatively with muscle mass, whereas the reverse holds for animal protein. All the associations between each two variables being independent after controlling for other variables included in the model. Longitudinal data are needed to validate these cross sectional findings and to determine optional amounts of both quantity and quality of protein intakes in the elderly population.

17.3 Introduction

Aging is associated with body composition changes, with an increase in fat mass[149] and a decrease in fat-free mass (mainly muscle), which is consistent with sarcopenia when it reaches a certain threshold. The accumulation of fat occurs more in the visceral region as omental and mesenteric fat depots[221], although muscle infiltration also occurs[222]. Increased visceral fat has been linked to the secretion of inflammatory markers such as adipokines and cytokines[223] and has been associated with an increased risk of insulin resistance (IR)[156]. For example, inflammatory markers such as TNF-α have been shown to act negatively on insulin action[157]. On the other hand, it has been proposed that the loss of skeletal muscle mass contributes to obesity and hence IR, since the reduction in the resting energy expenditure[158] that ensues will redirect the excess of dietary energy into fat accumulation. Sarcopenic, non-obese individuals have been found to have a high homeostatic model assessment of insulin resistance (HOMA-IR) score[220], implying that there are also other factors unrelated to obesity associating muscle loss with IR. Sarcopenia is accompanied by the loss of myocyte number preferentially affecting type 2 muscle fibers whereas type I fibers remain unchanged[71] or may decrease at a much slower rate than type 2 fibers [72]. Type 1 fibers contain most of the muscle mitochondria and aging is associated with damage to its structure that affects function[74]. This damage has been linked to insulin resistance[75]. The mitochondrial dysfunction in the elderly can also lead to a decreased fat oxidation in muscles[159]; as a consequence, there is intramyocellular lipid (IMCL) accumulation, which has been implicated in the development of IR [160]. Some studies have linked obesity to IMCL and to insulin resistance[161]. We propose that low skeletal muscle mass is linked to lower insulin sensitivity.

The decline in muscle mass can reach 40% by the eighth decade of life[7] which poses a significant public health burden [8]. There are several ways to prevent sarcopenia, but one of the easiest and most accessible ways consists in ensuring

adequate dietary protein intake. It has been proposed that the main effect of muscle protein synthesis (MPS) is attributable to amino acids[224],[225]. Protein types are usually divided into high quality or low quality, depending on their essential amino acids (EAA) content, with animal protein being assigned a high quality. It has been shown that a higher EAA content in the diet is linked to a higher MPS in both men and women[226]. Although total protein intake, particularly animal source, could have a beneficial effect on muscle mass, it appears however, that a high animal protein intake could increase the risk of IR. A number of studies have suggested that a diet high in protein of mainly animal origin may increase risk of IR development [44, 45, 227]. In fact, protein intake in most adults has increased in Western countries, attaining 150% of the recommended dietary allowances[228] (RDA; 0.8 g/kg/d), although it was found that 25% of elderly persons consumed less than 0.8 g/kg/d[229]. Optimal protein intake for the aged population is still highly controversial with some considering the RDA recommendation to be insufficient for maintenance of an optimal MPS[230].

Epidemiological data on long-term high protein intake support the notion that it might lead to impaired glucose metabolism, IR and type 2 diabetes[44, 45]. Many mechanisms have been proposed, including an increased glucagon secretion, gluconeogenesis and reduced glucose disposal [46]. For example, human studies of intravenous amino acids have induced lower uptake of glucose by the peripheral tissues[47]. It is still unclear however, if those negative effects of protein intake on IR could be attributed to all sources of proteins. While dietary intake of total and animal protein intake (mean intake of 1.1g/kg/day) increased the risk for type 2 diabetes after adjustments for fat intake and adiposity, plant protein intake, on the other hand, was not associated with IR in a population aged 21-70 years[44].

The above appears to suggest a paradox concerning the benefits of protein for health and that the optimal intake and sources of dietary protein to minimize IR or

muscle loss in older adults remain to be determined. The recommendations need to target a minimization of the effect of muscle loss while preventing IR development. Because of the opposing effects of protein on these two variables and because there is still no consensus on the optimal level of protein intake, we studied an initial model which takes into account protein intake of both quantity and quality, body composition, insulin resistance, physical activity, age, sex differences, chronic disease and smoking status. This study was as well motivated by results from a randomized controlled trial that attributed beneficial effects of amino acids on insulin sensitivity in sarcopenic individuals[48, 49]. To date, there is no consensus regarding many of these effects, and to our knowledge no study has yet determined the independent associations between muscle mass, protein intake and insulin resistance in one model.

To test our model, we performed a path analysis on a subsample of a healthy cohort of older adults to examine the interrelationships between the variables in our model and to test the associations of different sources of protein intake on IR and muscle mass, which could be implicated in reducing the risk of IR or sarcopenia development. We hypothesize that 1) muscle mass is negatively associated with insulin resistance independently from adiposity, 2) a dietary protein intake, and particularly animal protein intake, is positively associated with muscle mass, and 3) a high intake of protein, particularly animal protein, is positively associated with insulin resistance independently from adiposity.

We hypothesize and propose an initial model that suggests that dietary plant and animal protein as well as fat intake, all corrected for total energy intake, influence directly muscle mass, body fat and insulin resistance. Body composition components (muscle mass and body fat) would in turn also influence insulin resistance. In this way we would get direct effect of dietary intake of protein (plant vs. animal) and fat intake on both muscle mass and body fat and indirect effect of protein and fat intake on insulin resistance through body composition components. However, since other confounding factors might influence the variables included in the initial model, we decided to test separately two models:

one initial not taking into account age, sex, physical activity, smoking and the number of chronic diseases but only dietary intake of fat and protein, body composition components and HOMA-IR score. The second tested final model took into account all factors together. Both models were based on our hypotheses; however we wanted to test the fit statistics of our initial model to test the difference after including all effect modifiers.

17.4 Methods

The study population

Data for this study are from the Quebec Longitudinal Study on Nutrition and Successful Aging "NuAge"[200]. The NuAge study is a four year observational study of 1793 community-dwelling men and women aged 68-82 years at recruitment in general good physical and mental health, and functionally independent at recruitment. The participants were selected in two phases: a telephone survey and a clinical examination. The purpose was to generate 600 people per sex-age stratum equally divided between sexes. The recruitment of the population took place in Montreal and Sherbrooke areas. The methodology has been described in detail elsewhere[200]. Participants were French or English speaking and had to commit for a five-year period. They also had to be free of disabilities in activities of daily living (ADLs), without cognitive impairment, and able to walk 300 meters (one block) or to climb 10 stairs (one floor) without rest. Exclusion criteria were class II-IV heart failure, symptomatic chronic obstructive pulmonary disease, inflammatory digestive diseases or cancer in the past five years (except basal cell skin cancer). All participants signed an informed consent form approved by the Ethics Committees of Montreal and Sherbrooke Geriatrics University Institutes. At each annual interview, participants underwent extensive evaluation of body composition, dietary intake, physical performance, functional autonomy and provided a venous blood and urine sample for future analyses. The present study is based on data from 1062 non diabetic subjects. Participants suffering from diabetes were excluded based on reported diabetes mellitus or taking anti-diabetic medication, or fasting glucose > 7 mmol/L, because it has

been shown that diabetes has an effect on muscle mass[205]. Among these individuals, a total of 441 non- diabetic participants had a complete dataset of baseline data from the longitudinal study. The present study protocol was also reviewed by the Research Ethics Board of the McGill University Health Centre.

The proposed model (Figure 1.1; black arrows)

Our proposed initial model hypothesized that dietary intake from different protein sources and fat intake, all corrected for energy intake would affect body composition and that body composition measures would in turn affect HOMA-IR score.

Protein intake and fat intake were hypothesized to impact directly on body composition measures and HOMA-IR score. Body composition measures would impact directly on HOMA. Protein intakes would also indirectly impact on HOMA through both MMI and % body fat.

We combined both protein sources into one category for model representation purposes; however both variables (plant and animal sources of protein) were analyzed separately with both having identical paths to those represented in the model.

Personal factors such as age, sex, physical activity, smoking and presence of chronic conditions were considered as effect modifiers or confounders, and were entered into the final model as exogenous variables.

Insulin sensitivity was estimated based on the HOMA-IR score that requires only determination of fasting plasma glucose and insulin concentrations; it is calculated using the following formula: [insulin (μU/ml) x glucose (mmol)/22.5]. The HOMA-IR score is very practical for use in large populations[16] and has been compared with the gold standard, the hyperinsulinemic, euglycemic clamp in different age groups with good correlations (r=0.69-0.83)[231]. The hyperinsulinemic euglycemic clamp technique is a direct measure of IR and determines the amount of glucose necessary to compensate for an increased insulin level without causing hypoglycemia[192].

We did not dichotomize the HOMA score to assess IR in our study sample or determine individuals with IR, we modeled however HOMA as a continuous

variable and tested its association with all variables of interest. Fasting insulin concentration was determined using a human insulin radioimmunoassay (RIA) kit (detection limit: 12 pmol/L per tube; intra-assay coefficients of variation: 1.1% to 8.3%; Linco Research Inc, St. Charles, MO).

Personal factors of age, sex, smoking exposure, physical activity and comorbidity were available and included in the analyses. We hypothesized that all the above personal factors would affect dietary intake of protein and fat, body composition components and HOMA-IR score. Chronic diseases were reported using a modified version of the Older American Resources and Services questionnaire[207]. Participants were asked to answer ‘‘no’’ or ‘‘yes’’ if they had been diagnosed by a physician for 25 common diseases (e.g., diabetes, osteoporosis, high blood pressure). The total number of diseases was used as an indicator for comorbidity. Lifetime tobacco use is presented as pack-years smoked, based on 20 cigarettes per pack. Current physical activity was quantified using the Physical Activity Scale for the Elderly (PASE) questionnaire[232].

