FACULTY OF MEDICINE AND HEALTH SCIENCES

The determinants of body composition in healthy Flemish young and middle-aged men: a longitudinal study

Annelies Vanhove

Student number: 01509796

Supervisors: Prof. Dr. Lapauw, Prof. Dr. Shadid, Prof. Dr. Goemaere, Dr. Banica

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Preface

Het verwezenlijken van deze thesis was een grote uitdaging waar ik mezelf geregeld tegengekomen ben. Het was een lang en uitgebreid maar ontzettend leerrijk proces dat nooit tot stand had kunnen komen dankzij een aantal speciale mensen die ik hier graag zou willen bedanken.

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Abstract

Background

Over the past 40 years, obesity has doubled worldwide in both men and women and is still on the rise. Obesity is a multifactorial disease and is the most important lifestyle-related risk factor for early death. Knowledge about the exact distribution of body fat is important since certain types of adiposity distribution, especially abdominal adiposity, are strongly associated with several comorbidities such as cardiovascular diseases and diabetes type 2. The epidemic rise of obesity can partly be explained by environmental, lifestyle and genetic factors. Our main aim was to investigate to what extent lifestyle-related factors are associated with changes in body composition in adult men. Furthermore, we are also interested the interrelationship between hormones and body composition measurements.

Method

This study is part of a population-based study in Ghent with the aim to investigate the determinants of peak bone mass in healthy men between 25 and 45 years of age. At the beginning of the study, 999 healthy male participants were recruited. Between May 2014 and December 2019, 709 participants have been re-examined. Body composition measurements, including subtotal body fat, subtotal lean mass and truncal fat mass were quantified by using Dual-energy X-ray absorptiometry (DXA). Body weight, height, waist circumference, hip circumference and waist-hip-ratio (WHR) were measured, and life-style factors were assessed using questionnaires. Blood hormone levels (glucose, insulin, leptin and adiponectin) were determined by venous blood samples. HOMA-IR was used to evaluate insulin resistance. All statistical analyses were completed using IBM SPSS statistics (version 26, Chicago, IL, USA). To assess cross-sectional and longitudinal associations between the covariates and body composition parameters, linear mixed-models were used.

Results

In general, our subjects were at baseline on average 34.5 years old with a mean BMI of 25.1 kg/m^2, a subtotal fat percentage of 19.5% and a subtotal lean mass percentage of 77.4%. At follow-up, the men were on average 46.4 years old with a mean BMI of 26.3 kg/m^2, a subtotal fat percentage of 21.7% and a subtotal lean mass percentage of 75.4%. Our main findings include that age at baseline, smoking habits, alcohol consumption, physical activity, level of education and level of hormones at baseline are associated with certain body composition

parameters. Age at baseline was positively associated with BMI, subtotal fat, truncal fat, WHR and negatively to with subtotal lean mass percentage. Level of education was associated with a lower BMI, truncal fat and WHR. Smoking was positively correlated to subtotal fat and truncal fat while negatively correlated to WHR and subtotal lean mass percentage. Next, both non-drinkers and moderate drinkers were associated with lower truncal fat in comparison to heavy drinkers. However, moderate drinking was associated with a higher WHR in comparison to heavy drinkers. Furthermore, a negative association could be observed between physical activity and subtotal fat, truncal fat and WHR while a positive association was found for subtotal lean mass percentage. HOMA-IR was correlated to a higher BMI, subtotal fat, truncal fat and WHR. Leptin was positively associated with BMI, subtotal fat, truncal fat and WHR while a negative association was found for subtotal lean mass percentage. Adiponectin was correlated to a lower BMI, subtotal fat, truncal fat and WHR. Finally, no associations were found for coffee with any of the body composition parameters.

Longitudinally, in our full model, BMI, subtotal fat and truncal fat increase with ageing while subtotal lean mass percentage decreases. WHR did not change significantly with ageing. Besides, we observed that alcohol status, physical activity, HOMA-IR, leptin at baseline and adiponectin at baseline have an association with the evolution of several body composition parameters over time. Coffee intake, smoking status and level of education did not have a significant impact on the change of body composition over time.