Dietary variables comprised total energy (kilocalories), fat (g) and protein (g) intake (quantity and quality: plant versus animal protein). Dietary intake was measured by three non-consecutive 24-hour dietary recalls in one face-to-face interview and 2 telephone interviews. The 24-hour dietary recall has been shown to be an effective tool to measure dietary intake in such large populations, providing precise quantitative estimates of intake in healthy elderly people[233]. The 24-hour recalls were analyzed using CANDAT (©Godin, London ON) software based on the 2001b Canadian Nutrient File. We obtained values of total energy intake, protein and fat intake to be used in our model. We then conducted the analysis of sub groups of protein intake, classified as animal protein (all proteins from animal sources including fish), and plant proteins (all proteins from plant sources), to test their exclusive putative role in sarcopenia and insulin action. We were able to classify animal proteins into processed meats (cold cuts, sausages, cold cuts, cured beef and ham etc.) and unprocessed meat proteins. In

our analyses, we excluded processed meat proteins to test the effect of animal protein per se, as processed proteins containing nitrites have been shown to be have negative effects on insulin sensitivity [234, 235]. Dietary animal and plant protein intakes and fat intake were corrected for total energy intake and entered into our model as percentages of total energy intake. We did not enter carbohydrate intake into our model since protein and fat intake were corrected for total energy intake and carbohydrates constitutes ~ 50-60% of total energy intake[236].

Body composition was assessed by dual energy X-ray absorptiometry (DXA; GE Lunar Prodigy; Madison, WI) for subjects in the Sherbrooke area and by bio-impedance spectrum analyzer (model 4000B, Xitron Technologies, San Diego, CA) for subjects in the Montreal area. The muscle mass (kg) of the subjects undergoing BIA was estimated by entering values of reactance with a resistance at 50khz and a 800 mA current in the formula proposed by Janssen et al.[145] This formula was validated against MRI (magnetic resonance imaging), the gold standard for body composition assessment and the muscle mass value was divided by the individual's height squared to derive the muscle mass index [MMI= muscle mass (kg) / height $(m)^2$]. Results of appendicular muscle mass obtained by DXA were extrapolated to total muscle mass using the equation of Kim et al[209], which was also validated against MRI. MMI was then calculated, thus rendering the whole sample similar with regard to this variable, which was then entered into our model. Body fat mass was estimated directly by DXA, while for those assessed using BIA, we subtracted the fat-free mass from the weight. We then calculated percent body fat, which was entered into our model.

Basic descriptive statistics and bivariate relationships were used to describe our sample and test relationships between variables. All direct and indirect effects between variables were modeled by path analysis to be able to estimate the interrelationships among them. Path analysis combines factor analysis, correlation and regression but all variables are observed; it is used to test a confirmatory

versus an exploratory hypothesis or theory. The main purpose to find out if the model fits with the analyzed dataset[198]. All variables in our model were continuous.

Path analysis involves several steps: (i)-Specification of the model based on theories and hypotheses; (ii) Identification of the model to check if it is theoretically possible for the software to derive the model for a specific set of data; (iii)- Estimation technique, for which we selected the maximum likelihood estimation; (iv)- Evaluation of the fit statistics for the data; (v)- Modification of the model based on rationale and hypotheses, by adding paths that allow for a better fit of the data[199].

Several fit statistical indices were used to evaluate our model. The chi square test was used to verify the null hypothesis that the model does fit the data. The statistical indices used included the Normed fit index (NFI), the Root mean square error of approximation (RMSEA) the Comparative fit index (CFI), and Standardized root mean square residual (SRMR). The NFI may range in value from 0 to 1, where 0 represents the goodness of fit associated with a 'null' model. Values on the NFI and the CFI >0.9 indicate an acceptable fit between the model and the data. The SRMR is the average of all residuals and should not exceed 0.1. RMSEA is favorable at a value of less than 0.05[212]. For model identification purposes, we rescaled the PASE score to 1/10 in other terms, the values of the variable PASE were all divided by 10 for mathematical purposes. Estimates presented in the results and table sections are from rescaled variables entered into the model. Significance was set at $p<0.05$. The Statistical Analyses Software (SAS) version 9.3 was used for analyses. The SAS PROC CALIS procedure was used for path analysis.

17.5 Results

Characteristics of the study population are presented in Table 17.1 by sex. There were no differences between men and women in age, BMI, dietary intakes (%

protein and % fat) and HOMA score. The expected differences between men and women were observed for components of body composition and total energy intake. Men smoked more and were more physically active, whereas women reported a higher burden of chronic diseases.

We report the bivariate analyses in Table 17.2. Only weak correlations were observed between the variables included in our model and only those with $p \leq 0.0001$ were retained as significant due to the large population sample. Significant negative correlations were observed for MMI and PASE with age, MMI and chronic diseases. Significant negative correlation were as well observed between MMI and % body fat. Plant protein intake was also negatively correlated with fat intake. Positive correlations were observed between % body fat and HOMA-IR and between % body fat and chronic diseases. Positive correlations were observed between MMI and HOMA-IR and MMI and physical activity.

Goodness of fit indices for the initial and revised models are presented in Table 17.3. Chi square, testing the exact fit hypothesis, was significant on the initial model, indicating that the model was to be rejected without including effect modifiers. Values of CFI and NFI however were good fits; RMSEA was 0.34 and SRMR 0.12. These values suggested that our model could be improved. We thus added paths from effect modifiers consistent with theories and hypotheses[213] to construct a revised model (Figure 1; gray arrows added).

The fit statistics for the revised model included a non-significant chi square test, indicating that we failed to reject the null hypothesis and our model had thus a good fit in our population. All other fit statistics applied were favorable and indicated that our model fitted with the data (Table 17.3). Not all the paths in our revised model were significant and we retained the non-significant paths since they were part of our initial hypothesis.

As Table 17.4 and figure 17.1 show, several direct significant associations were observed between the variables in our model. All associations reported are

independent of all other factors included in the model. Muscle mass index and HOMA-IR score were positively associated, and for every 1 unit increase in MMI there was a 0.42 unit increase in HOMA-IR score independently from other variables (Table 4). HOMA-IR score was also positively associated with % body fat and number of chronic diseases. % body fat was positively associated with female sex and dietary fat, and negatively with physical activity score. MMI on the other hand was positively associated with male sex and negatively associated with plant protein intake.

There were also some non-significant direct associations between animal protein and MMI, between animal protein and HOMA-IR and between HOMA-IR and plant protein intake.

Physical activity measured by PASE questionnaire was significantly positively associated with MMI and significantly negatively associated with % body fat. This suggests that in our model, physical activity significantly modulated body composition in a favorable manner. There were no associations between physical activity and dietary fat in our model independently of all other factors.

Path analysis also provided indirect effects and standard errors of the interrelationships of the variables included in our model (Table 17.4). The following significant indirect associations were found: animal protein intake was positively associated with HOMA-IR whereas plant protein was negatively associated with HOMA-IR, both through body composition (MMI and %body fat), and this independently from all other variables included in our model.

17.6 Discussion

The present study employed a model comprising many variables commonly associated with sarcopenia and insulin resistance to understand the direct and indirect effects of different sources of protein intake on MMI and HOMA score. The initial model not taking into account personal factors did not get a good fit in the data showing the importance of personal factors in our model. To our

knowledge, this is one of the first studies to investigate these associations using path analysis. The well established associations of % body fat and fat intake with HOMA were reproduced, however, we found an unexpected positive association between muscle mass and HOMA-IR score, suggesting that increased muscle mass in the elderly population is associated with higher insulin resistance. Our results are consistent with those showing that sarcopenic women had lower levels of insulin resistance than non sarcopenic ones[237]. Fukagawa et al.[238] have reviewed the effect of loss of muscle on glucose tolerance and found that loss of muscle mass plays a minor role in the development of glucose tolerance; it is mainly the abdominal visceral fat that is implicated primarily in the risk of developing insulin resistance with aging. However higher muscle mass has been associated with better insulin sensitivity in analyses using data from the National Health and Nutrition Examination Survey data III (NHANES III)[220]. The discrepancy between our results and those from the NHANESIII study may lie in the different methods of assessing muscle mass index since we divided muscle mass by height^2 whereas it was divided by total body weight in NHANES III[220]. In order to understand whether this difference in the assessment method could be responsible for the discrepancy, we calculated MMI per body weight and compared results by general linear models quartiles of MMI with those of MMI per height^2 for HOMA-IR score (results not shown). We found that those having the highest quartile of MMI per body weight had the lowest HOMA-IR score whereas of the reverse was so for MMI per height^2. The definition of MMI based on weight may actually artificially lower the MMI for the same skeletal muscle mass if an individual is heavier and has a higher fat mass. In this circumstance, % body fat should be considered responsible for an increased HOMA-IR score rather than a lower MMI value. Since MMI is a composite variable in our model including height, height could possibly be associated with HOMA and distort the findings. However, no associations were found between height and HOMA making this assumption invalid.

Our results do not however suggest that older adults should lose muscle mass to prevent insulin resistance. The relationship between higher muscle mass and insulin resistance is likely to stem from the type 1 muscle fibers containing most of the muscle mitochondria, which are negatively affected by aging as older age has been related to increased mitochondrial dysfunction. Another explanation may result from muscle being infiltrated by fat with aging[160] which in turn increases the risk of inflammation and predisposes to insulin resistance; thus, having low muscle mass may attenuate this risk which has been seen in one study[119].

We found that different protein sources had different effects on the HOMA score and MMI. The only direct effect was the negative association of plant protein with MMI. The literature supports such a relationship since it has been shown that protein intake from animal sources was the only independent predictor of MMI, explaining 19% of its variance[226].

One of the advantages of the path analysis over simple linear regression is the observation of indirect effects rather than direct ones alone. We observed that the significant indirect effects of protein sources were mediated through MMI and % body fat on HOMA-IR and that the associations were in the same directions as we hypothesized, i.e. plant protein intake negatively associated with both MMI and HOMA and animal protein intake positively associated with both MMI and HOMA.

The different significant indirect effects of proteins (animal vs. plant) on HOMA could be explained by several factors. Animal protein was positively associated with insulin resistance but this was seen only indirectly through both MMI and % body fat. It is noteworthy that this effect is not independent from % body fat but independent from fat intake, and since protein was included as % of calories it was also independent of total energy intake. One plausible explanation apart from the effect of % body fat for worsening insulin sensitivity would be that animal protein is rich in iron which has been shown to act as a pro-oxidant and to increase the risk of oxidative stress and insulin resistance[239]. Another is that

processed meat is rich in nitrites and sodium, both having been implicated in increasing the risk of type 2 diabetes[240]. In our model however, the animal protein, did not include processed meats proteins as we wanted to test for the effect of animal protein but without any cured meats (cold cuts or sausages), which are also high in saturated fat and cholesterol. Our results show that even without the presence of processed meats, animal protein intake (unprocessed meats, poultry, fish, and dairy products) was associated positively but indirectly with HOMA-IR score. It is still unclear however, if those negative effects of protein intake on IR could be attributed to all sources of non processed meat proteins. In fact, Lavigne et al.[184] on a study performed on rats fed a high fat diet showed that cod protein intake adjusted for omega 3 intake in fish oil, decreased the risk of IR development in contrast to casein (major milk protein) and soy protein (vegetable source). Those beneficial effects of cod protein intake were found without any weight loss in the population, suggesting that the effect of protein per se independently from adiposity is an important determinant of insulin action. We did not dissociate fish from red meat or dairy products however in our study, so we cannot assume which type of protein was driving the positive association between animal protein intake and HOMA-IR. On the other hand, plant protein intake was indirectly through %body fat and MMI negatively associated with insulin resistance. One explanation may rest in the low essential amino acids content of vegetable proteins and their low leucine content; the low leucine intake has been independently associated with glucose uptake impairment in rats even in the presence of high insulin levels[241]. Another explanation may be that plant protein foods have higher fiber and antioxidant content than animal proteins that could foster, in part, maintenance of insulin sensitivity[242]. It is important to state however that indirect effects do not exclude the possibility that the association of both sources of protein with HOMA-IR score may be driven by % body fat and/or MMI.

The finding of the favorable effect of physical activity on both MMI and % body fat is also of interest, in that it was positively associated with MMI but negatively

with % body fat. In our study however, physical activity did not have a significant association with HOMA-IR, which is recognized as an important determinant of insulin sensitivity [84].

Our study has several limitations including the path analysis statistics for our cross sectional analysis of associations, which cannot assume causality since causality is a design issue (longitudinal versus cross sectional) and not a type of analysis. We are also cognizant that our model could include other relationships providing good fit with other or different paths added. However, we conducted thorough analysis and we consider that both the initial and the revised model provided at the very least, a minimally acceptable fit to the data. The revised model provided the best fit with a good model chi-square value. In addition, the revised model demonstrated very high values in excess of 0.99 on the NFI, NNFI and CFI.

We added paths in our revised model, however according to MacCallum et al.[213], changes must only be made when they are theoretically meaningful. Still, the revised model is itself of admittedly questionable validity, since it results from data-driven modifications made to a rejected initial model, and it is based on a single sample. It is therefore possible that the model will not generalize to other samples from a different population. Future path analytic studies should be conducted to test the validity of this model, preferably by comparing the two models investigated in this study as a priori models, to determine which provides best fit to the data in new samples. Our analyses were limited to a healthy population without disability at baseline and our findings may not be applicable to subpopulations such as the frail elderly. However we detected associations in a healthy sample which may be even stronger in disabled or diseased participants. Associating these variables and significant paths to diseases and outcomes, requires longitudinal analyses but our model using path analysis could at least help us in part understand the complex relationships that occur with aging in regard to protein intake, muscle mass and insulin resistance.

In conclusion, our model indicated that MMI based on height^2 but not on weight was positively associated with HOMA-IR score independently from adiposity. This suggests that different methods of assessing muscle mass would have different interpretations on its associations with the outcomes of interest. Animal protein intake was indirectly positively associated with HOMA-IR score while plant protein intake had the opposite indirect association. Direct effects of dietary protein on HOMA-IR score were not observed but we observed a significant negative association between MMI and plant protein intake. Indirect effects provided significant results showing the effects of the intermediate variables MMI and body fat % on the association between protein intake and HOMA score.

Physical activity significantly modeled body composition favourably, by its positive association with MMI and negative association with % body fat.

Table 17.1: Characteristics of study population

Participants	Women	Men	P-value
N	224	217	
Age (y)	73.5 ± 4.1	73.2 ± 4.2	0.41
Chronic diseases (total number of diseases)	3.8±2.0	2.8 ± 1.9	<0.0001
Smoking (packs-year)	7.0±45.6	12.9 ± 62.0	0.24
BMI (kg/m^2)	27.5±4.6	27.5±4.1	0.87
Energyintake (kcal/day)	1692±417	2059±476	<0.0001
Animal protein (% total calories/d)	8.2 ± 3.4	7.7±3.3	0.14
Plant protein (% total kcal/d)	5.1±1.3	5.1±1.4	0.95
Fat intake (% total calories/d)	34.3±6.2	34.4±5.6	0.88
CHO intake (% total calories/d)	49.37±6.9	51.07±6.5	0.006
Fat mass (%)	38.1 ± 7.2	26.4 ± 7.1	<0.0001
Muscle mass index (MMI; kg/m^2)	7.8 ± 0.9	10.0± 1.0	<0.0001

PASE (score)	94.6 ± 47.1	128.0 ± 59.7	<0.0001
HOMA-IR (score)	3.2±1.9	3.4±2.1	0.27

Results are mean ± SD

Significance at p<0.05

Table 17.2: Significant Pearson correlations between variables included in the model

	Age	% body fat	MMI	PASE	Plant protein intake
MMI	-0.22	-0.44			
HOMA		0.25	0.18		
PASE	-0.20	-0.30	0.34		
Fat intake %					-0.26
# Diseases		0.27	-0.19		-0.27

Results are r

Correlations are significant at the 0.0001 level (2-tailed)

Table 17.3: Goodness of Fit Indices for initial and revised models

Model	Chi-square	Df	Pr	NFI	RMSEA	95% CI RMSEA	CFI	SRMR
Initial model	57.98	2	<0.0001	0.58	0.34	0.29-0.4	0.57	0.12
Revised model	4.83	7	0.68	0.99	0.000	0-0.044	1.00	0.0086

Note:

N= 441. NFI= Normed fit index; RMSEA= Root mean square error of approximation; CFI= Comparative fit index, SRMR= Standardized root mean square residual.

The NFI may range in value from 0 to 1, where 0 represents the goodness of fit associated with a 'null' model. Values on the NFI and the CFI >0.9 indicate an acceptable fit between the model and the data.

The SRMR is the average of all residuals and should not exceed 0.1.

RMSEA: favorable at a value less than 0.05.

Table 17.4: Estimates and standard errors of the direct and indirect effects of path analysis of the revised model

Direct effects of animal protein (%) on: (dependent variables)

Independent variables	**Estimate**	**Standard error**	**t-test[1]**
Age	0.005	0.040	0.14
Sex	-0.52	0.34	-1.54
PASE	0.0009	0.003	0.29
# Chronic diseases	-0.0361	0.084	-0.42
Fat intake (%)	-0.08	0.027	**-2.91***
Direct effects of plant protein (%) on:			
Age	-0.02	0.015	-1.3
Sex	-0.06	0.12	-0.48
PASE	0.001	0.001	1.2
# Chronic diseases	0.025	0.031	0.79
Fat intake (%)	-0.064	0.01	**-6.19***
Animal protein (%)	-0.06	0.01	**-3.71***
Direct effects of fat intake (%) on:			
Age	0.084	0.07	1.2
Sex	-0.24	0.59	-0.41
PASE	0.0002	0.005	0.05
# Chronic diseases	-0.36	0.14	**-2.43***
Direct effects of muscle mass index on:			
Animal protein (%)	0.019	0.013	1.48
Plant protein (%)	-0.068	0.033	**-2.04***
Age	-0.066	0.011	**-5.94***
Sex	2.13	0.09	**22.47***
PASE	0.0028	0.0008	**3.32***

# Chronic diseases	0.03	0.02	1.29
Direct effects of % body fat on:			
Age	0.023	0.082	0.27
Sex	-10.64	0.70	**-15.09***
PASE	-0.01	0.006	**-2.43***
Animal protein (%)	0.14	0.09	1.42
Plant protein (%)	-0.47	0.26	-1.82
Fat intake (%)	0.13	0.058	**2.36***
# Chronic diseases	0.55	0.17	3.2*
Direct effects of HOMA on:			
Fat intake (%)	-0.006	0.015	-0.43
Age	0.031	0.022	1.41
Sex	0.48	0.30	1.62
PASE	0.0008	0.001	0.46
# Chronic diseases	0.12	0.046	**2.60***
Animal protein (%)	0.0092	0.02	0.35
Plant protein (%)	-0.08	0.06	-1.18
Muscle Mass index	0.42	0.09	**4.59***
Smoking	-0.001	0.0015	-1.14
% body fat	0.094	0.012	**7.54***
Indirect effects of HOMA on:			
Animal protein (%)	0.0321	0.01	**2.6***
Plant protein (%)	-0.07	0.02	**-2.4***