Conclusion

The main objective of this present study was to investigate to what extent lifestyle factors and age have an impact on body composition and adiposity. Especially level of adiponectin at baseline, ageing, change in HOMA-IR and change in physical activity have an association with the evolution of almost all body composition parameters over time. Alcohol intake was only correlated to the change of BMI and WHR over time. Moreover, the level of leptin at baseline was only associated with the change of subtotal fat and subtotal lean mass percentage over time and not with the other body composition parameters. Level of education, coffee intake and smoking status did not modulate the change of any of the body composition parameters. We can thus conclude that these variables do not have a significant impact on evolution of body composition and adiposity.

Samenvatting

Achtergrond

De afgelopen 40 jaar is de prevalentie van obesitas bij zowel mannen als vrouwen verdubbeld en is nog steeds aan het stijgen. Obesitas is een multifactoriële aandoening en is de belangrijkste levensstijl gerelateerde risicofactor voor vroegtijdige sterfte. De exacte verdeling van lichaamsvet is belangrijk sinds bepaalde vormen van adipositas, zoals abdominale adipositas, geassocieerd zijn met verschillende comorbiditeiten zoals cardiovasculaire ziekten en diabetes type 2. De stijgende prevalentie van obesitas kan deels worden verklaard door zowel omgevingsfactoren als levensstijl en genetische factoren. Het hoofddoel van deze thesis was om te onderzoeken in welke mate levensstijl gerelateerde factoren geassocieerd zijn met veranderingen in lichaamssamenstelling. Daarbovenop zijn we ook geïnteresseerd in de onderlinge relatie tussen hormonen en lichaamssamenstelling.

Methode

Deze studie is deel van een populatiestudie die de determinanten van piekbotmassa in gezonde mannen tussen 25 en 45 jaar onderzocht. In het begin van de studie werden 999 gezonde mannelijke deelnemers gerekruteerd waarvan 709 opnieuw werden onderzocht tussen mei 2015 en december 2019. Lichaamssamenstelling parameters zoals de subtotale vetmassa, de vetmassa van de romp en de subtotale vetvrije massa werden gemeten met een DEXA-scan. Lichaamsgewicht, lengte, tailleomtrek, heupomtrek en waist-hip ratio (WHR) werden opgemeten en levensstijl factoren werd bevraagd aan de hand van vragenlijsten. De hoeveelheid hormonen (glucose, insuline, leptine en adiponectine) werden gemeten in veneus bloed. De HOMA index werd gebruikt om insuline resistentie te bepalen. Alle statistische analyses werden uitgevoerd met behulp van IBM SPSS statistics (versie 26, Chicago, IL, USA). Lineaire mixed-modelling werd gebruikt om de cross-sectionele en longidutinale associaties tussen de covariaten en de lichaamssamenstelling parameters te onderzoeken.

Resultaten

Bij baseline waren de proefpersonen gemiddeld 35.5 jaar oud met een gemiddeld BMI van 25.1 kg/m^2, een subtotaal vet percentage van 19.5% en een percentage subtotale vetvrije massa van 77.4%. Bij follow-up waren de mannen gemiddeld 46.4 jaar met een gemiddeld BMI van 26.3 kg/m^2, een subtotaal vet percentage van 21.7% en een percentage subtotale vetvrije massa van 75.4%. De belangrijkste bevindingen van deze studie zijn dat leeftijd,