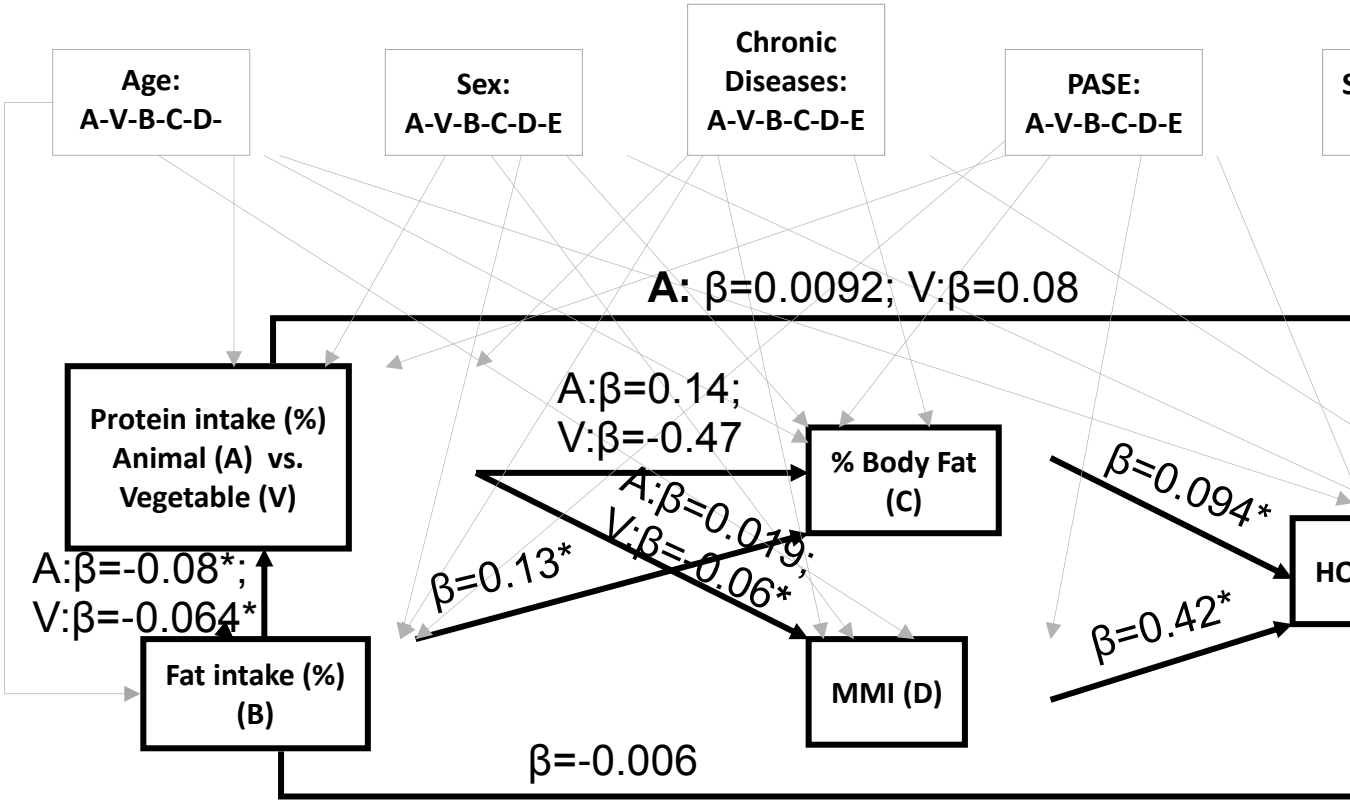
[1]: statistical test to determine the independent association between each two variables

**: significant at t in absolute value>2.*

Value for men entered as 1, women 0.

Results are presented as Y on X.

Figure 17.1: Revised model of protein on MMI and HOMA score



Note:

Endogenous variable: variable receiving an effect from a predictor and acting as an outcome.

Exogenous: predictor.

Single headed arrow: path from an exogenous to an endogenous variable.

Protein intake divided into percentage of animal protein vs. vegetable protein in the analyses, but presented here in combination for simplification purposes.

____: proposed model.

____: added path and which complement the proposed model to attain the revised model.

Please refer to methodology for description of variables in the model.

The covariances between the effect modifiers are not included in the figure for simplification purpose but were taken into account in the analyses.

Variables were given letters to better clarify the model.

**: Significant beta*

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The aim of the second manuscript was to understand cross sectional relationships among dietary protein intake from different sources (animal versus plant protein), body composition and insulin resistance using the HOMA-IR score.

We were able to detect differences of relationships between protein sources and insulin resistance. The most surprising relationship was the positive association between HOMA-IR scores, an indicator of insulin resistance, and muscle mass index. Since the previous analyses were cross sectional, our next aim was to conduct longitudinal analyses to better understand these relationships. However, since we do not have cut-offs for MMI and HOMA-IR score to define sarcopenia or insulin resistance, respectively, we decided to analyze these two variables continuously. We found that the best way to understand and describe our population was to run trajectory analyses that would provide a better comprehension of the data.

Trajectory analyses enabled one to separate insulin resistant versus non-insulin resistant subjects who were then compared for baseline variables to assist in defining risk factors associated with developing insulin resistance.

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19.1 Title: Trajectory model of the Homeostatic Model Assessment of insulin resistance (HOMA-IR) score and its association with body composition measures in elderly participants of the Quebec Longitudinal Study on Nutrition and Aging NuAge

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19.2 Abstract

Background: Aging is associated with a gain and central redistribution of fat depots and a loss of lean tissue, mainly skeletal muscle. The loss of muscle mass has been implicated in the risk of developing insulin resistance. Determining insulin resistant subjects in epidemiological studies is challenging because of the lack of cut-off for scores used to assess insulin resistance.

Objective: To identify insulin resistant subjects over a 3 year period and compare them to insulin sensitive subjects in regard to body composition measures and other baseline characteristics.

Participants: A total of 649 non- diabetic participants of the Quebec Longitudinal Nutrition and Successful Aging Study (NuAge Study) with the complete dataset.

Methods: Muscle mass index (MMI; kg/height in m^2) and % body fat were derived from DXA and bio impedance analysis. Insulin resistance was estimated based on the HOMA-IR score. Physical activity was assessed by the PASE questionnaire. Protein intakes and sources were obtained from three 24h-food recalls and analyzed with the CANDAT software. Developmental trajectories over 4 time points were used to determine insulin sensitive versus non-insulin sensitive subjects. Logistic regression analyses were used to determine baseline variables that affected insulin sensitive versus non-insulin sensitive participants over time.

Results: 7 group based trajectories were identified and good posterior probabilities were obtained. A visual inspection of the curves allowed for determination of insulin sensitive subjects and insulin resistance subjects classification. The logistic regression with the most parsimonious model provided only 3 significant predictors of insulin resistance: MMI= muscle mass (kg) / height $(m)^2$ [OR (95% CI): 1.72 (1.26-2.3)]; body fat% [OR(95%CI): 1.18 (1.12-1.25)]; male sex [OR for women versus men (95%CI): 0.145 (0.04-0.45)].

Conclusion: Our analyses showed that a higher muscle mass and % body fat contribute to a higher odd of insulin resistance with aging. Being a female decreases the odds of being insulin resistant. This relationship is counterintuitive since it suggests that maintenance of muscle mass with aging contributes to the development of insulin resistance. Our determination of insulin resistant versus non-insulin resistant subjects remains a probabilistic approach but addresses one of the challenges in determining insulin resistant subjects in epidemiological studies. Further research is required to compare results with other approaches.

19.3 Introduction

Aging is associated with an increase in disease risk, especially chronic diseases such as diabetes and cancer, among others. Data from the Canadian Community Health Survey shows that in Canada, the prevalence of the population afflicted with diabetes increases from 4.4% at age 30-64 years to 13.5% at 65 years and over[3]. Insulin is an anti-catabolic hormone regulating different substrates that stimulates glucose uptake from the bloodstream into adipocytes and myocytes while suppressing glucose production from the liver[15]. A dysregulation of insulin action, a condition named insulin resistance (IR) is responsible for increases in blood glucose and lipid concentrations, hyperinsulinemia and eventually overt type 2 diabetes[16]. IR increases with age as data from NHANES III showed a prevalence of type 2 diabetes in the elderly > 75 y of 13% with another 14% showing impaired glucose tolerance, that is twice that of the younger population[17]. With aging, there is a change in body composition, with an increase in fat mass and a decrease in fat-free mass[7]. The greater adiposity has been associated with an increased risk of IR development[156]; as visceral fat increases so is the risk of inflammation and the availability of free-fatty acids[223]. In contrast, the decrease in fat-free mass; mainly muscle, could also be independently associated with IR since skeletal muscle is the major organ for insulin mediated glucose disposal[158]. Muscle loss with aging, called sarcopenia is accompanied by the loss of myocyte number and the decrease in its protein

content, contributing to loss of muscle quality[147]. There is evidence that type 2 muscle fibers size decreases whereas type I fiber size remains unchanged[147]. Type I fiber contains most the muscle mitochondria and aging affects skeletal mitochondrial function[74]. It has been proposed that the by-products of the mitochondrial oxidative metabolism not concealed by our antioxidant defense mechanisms, interfere with the modulation of insulin action, and thus lead to its resistance[75]. Furthermore, the decrease in muscle protein in elderly persons has been linked to elevated markers of inflammation, which are implicated in insulin resistance[76].

The mitochondrial dysfunction in the elderly can also lead to a decreased fat oxidation in muscles[159]; as a consequence, there is an accumulation of intramyocellular lipid (IMCL) which has been implicated in insulin resistance development[160]. Some studies have linked obesity to IMCL and to insulin resistance[161]. In addition, a study by Petersen et al.[160] has demonstrated that mitochondrial dysfunction has been associated with reduced insulin mediated glucose disposal and increased IMCL in elderly compared to young persons and this finding was independent of overall adiposity. Because usually IR precedes diabetes, it becomes important to understand the factors that affect IR.

In epidemiological studies, insulin resistance can be determined using the Homeostatic Model Assessment (HOMA-IR) score that requires only determination of fasting plasma glucose and insulin concentrations.

The HOMA score is a very useful tool to use in large populations[16]. It had good correlations (r=0.69-0.83) compared with the gold standard, euglycemic hyperinsulinemic clamp in different age groups [231]. Based on the above, we hypothesized that, 1-HOMA-IR score would progress over time in elderly subjects; and 2- baseline skeletal muscle mass, independently from obesity is negatively associated with the development of HOMA-IR score. Using a longitudinal data from a healthy elderly cohort of the NuAge Study, our first objective was to describe the developmental changes that occur with the HOMA-

IR score over four annual time points. Our second objective was to compare sensitive versus non sensitive subjects using the HOMA-IR score, in regard to several baseline variables, including body composition. Among other factors, dietary protein intake and physical activity were taken into consideration as epidemiological data concerning long-term high protein intake shows that this might lead to an impaired glucose metabolism and possibly to IR development and type 2 diabetes[44, 45]. Since we have no cut-off value for abnormal HOMA-IR score, the depiction aspect of the developmental trajectories of the data by visual inspection and using objective analytical methods served to determine sensitive versus non sensitive subjects over time. In addition, since there is a paucity of longitudinal data in relation to development of insulin resistance with age, our study is even more justified.

19.4 Methods

The study population

Source of data for this study comes from the Quebec Longitudinal Study "NuAge"[200]. The NuAge study is a four year observational study of 1793 community-dwelling men and women aged 68-82 years in general good physical and mental health, and functionally independent at recruitment. The participants were selected in two phases: a telephone survey and a clinical examination. The purpose was to generate 600 people per sex-age stratum equally divided between sexes. The recruitment of the population took place in Montreal and Sherbrooke areas. Methodology has been described elsewhere[200]. Participants were French or English speaking and needed to commit for a five-year period. They also had to be free of disabilities in activities of daily living (ADLs), without cognitive impairment, and able to walk 300 meters (one block) or to climb 10 stairs (one floor) without rest. Exclusion criteria consisted of people suffering from class II-IV heart failure, symptomatic chronic obstructive pulmonary disease, inflammatory digestive diseases or cancer in the past five years (except basal cell of the skin). All participants signed an informed consent document approved by

the Ethics Committees of Montreal and Sherbrooke Geriatrics University Institutes. Participants underwent extensive evaluation of body composition, dietary intake, physical performance, functional autonomy and provided a venous blood and urine sample for future analyses. For the purpose of this article we include data from 1062 non diabetic subjects. Participants suffering from diabetes were excluded based on reported diabetes mellitus or taking anti-diabetic medication, or fasting glucose > 7 mmol/L, because it has been shown that diabetes has an effect on muscle mass[205]. From those, a total of 649 non-diabetic participants had a complete dataset. This article uses baseline and longitudinal data of the longitudinal study. The present study's protocol was also reviewed by the Research Ethics Board of the McGill University Health Centre.

The proposed hypotheses and model

We propose that HOMA-IR score changes over time with aging. To determine and describe the developmental change of HOMA, we used trajectories for HOMA-IR scores over four time points. We then compared sensitive versus non sensitive subjects based on trajectories to baseline variables of interest. We proposed a model hypothesizing that dietary intake from different protein sources and fat intake, all corrected for energy intake and body composition would all affect HOMA-IR score. Personal factors such as age, sex, physical activity, smoking and presence of chronic conditions were considered as covariates, and were entered into the model.

Measurements and data collection

Insulin sensitivity was estimated based on the HOMA-IR score that requires only determination of fasting plasma glucose and insulin concentrations and calculated using the following formula: [insulin (μU/ml) x glucose (mmol)/22.5]. The HOMA-IR score is very practical to use in large populations[16] and has been compared with the gold standard, the hyperinsulinemic, euglycemic clamp, in different age groups with good correlations (r=0.69-0.83)[231]. To assess the IR

in our sample of the population, no cut-offs were made and we modeled HOMA as a continuous variable. Insulin concentration was determined using a human insulin radioimmunoassay (RIA) kit (detection limit: 12 pmol/L per tube; intra-assay coefficients of variation: 1.1% to 8.3%; Linco Research Inc, St. Charles, MO).

Personal factors of age, sex, smoking exposure, physical activity and comorbidity were available. Chronic diseases were reported using a modified version of the Older American Resources and Services questionnaire[207]. Participants were asked to answer ‘‘yes’’ or ‘‘no’’ if they had been diagnosed by a physician for 25 common diseases (e.g., diabetes, osteoporosis, high blood pressure). Participants also had the possibility of adding other diseases at the end of the questionnaire. The total number of diseases was used as an indicator for comorbidity. Lifetime tobacco usage is presented as pack-years smoked, based on 20 cigarettes per pack. Current physical activity was quantified using the Physical Activity Scale for the Elderly (PASE) questionnaire[232].

Dietary assessment comprised total energy, fat and protein intake (quantity and quality). Dietary intake was measured annually by three non-consecutive 24-hour dietary recalls by one face-to-face interview and 2 telephone interviews. The 24-hour dietary recall has been shown to be an effective tool to measure dietary intake in such large populations, providing good estimates of intake in healthy elderly people[233]. The 24-hour recalls were analyzed using CANDAT program (©Godin, London ON), which uses the 2005 Canadian Nutrient File, Health Canada. The nutrient analyzed using the CANDAT program included total energy intake, protein, fat, carbohydrates, fiber, and micronutrients (vitamins and minerals). We conducted the analysis of sub groups of protein intake, classified as animal protein (all proteins from animal sources including fish), and vegetable proteins (all proteins from vegetal sources, to test their exclusive putative role on sarcopenia and insulin action. We were able to classify animal proteins into preserved and regular meat proteins. In our analyses, we excluded preserved meat

proteins to test the effect of animal protein per se, as preserved proteins containing nitrites have been shown to be even worse on insulin resistance development[234, 235]. Dietary protein intake (from both sources) and fat intake were corrected for total energy intake and entered into our model as percentages of the latter.

Body composition was assessed by dual energy X-ray absorptiometry (DXA; GE Lunar Prodigy; Madison, WI) for subjects from Sherbrooke area and by bio-impedance spectrum analyzer (model 4000B, Xitron Technologies, San Diego, CA) for subjects from Montreal area. The muscle mass (kg) of the subjects undergoing BIA was estimated by entering values of reactance with a resistance at 50khz and a 800 mA current in the formula proposed by Janssen et al.[145] This formula was validated against magnetic resonance imaging (MRI), the gold standard for body composition assessment. This muscle mass value was divided by the height squared to derive the muscle mass index [MMI= muscle mass (kg) / height $(m)^2$]. Results of appendicular muscle mass obtained by DXA were extrapolated to total muscle mass using the equation of Kim et al[209], which was also validated against MRI. MMI was then calculated, rending thus the whole population similar in regard to this variable, which was entered into our model. Body fat was estimated directly by DXA, while for that from BIA, we subtracted the fat-free mass from the weight. We then calculated percent body fat, which was entered into our model.

Statistical analyses

To estimate the change in HOMA-IR score over 4 time points (3 years), we used developmental trajectories. We used the Statistical Analysis Software (SAS version 9.2) procedure TRAJ which is based on a semi parametric group-based modeling strategy[243]. The model is a mixture of probability distributions that are suitable for the data to be analyzed. This SAS procedure complements hierarchical modeling and latent growth curve modeling. Since TRAJ provides the option of modeling three different distributions for probabilities, we used the censored normal model (CNORM). The CNORM model is appropriate for data

that are approximately normally distributed with or without censoring. The censoring is used because the data tends to cluster at the minimum and at the maximum of the scale. The group-based trajectory is designed to identify clusters of individuals following the same progression over time[243]. The calculated HOMA-IR score was the outcome regressed over time. Fit statistics and posterior probabilities of group membership were used to compare models with different trajectory groups.

The best model of probability identified 7 groups for HOMA. They were further assembled into two groups based on their HOMA scores that we called insulin sensitive and non-insulin sensitive groups. We then calculated the probabilities of being assigned in the insulin-sensitive versus non-insulin-sensitive group.

To test our second hypothesis, predictors of HOMA were examined using logistic regression. We compared the two groups (insulin sensitive versus non-insulin sensitive) as dependent variables for baseline independent variables and included all variables of interest; specifically body composition measures, age, physical activity, sex, smoking, chronic diseases, fat intake, plant protein intake, animal protein intake. All variables were entered continuously in our model except sex which was categorized. Participants with all available measures were included in the analyses. Odd ratios were used for interpretation of the logistic regression.

Basic descriptive statistics and bi-variate relationships were used to describe the two groups and test relationships between variables. We used the Statistical Analysis Software (©SAS) version 9.2 for analyses.

19.4.1 Results

The trajectory estimation method identified 7 groups. The probabilities obtained for being assigned in each group as well as the mathematical determination of 2 groups, are shown in Table 19.1. The trajectories of HOMA-IR score over time are shown in Figure 19.1. We did not find change over time for groups 1,2,3,4 and 5. Groups 6 and 7 showed some variation but already started at much higher

values then groups 1, 2 and 3. Group 6 tended to increase its HOMA-IR score. Even though group 7 (comprising 0.66% of the population) showed a decrease in HOMA-IR score, the final value was still considered to be high. Our aim was to determine subjects who developed insulin resistance, but we were not able to see any significant changes over a 3-year period. The visual inspection of the curves suggested 3 patterns (no change, increase, decrease). However, since groups 6 and 7 contained together only 1.3% of the population, we did not have enough power to compare them with the third group of unchanged pattern.

We then proceeded with a mathematical determination of insulin sensitive versus non-insulin sensitive subjects over time by grouping curves subjects falling in groups 1, 2 and 3 versus 4, 5, 6, and 7. We decided to group those in the lowest trajectories (1, 2 and 3) based on the rationale that they had a lower HOMA versus those in the higher trajectories (4, 5, 6, 7) which had higher HOMA scores. The trajectory method helped us find the difference in the population in regard to HOMA-IR score and group subjects that approximately had the same level of HOMA into one trajectory to get 7 trajectories at the end.

Probabilities of participants falling in the insulin-sensitive group was very high (99%) as well as the probability in being assigned in the non-insulin-sensitive group (92%) showing that the participants had a high chance of being assigned correctly to either one of the groups.

Descriptive characteristics at baseline for insulin sensitive versus non-insulin sensitive groups are presented in Table 19.2. We only found significant differences for body composition measures between the two groups (%body fat and muscle mass index, $p<0.05$). We report the bivariate analyses of the baseline variables included in our model in Table 19.3. Weak but significant negative correlations were observed between body fat% and physical activity; body fat% and MMI; animal protein intake in percent of total energy and fat percent intake. A significant positive correlation was obtained between MMI and PASE score.

Other weak correlations were observed for the remaining variables and these are presented in Table 19.3.