rookgewoonten, alcohol gebruik, fysieke activiteit, opleidingsniveau en hormonen in het bloed geassocieerd zijn met bepaalde lichaamssamenstelling parameters. Leeftijd bij baseline is positief geassocieerd met BMI, subtotale vetmassa, vetmassa van de romp en WHR terwijl het negatief gecorreleerd is met het percentage subtotale vetvrije massa. Opleidingsniveau was negatief geassocieerd BMI, vetmassa van de romp en WHR. Roken was positief gecorreleerd met zowel subtotale vetmassa als vetmassa van romp maar negatief gecorreleerd met WHR en het percentage subtotale vetvrije massa. Vervolgens was zowel het niet drinken als het gematigd drinken van alcohol geassocieerd met een lagere vetmassa van de romp in vergelijking met zware drinkers. Bij WHR daarentegen was gematigd drinken juist geassocieerd aan een hogere WHR in vergelijking met zware drinkers. Verder vonden we een negatieve associatie tussen fysieke activiteit en subtotale vetmassa, vetmassa van de romp en WHR terwijl we een positieve associatie vonden tussen fysieke activiteit en het percentage subtotale vetvrije massa .De HOMA index was gecorreleerd met een hogere BMI, subtotale vetmassa, vetmassa van de romp en WHR. Leptine was ook positief geassocieerd met BMI, subtotale vetmassa, vetmassa van de romp en WHR maar negatief geassocieerd met het percentage subtotale vetvrije massa. Adiponectine was gecorreleerd met een lagere BMI, subtotale vetmassa, vetmassa van de romp en WHR. Tot slot werden er geen relaties gevonden tussen het drinken van koffie en de lichaamssamenstelling parameters. Longitudinaal zagen we in ons volledige model dat BMI, subtotale vetmassa en vetmassa van de romp stegen met de leeftijd terwijl het percentage subtotale vetvrije massa juist daalde. Hierna werd er vastgesteld dat dat alcohol gebruik, fysieke activiteit, HOMA index, leptine op baseline en adiponectine op baseline een invloed hebben op de evolutie van verschillende lichaamssamenstelling parameters over de tijd heen. Het drinken van koffie, rookgewoonten en opleidingsniveau hebben geen invloed op de verandering van lichaamssamenstelling.

Conclusie

We hebben vastgesteld dat voornamelijk adiponectine op baseline, veroudering, verandering van HOMA index en verandering in fysieke activiteit een invloed hebben op de evolutie van bijna alle lichaamssamenstelling parameters over de tijd. De consumptie van alcohol was enkel geassocieerd met de verandering van BMI en WHR over tijd. Ook leptine op baseline was enkel geassocieerd met de verandering van de subtotale vetmassa en het percentage subtotale vetvrije massa en niet met de andere parameters. Opleidingsniveau, het drinken van koffie en rookgewoonten hadden geen invloed op de verandering van geen enkele van de lichaamssamenstelling parameters. Bijgevolg kunnen we concluderen dat deze variabelen geen significante invloed hebben op het verloop van lichaamssamenstelling en adipositas.

1. Introduction

Over the past 40 years, worldwide obesity has doubled in both men and women and is still on the rise. In 2015, 5% of the children and 12% of the adults was obese (1). Currently, about 39% percent of the worldwide population is classified as overweight or obese. In addition, Europe and America are believed to have the highest numbers of overweight citizens (2).

Obesity is associated with increased mortality and is related to many comorbidities such as type 2 diabetes mellitus, cardiovascular diseases, several types of cancer, non-alcoholic fatty liver disease, osteoarthritis, sleep apnea, depression and stroke (1, 3-5). Because of this, it is the most important lifestyle-related risk factor for early death (5). Obesity does not exclusively lead to medical consequences. Obese individuals are also faced with increased unemployment, social impairment, a lower quality of life and psychological problems such like depression (4, 5). Furthermore, the medical expenditures of the obese proportion of the population appears to be around 30 percent higher than their peers in the normal weight category (6).

Obesity is a multifactorial disease that is caused by a greater calorie intake than output (7). It is defined by the World Health Organization (WHO) as an accumulation of fat which may lead to an impairment of health (8). Obesity is often described by the use of body mass index (BMI) which is calculated by body weight (kg) divided by the square of the height (m). WHO defines being overweight as a BMI greater than 25 kg/m² and obesity as a BMI greater than 30 kg/m² (4, 8)

Table 1: WHO weight classification (4)

WHO weight classification	BMI (kg/m²)
Underweight	<18,5
Normal weight	18,5-24,9
Overweight	25,0-29,9
Obesity class I	30,0-34,9
Obesity class II	35-39,9
Obesity class III	>40,0

Despite the fact that BMI is the most popular marker to classify obesity, it does not make a distinction between fat mass and muscle mass. Therefore, muscular individuals can be given a higher BMI than individuals with the same weight but with a higher fat percentage (3). Since BMI cannot predict levels of adiposity accurately, markers of body composition such as waist-hip ratio (WHR) and fat mass are preferred as an indicator for obesity (4, 9).