Results of the logistic regression are presented in Table 19.4. Independent baseline variables included body composition measures, age, gender, number of chronic diseases, and smoking, physical activity, fat and protein intakes from different sources. Significant odd ratios were obtained for muscle mass index, % body fat and sex, after controlling for our panel of covariates in the model. For every one unit increase in muscle mass, the odds of having insulin resistance increased by 1.72 independently of % body fat. For every one unit increase in % body fat, the odds of having insulin resistance increased by 1.18. For gender, the odds of having insulin resistance are 6.9 times greater for men than women.

19.4.2 Discussion

To our knowledge this is one of the first studies to analyze the developmental trajectories that occur with HOMA-IR score over time. We did not disclose any significant changes for insulin resistance over 3 years; however some subjects falling in the non-insulin sensitive group had already started at high HOMA-IR values (9% of the overall population). We propose that our subjects were healthy at baseline, and that changes of HOMA-IR may not be detected within a 3-year period. This paper addresses one of the challenges associated with determining insulin resistance subjects in epidemiological studies. We chose the developmental trajectories because of the lack of HOMA-IR score cut-off. It was not possible to identify insulin sensitive subjects versus non-insulin sensitive ones based on their score as this may have led to a misclassification. To conduct the trajectory analysis, we started with fewer groups at first and then progressed to 6 and finally reached the best model which identified 7 trajectories. The validity of the model is supported by the high posterior probabilities obtained (Table 19.1). Based on visual inspection of the curves, we were able to separate insulin sensitive versus non-insulin sensitive subjects. Although, we did not have a cut-off to separate participants based on their HOMA-IR score, we defined our

population with the lowest HOMA-IR values to be sensitive in contrast to higher HOMA-IR score participants. Next, we ran posterior probabilities of the determined groups, and obtained high values (Table 19.1). It is worth mentioning that 2 groups (six and seven) showed some change patterns, but included only 1.3% of the total population. Besides, we tried running a logistic regression to compare groups 6 or 7 to other insulin sensitive groups, the validity of our results were questionable because of lack of power (results not shown).

After determination of the two groups, our second objective was to compare subgroups identified from trajectories for baseline variables. The logistic regression provided only 3 significant odd ratios for body composition measures (% body fat and MMI) as well as sex differences. We were not surprised by sex or % body fat impact on HOMA-IR score over time. However, we found an independent counterintuitive relationship between MMI and HOMA-IR score over time suggesting that higher muscle mass predicts an increased insulin resistance risk independently from % body fat. Our results are discordant with some other studies. Results from the Florey Adelaide Male Ageing Study showed that muscle mass and strength were strong predictors of the metabolic syndrome independently from abdominal fat and insulin resistance[244]. The discrepancy with the present results may be due to different variables of body composition used as well as the type of conducted analyses. Indeed, they have calculated muscle mass as whole body % lean mass and fat as abdominal % fat mass. We have corrected for height while calculating muscle mass index which may account for the discrepancy in results. Data from the NHANES III Study have shown cross sectionally that sarcopenia independently of obesity is associated with insulin resistance risk when sarcopenia was measured as height divided by weight[220]. However the association was strongest in younger individuals (less than 60 years of age). This suggests that there is a difference on the effect of muscle mass on insulin sensitivity among younger versus older individuals. Our results are consistent with those showing that sarcopenic obesity (a condition combining low muscle mass and high % body fat) was associated with better

metabolic outcome than obese non sarcopenic individuals even after correcting for visceral fat mass, which may possibly link sarcopenia to a better metabolic profile[245]. Our results do not encourage however loss of muscle mass in the elderly population to prevent insulin resistance; this relationship between higher muscle mass and insulin resistance stems from the fact that the remaining muscle (mainly type 1 muscle fibers) in the elderly is contributing to an increased risk of insulin resistance with aging. Type 1 muscle fibers contain most of the muscle mitochondria and aging affects skeletal mitochondrial function[74]. It is envisioned that since there is a decrease in type 2 muscle fibers with aging, which are less linked to insulin function, so the remaining type I fibers (involved in insulin action) would render the muscles more insulin resistant. However, since one other study showed that the increase in type 2 fibers in mice improved overall glucose disposal, this justification remains controversial[246]. Another explanation may be that with aging, muscles may undergo fat infiltration, which in turn increases inflammation risk and predisposes to insulin resistance[160]. Therefore, having low muscle levels may attenuate this risk. Further studies taking into consideration muscle fiber phenotype while associating muscle loss with insulin resistance in aging individuals are needed to elucidate this issue. Since other variables included in our model (physical activity, protein and fat intake, among others) did not differ between the insulin sensitive and the non-insulin sensitive groups, we ran the most parsimonious model including body composition measures and gender. The fit statistics were good (results not shown). This shows that over time, the only baseline variables affecting HOMA-IR in a 3-year period were mainly MMI, % body fat and sex.

Our study has several limitations. We have defined our subjects to belong to the insulin sensitive versus non-insulin sensitive groups based on developmental trajectories. Since trajectories are a probabilistic determination of individuals being assigned to a particular group, some individuals might belong to a different faction. The posterior probabilities obtained with the 7 assigned groups were high, which adds validity to our model. However, since groups 3 and 4 obtained in the

middle of the trajectories might overlap (individuals assigned to group 3 could also be assigned to group 4 and vice versa), we tried running the regression by grouping the highest and lowest trajectories only to minimize misclassification. We grouped trajectories 1 and 2 versus 5, 6 and 7 and ran the logistic regression. The results provided the same interpretations (data not shown). Another limitation comes from the different body composition measures used in our population. Some individuals had their body composition measured by BIA whereas others had it by DXA. DXA provides a good estimate for the measure of whole body composition while providing a three compartment model: bone mineral density, fat, and bone-free lean masses [134]. BIA was also shown to be a good tool of assessing body composition based on a 2-compartment model; it was validated against densitometry where good correlations were obtained (0.907-0.952). We have rendered the whole population similar in regard to MMI which should minimize errors due to the technique.

Furthermore, we have restricted the analyses to individuals having a minimum of three time points of the HOMA-IR score, decreasing the power of our study. Nevertheless, an advantage of the trajectory method is that people with missing data can be modeled.

In conclusion, since it is hard to identify subjects who developed insulin resistance by only using cut-off values with the inherent risk of misclassification, the developmental trajectories provided a way to solve this challenge. The trajectory analysis can detect and identify subgroups of the population. Our study showed that % body fat and muscle mass can predict the development of insulin resistance, as well as being a man. We did not observe any changes for HOMA-IR score over time suggesting that it may not be detected in healthy individuals in a short time period as ours. Future longitudinal research with more data over longer periods of time is warranted.

Table 19.1: Description of HOMA-IR score trajectory groups and grouping of the 7 groups into 2 subgroups (insulin sensitive versus non-insulin sensitive)

				Posterior probabilities	
Group	**Intercept**	**Number**	**%**	**Mean (SD)**	**Minimum-Maximum**
6 group model					
1 insulin-sensitive	2.40	279	43.03	0.82(0.15)	0.49-0.99
2 insulin-sensitive	3.52	213	32.76	0.71(0.12)	0.47-0.96
3 insulin-sensitive	5.23	96	14.80	0.78(0.14)	0.5-0.99
4 non-insulin-sensitive	7.39	40	6.24	0.87(0.15)	0.52-0.99
5 non-insulin-sensitive	7.75	16	2.46	0.97(0.04)	0.82-1.00
6 non-insulin-sensitive	19.86	5	0.69	0.92(0.16)	0.56-1.00
7 group model (used model)					
1 insulin-sensitive	2.35	261	40.21	0.82(0.14)	0.50-0.99
2 insulin-sensitive	3.45	221	34.00	0.72(0.13)	0.46-0.96
3 insulin-sensitive	5.15	102	15.80	0.80(0.14)	0.47-0.99
4 non-insulin-sensitive	6.84	41	6.37	0.86(0.15)	0.50-0.99
5 non-insulin-sensitive	10.85	15	2.23	0.87(0.15)	0.50-1.00

6 non-insulin-sensitive	3.33	5	0.70	0.99(0.006)	0.98-1.00
7 non-insulin-sensitive	20.06	4	0.66	0.95(0.12)	0.69-1.00

Note:

Bayesian information criterion (958 df) was -4252.67 for 6 group model and -4211.56 for 7 group model. Akaike information criterion -4208.89 for 6 group model and -4160.48 for 7 group model.

Lower values indicate better fit. Proportions are calculated based on assignment to the group with the highest probability.

Groupings of 7 groups into 2 subgroups	*Posterior probabilities: Mean (SD)*
Groups 1, 2, &3 (insulin-sensitive)	*0.99 (0.04)*
Groups 4,5,6 &7 (non insulin-sensitive)	*0.92 (0.13)*

Table 19.2: Descriptive characteristics at baseline of insulin sensitive and non-insulin sensitive groups over time

Participants:	**Insulin-sensitive group**	**Non-insulin-sensitive group**	**P-value**
N	594	55	
Sex	M=270, W=324	M=37; W=18	
Age (y)	73.5± 3.9	73.1 ± 3.7	0.56
Chronic diseases (total number of diseases)	3.2±1.9	3.2 ± 2.0	0.81
Smoking (packs-year)	9.5±52.7	3.7± 21.0	0.41
Animal protein (% total calories/d)	5.1±1.3	7.6±2.9	0.39
Plant protein (% total calories/d)	5.1±1.3	5.1±1.4	0.69
Fat intake (% total kcal/d)	33.4±6.0	33.6±6.2	0.77
Body fat (%)	32.8 ± 9.2	36.7 ± 7.2	0.0019
Muscle mass index (MMI; kg/m^2)	8.6 ± 1.4	9.6± 1.4	<0.0001
PASE (score)	107.8 ± 51.4	98.9 ± 67.7	0.23

Table 19.3: Pearson correlation between all baseline variables

	Age	%Body fat	MMI	PASE	Smoking	Plant protein (%)	Animal protein (%)	Fat intake (%)	#Diseases
Age	1								
%Body fat	0.06**	1							
MMI	-0.19*	-0.49*	1						
PASE	-0.21*	-0.28*	0.28*	1					
Smoking	-0.02	-0.04	0.03	0.005	1				
Plant protein intake (%)	-0.01	-0.02	-0.08**	0.03	-0.02	1			
Animal protein intake (%)	-0.03	0.12**	-0.1*	-0.06	-0.01	-0.12*	1		
Fat intake (%)	-0.003	0.007	0.06	0.05	0.04	-0.16*	-0.28*	1	
#Diseases	0.14*	0.20*	-0.15*	-0.19*	0.002	0.01	0.02	-0.04	1

Results are r.

**.Correlation is significant at p<0.001 level (2-tailed).*

***. Correlation is significant at the 0.05 level (2-tailed).*

Table 19.4: Logistic regression Odds Ratios and most parsimonious model

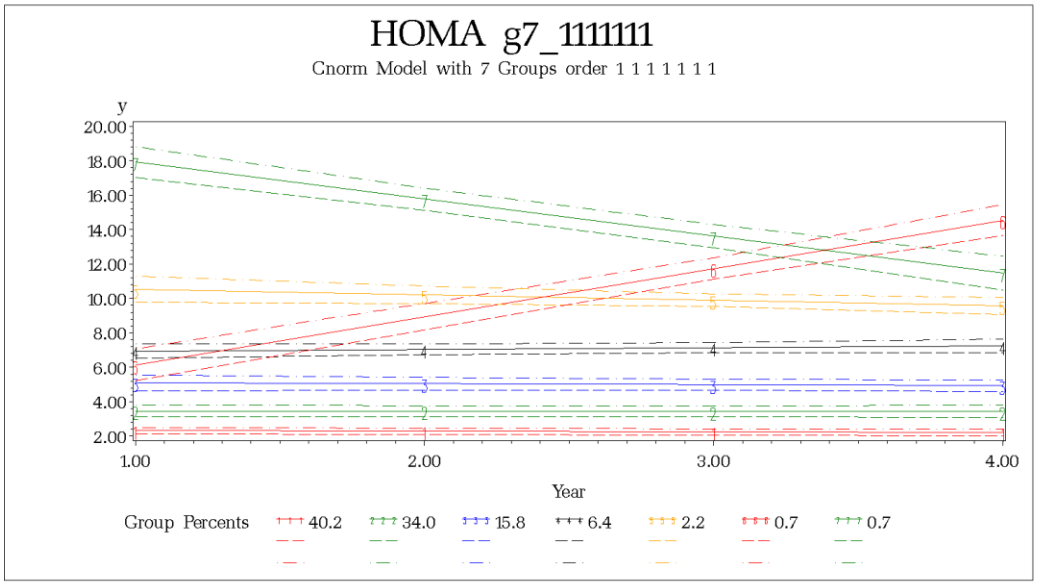
Baseline variables	**Estimate** ***B***	**Standard error of *B***	**Odd ratios (95%CI)** **(exponentiated *B*)**	**P value**
Body fat %	0.16	0.02	1.18 (1.12-1.25)	<0.0001
MMI	0.54	0.15	1.72 (1.26-2.3)	0.0006
PASE	-0.003	0.003	0.99 (0.99-1.0)	0.19
Age	-0.003	0.04	0.99 (0.91-1.0)	0.93
Sex (women versus men)	-0.96	0.29	0.145 (0.04-0.45)	0.0009
Smoking	-0.006	0.005	0.99 (0.98-1.0)	0.24
Chronic diseases (number)	-0.005	0.08	0.99 (0.84-1.17)	0.95
Fat intake (% total energy)	-0.01	0.026	0.98 (0.93-1.04)	0.67
Animal protein intake (% total energy)	-0.03	0.04	0.96 (0.87-1.06)	0.45
Plant protein intake (% total energy)	-0.06	0.12	0.93 (0.73-1.2)	0.61

Dependent variable: HOMA-IR score (insulin-sensitive) versus non insulin-sensitive).

Sexe coded 0 for women and 1 for men.

Kendall's Tau-a= 0.102; Sommer's D=0.657; c-statistic= 82.9%.

Figure 19.1: Trajectories of HOMA-IR score over 4 time points with CI



Note: Predicted values of HOMA in each group at different time points:

Predicted 1	***Predicted 2***	***Predicted 3***	***Predicted4***	***Predicted5***	***Predicted6***	***Predicted7***
2.31	*3.45*	*5.09*	*6.94*	*10.53*	*6.13*	*17.91*
2.28	*3.45*	*5.04*	*7.04*	*10.21*		*15.77*
2.24	*3.44*	*4.98*	*7.14*	*9.89*	*11.74*	*13.62*
2.21	*3.44*	*4.93*	*7.24*	*9.57*	*14.54*	*11.47*

20 Discussion and conclusions

20.1 Discussion

We have combined all available complex interrelated factors that occur with aging obtained from the NuAge dataset. One of the most important objectives of the present work was to understand the role of different protein sources on muscle mass and insulin resistance. We were able to dissociate the association of plant sources of protein from that of animal sources on both muscle mass and insulin sensitivity. We were also able to associate, by path analysis, muscle mass and insulin resistance, and understand that high muscle mass in elderly individuals was in fact associated with higher insulin resistance score based on HOMA-IR. Furthermore, this relationship was also observed longitudinally using trajectory analysis. Our study do not however promote the loss of muscle mass with aging, but targets a decrease in % body fat and/or a better quality of muscle mass that could be achieved by physical activity.

Our first manuscript showed that physical performance measures could be grouped into two separate components: strength and mobility, which could be used in clinical settings. We have as well found a significant negative association between % body fat and mobility such that for each unit increase of fat mass, there was a 0.2 unit decrease in mobility score. Parts of our results are consistent with previous analyses conducted within the framework of the NuAge study. Bouchard et al.[150] reported that fat mass but not lean body mass is related to physical capacity in the elderly participants, when physical capacity was measured by one leg balance and walking speed. We found a significant positive association between strength and mobility in our model showing for each unit increase in strength there was a 0.37 unit increase in mobility. Within the context of the NuAge study, individuals with a low relative strength measured by handgrip and quadriceps strength were more likely to have lower mobility scores[108]. By including subjects of both sites (Montreal and Sherbrooke), we are thus confirming the solidity of previous findings and this by including all

aspects at once. We obtained significant negative associations between muscle mass and mobility indicating a 1.1 unit decrease in mobility score with every one unit increase in muscle mass. The counterintuitive negative association between muscle mass and mobility score could be explained by the concept of muscle quality with aging. In our study, we did not measure muscle density and fat infiltration into the muscle tissue, which have been linked with decreased mobility, even after correction for % body fat[120]. As stated previously, mobility requires more than muscle mass alone and the usefulness of the latter rests in the development of strength, a marker of its quality. The association between the concept of muscle quality and muscle density[58] may explain some of the apparent discrepancy found between muscle mass and mobility. The concept of muscle quality extends also to metabolic aspects as it has been shown that insulin resistance was exacerbated with increased lean body mass in postmenopausal women[119]. Although the latter findings may not be related to the association we report between mobility and muscle mass, they further illustrate the concept of worsening muscle quality in aging. The model in our first manuscript suggested that muscle mass is b\negatively associated with mobility, this suggests that quality of muscle mass might be taken into consideration with aging. As with other studies [110-112, 150] % body fat was negatively associated with mobility. Body fat % on the other hand was not significantly associated with strength score. Physical activity in our model was negatively associated with % body fat but positively so with muscle mass (physical activity being a predictor). These associations suggest that physical activity is the right approach to improve physical performance since it was associated with mobility, independently from the effect on % body fat and it is envisioned that this is mediated through better quality of muscle mass with exercise[152].

In our second manuscript, our main objective was to understand the direct and indirect effects of different sources of protein intake on MMI and HOMA score. To our knowledge, this is one of the first studies to investigate these associations between different interrelated variables of this kind in one model by using path

analysis. We found an unexpected positive association between muscle mass and HOMA-IR score suggesting that an increased muscle mass in the elderly population is associated with a higher insulin resistance. Our results are consistent with those of Messier et al. who showed that sarcopenic women had lower levels of insulin resistance than non sarcopenic women[237]. Srikanthan et al. [220] showed however that higher muscle mass was associated with better insulin sensitivity using data from the third National Health and Nutrition Examination Survey data III[220]. The discrepancy between our results and those by Srikanthan et al. may lay in the different methods of assessing muscle mass index. We have calculated muscle mass index based on measures of muscle mass divided by height^2 whereas Srikanthan et al. divided it by total body weight[220]. We have tried to understand if this difference in the assessment method would be responsible for this discrepancy. We thus calculated MMI per body weight and compared results by general linear models quartiles of MMI with those of MMI per height^2 for HOMA-IR score (results not shown). We found that those having the highest quartile of MMI per body weight had the lowest HOMA-IR score whereas those having the highest quartile of MMI per height^2 had the highest HOMA-IR score. The definition of MMI based on weight may actually artificially lower the MMI for the same skeletal muscle mass if an individual has a heavier weight and thus a higher fat mass. In this circumstance, fat mass should be considered responsible for an increased HOMA-IR score rather than a lower MMI value. Our results do not encourage however loss of muscle mass in the elderly population to prevent insulin resistance; this relationship between higher muscle mass and insulin resistance stems from the fact that the remaining muscle (mainly type 1 muscle fibers) in the elderly is contributing to an increased risk of insulin resistance with aging. In fact, type 1 muscle fibers contain most of the muscle mitochondria and aging affects skeletal mitochondrial function. Another explanation may be that with aging, muscle may undergo fat infiltration which in turn increases risk of inflammation and predisposition to insulin resistance; having low muscle levels may attenuate this risk. Our second objective of the

second manuscript was to study the effect of different protein sources on HOMA score and MMI. The only direct effect that reached statistical significance was the negative association of plant protein with MMI suggesting that vegetable source of dietary protein intake is associated with lower muscle mass levels. There is literature supporting such a relationship since one study has shown that protein intake from animal sources was the only independent predictor of muscle mass index explaining 19% of its variance[226]. One of the advantages of the path analysis over simple linear regression is the observation of indirect effects rather than direct ones only. We observed that indirect effects of protein sources through the intermediate variables MMI and % body fat on HOMA-IR reached statistical significance and associations were in the same directions as hypothesized. The different effects of proteins (animal vs. plant) on MMI and HOMA could be explained by several factors. In fact, animal proteins have been considered to be the best source for muscle protein synthesis due to their essential amino acids content. Leucine, a branched-chain amino acid has been considered to act as both a substrate and a signaling molecule for MPS[40]. On the other hand, animal protein seemed to be negatively associated with insulin sensitivity. Of note is that this effect is independent from fat intake and % body fat and since protein was included as % of calories it also takes account of the total enegy intake. One plausible explanation for worsening insulin sensitivity would be that animal protein is rich in iron which has been shown to act as a prooxidant and increase the risk of oxidative stress and insulin resistance[239]. Another explanation comes from the fact that animal protein intake may be high in processed meat, which also is high in nitrites and sodium, both having also been implicated in increasing the risk of type 2 diabetes[240]. In our model however, the animal protein that we entered as a continuous variable, did not include processed proteins as we wanted to test the effect of animal protein per se without any processed meats (cold cuts or sausages) which are also high in saturated fat intake and cholesterol. Our results show that even without the presence of processed meats, animal protein intake (regular meats, milk and dairy products) was associated positively with

HOMA-IR score. Plant protein intake seemed to be negatively associated with insulin resistance. One explanation may rest in its low essential amino acids content, vegetable proteins being low in essential amino acids are consequently low in leucine; the latter having been independently associated with glucose uptake impairment in rats even with high insulin levels[241]. Another explanation may be due to their higher fiber and antioxidant content that could drive in part the increased insulin sensitivity[242]. Physical activity was positively associated with MMI and negatively associated with % body fat.

The third part of our study was to determine insulin resistant versus non-insulin resistance subjects over time using trajectory analysis. To our knowledge this is the first study to analyze the developmental trajectories that occur with HOMA-IR score over time. We did not disclose any significant changes for insulin resistance over 3 years; however some subjects falling in the non-insulin sensitive group had already started at high HOMA-IR values (9% of the overall population). We propose that our subjects were healthy at baseline, and that changes of HOMA-IR may not be detected within a 3-year period. Our third study addresses one of the challenges associated with determining insulin resistance subjects in epidemiological studies. We chose the developmental trajectories because of the lack of HOMA-IR score cut-off. It was not evident to determine insulin sensitive subjects versus non-insulin sensitive ones based on their score as this may have led to a misclassification. To carry the trajectory analysis, we started with fewer groups at first and then progressed to 6 and finally reached the best model which identified 7 trajectories. The validity of the model is supported by the high posterior probabilities obtained. Although, we did not have a cut-off to separate participants based on their HOMA-IR score, we defined our population with the lowest HOMA-IR values to be sensitive in contrast to higher HOMA-IR score participants. After determination of the groups, our second objective was to compare subgroups identified from trajectories to baseline variables. The logistic regression provided only 3 significant odd ratios for body composition measures (% body fat and MMI) as well as sex. We were not surprised by sex or % body fat

impact on HOMA-IR score over time, but we found an independent counterintuitive relationship between muscle mass index and HOMA-IR score over time suggesting that higher muscle mass could predict an increased insulin resistance risk independently from fat mass percent. Our results are discordant with some other studies. Atlantis et al.[244] using data from the Florey Adelaide Male Ageing Study showed that muscle mass and strength were strong predictors of the metabolic syndrome independently from abdominal fat and insulin resistance. The difference of the results obtained may be due to different variables of body composition used as well as the type of conducted analyses. Atlantis et al.[244] have calculated muscle mass as whole body lean mass percent and abdominal fat mass percent. We have corrected for height while calculating muscle mass index which may account for the discrepancy in results. Srikanthan et al. [220] have shown by cross sectional analyses, that sarcopenia independently of obesity is associated with insulin resistance risk using data from the National Health and Nutrition Examination Survey; however the association was strongest in younger individuals (less than 60 years of age). This suggests that there is a difference on the effect of muscle mass on insulin sensitivity among younger versus older individuals. Our results are consistent with those of Aubertin Leuheudre et al. [245]. The latter authors showed that sarcopenic obesity (a condition combining a low muscle mass and a high body fat percent) was associated with better metabolic outcome than obese individuals even after correcting for visceral fat mass; which may possibly link sarcopenia to a better metabolic profile. Aside from the fact that type 1 muscle fibers contain most of the muscle mitochondria and aging affects skeletal mitochondrial function, a possibility may be that with aging there is a decrease in type 2 muscle fibers which are less linked to insulin function, so the remaining of type I fibers (involved in insulin action) would render the muscles more insulin sensitive. However, since one other study showed that the increase in type 2 fibers in mice improved overall glucose disposal, this justification remains controversial. Another explanation may be that with aging, muscles may undergo fat infiltration

which in turn increases risk of inflammation and predisposition to insulin resistance; having low muscle levels may attenuate this risk.

20.1.1 Strengths and limitations

Our study has several limitations including the path analysis statistics for our cross sectional analysis of associations, which cannot assume causality. We are also cognizant that our models could include other relationships providing good fit with other or different paths added. However, we conducted thorough analysis and we consider that both the initial and the revised models provided at the very least, a minimally acceptable fits to the data. We added paths in our revised models, however according to MacCallum et al.[213] changes must only be made when they are theoretically meaningful. In our view, the paths we added are clearly interpretable within theories. Still, the revised models were themselves of admittedly questionable validity, since it results from data-driven modifications made to a rejected initial model, and it is based on a single sample. It is therefore possible that the models will not generalize to other samples from a different population.

We included men and women together in our sample, despite known differences in body composition. However, we also tested them separately to fit our models by gender and the results did not change; the fit statistics were the same and as good as those provided in the present analyses. This adds to the validity of our results. In this study, we have merged body composition measurements of participants from the two recruitment sites of the NuAge Study despite the different techniques being employed. For this latter aspect, we took great care of assembling the data on muscle mass by adjusting quantities based on the same gold standard, i.e. magnetic resonance imaging. Furthermore, the equations used to determine fat mass by BIA in Montreal site was validated against DXA in the same subjects that underwent both tests in Sherbrooke site. Finally, our analyses were limited to a healthy population without disability at baseline and our findings may not be applicable to other elderly populations such as the frail elderly. However this could be considered as an advantage as we detected associations in a healthy sample which may be even stronger in disabled or

diseased participants. Associating these variables and significant paths to diseases and outcomes requires longitudinal analyses but our model, using cross sectional data at this stage, can assist in better defining ways of counteracting the expected decrease in physical performance associated with aging as well as understanding the association between muscle mass, insulin resistance and protein intakes with aging.

In our third manuscript, we have defined our subjects to belong to the insulin sensitive versus non-insulin sensitive groups based on the developmental trajectories. Since trajectories are a probabilistic determination of individuals being assigned to a particular group, some individuals might belong to a different faction. The posterior probabilities obtained with the 7 assigned groups were high, which adds validity to our model. However, since groups 3 and 4 obtained in the middle of the trajectories might overlap (individuals assigned to group 3 could also be assigned to group 4 and vice versa), we tried running the regression by grouping the highest and lowest trajectories only to minimize misclassification. We grouped trajectories 1 and 2 versus 5, 6 and 7 and ran the logistic regression. The results provided the same interpretations (data not shown). Besides, we have restricted the analyses to individuals having three time points of the HOMA-IR score decreasing the power of our study. Nevertheless, an advantage of the trajectory method is that people with missing data can be modeled.

NuAge is a longitudinal study, but we have limited our analyses to cross sectional in both manuscripts 1 and 2. The main reason behind this is that we wanted to test the fits of our two hypothesized model in a cross sectional analysis to better understand what is happening at baseline in our population. Besides, we had missing information at the rest of the time points in regard to body composition measures which made it hard to conduct longitudinal analyses taking into account all variables included in our model.

20.2 Conclusions

Our first model of path analysis fitted with our data and indicated that mobility components influence PA. Mobility would in turn be influenced positively by strength and negatively by % body fat. Strength is influenced by MMI. PA influences % body fat. We proposed and confirmed a model taking into consideration PA, PP and body composition components among a national sample of Canadian community dwelling older men and women. This model suggests that many aspects must be taken into consideration when assessing factors association with PP and PA in older adults.

Our second model of path analysis indicated that MMI based on height^2 but not on weight was positively associated with HOMA-IR score independently from adiposity. This suggests that different methods of assessing muscle mass would have different interpretations on its associations with the outcomes of interest. Animal protein intake appeared to be associated with a higher MMI and HOMA-IR score while plant protein intake had the opposite effect on both variables. Direct effects of dietary protein on HOMA-IR score did not reach statistical significance; however indirect effects provided significant results showing the effects of the intermediate variables MMI and fat mass on the association between protein intake and HOMA score. Physical activity significantly modeled body composition favourably, by its positive association with MMI and negative association with % body fat.

Since it was hard to identify subjects who developed insulin resistance by only using cut off values and increasing misclassification risk, the developmental trajectories provided a way to solve this challenge. The used methodology appears to be useful in these types of studies in clinical sciences where no cut offs are available. The trajectory analysis could detect and identify subgroups of the population. Our study showed that fat mass and muscle mass can predict insulin resistance over time, as well as being a man independently of other covariates

included in our model. We did not observe any changes for HOMA-IR score over time suggesting that it may not be detected in healthy individuals in a short time period.

21 Future directions

Future path analytic studies should be conducted to test the validity of these models, preferably by comparing the models investigated in this study as a priori models, to determine which provides best fit to the data in new samples. More longitudinal data for longer periods are needed to validate our findings. If results of future studies are consistent with ours, it would then be appropriate to test in randomized controlled trial the effect of physical activity and protein intake and its sources on physical performance and insulin resistance. This would greatly increase our understanding of healthy aging and would provide sound data on which to base recommendation for successful aging.

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