To provide information about the distribution of fat, Dual-Energy X-Ray Absorptiometry (DXA) is mostly used (10). DXA is a very precise technique to measure both bone mass and soft tissue composition. It can perform a whole-body scan and subdividing the body in different regions by using cut lines. Subsequently, the different body composition parameters of every region can be determined (10, 11). However, DXA cannot precisely make a difference between visceral and subcutaneous fat. Therefore, computed tomography (CT) and magnetic resonance imaging (MRI) are generally used. An alternative for these methods can be a DXA for visceral adipose tissue (DXA-VAT), developed by Micklesfield *et al.* (12).

Knowledge about the fat distribution is important since the effects of abdominal fat are more harmful for health than those of peripheral fat. The exact distribution of body fat provides us with important information on comorbidities related to obesity. Abdominal or central fat is believed to be associated to an increased risk of cardiovascular diseases and diabetes type 2. Abdominal fat can be split into visceral fat and subcutaneous fat (13). Especially a higher visceral fat percentage is associated with an increased insulin resistance and glucose tolerance as well as an increased amount of free fatty acids in the blood, which are risk factors for diabetes type 2 and cardiovascular diseases (4). Subcutaneous abdominal fat is believed to have a lower impact on insulin resistance than visceral fat (13). Furthermore, peripheral fat is located in the lower body and is also associated with a lower risk of insulin resistance (4). Besides, it is correlated to a lower cardiovascular risk thanks to the more favorable lipid and glucose profile (3).

Several studies observed that an association between a higher amount of abdominal fat was positively associated with a greater cardiovascular risk (3, 4, 13). Central fat distribution is usually measured by using waist-hip ratio and waist circumference. In men, waist circumference measurements greater than 102 cm and waist-hip ratio measurements greater than 0,90 represent abdominal obesity. In women these thresholds are 88 cm for the waist circumference and 0,85 for the waist-hip ratio (4).

***Table 2**: definition central obesity by using WC and WHR (4)*

Central obesity	WC (cm)	WHR
Men	102	0,90
Women	88	0,85

1.1 Determinants of body composition and obesity

The etiology of obesity is very complex and is influenced by many different factors. The prevalence of obesity has risen extremely over the last few years. This epidemic rise cannot only be explained by genetic causes, environmental factors and epigenetics also play a role. These environmental factors can for example involve dietary habits, sedentary behavior, physical activity, socioeconomic status and many more, especially lifestyle has changed a lot the last years. Food intake is not limited anymore and sedentary behavior has increased tremendously by the growth in the amount of office jobs among other things and the drop in the activity rate of people in general (14). This thesis will be focusing mainly on the influence of age and lifestyle factors on body composition and obesity.

Influence of age

Ageing has a significant impact on body composition in both men and women. For both sexes, BMI and body mass increase with age until a certain age at which a small decline starts (15-19). However, research has not been able to agree on a specific age at which weight reduction starts. Jackson *et al*. estimates the age to be around 60, while Bemben *et al*. states this age to be around 70 (16, 19).

Generally, studies show that the fat mass keeps on increasing throughout the life of a person and stabilizes or starts decreasing after a certain age limit is reached. However, studies have not been able to agree on a specific age limit for this increase (15-20). Differences could also be observed between men and women. Bazzocchi *et al.* (15) showed that for women fat mass generally increases until the age of 40, after which it will remain stable for the remainder of their lives. For men, studies show this steady increase through lifetime, nonetheless there is no consensus about what happens after the peak is achieved around 70 to 80 year of age (16, 18).

Additionally, Vermeulen *et al*. makes note of the abdominal region being the body region most influenced by the increase in fat mass, which might pose a threat, since the risk of cardiovascular diseases increases with the amount of abdominal fat (20). Bemben *et al*. found likewise that the abdominal circumference increases with time until approximately 70 years of age, afterwards a small decline can be seen (19).

Fat free mass or lean mass consists of bone mass, body water, organs and mainly muscle mass (16). Research shows that lean mass in general decreases throughout the years (16, 18, 19). There is an overall consensus on the continuality of the decrease in lean mass, however, differences can be found between men and women. Multiple studies observed that women had a lower amount of fat free mass than men (15, 18). Rubin *et al*. even found that fat free mass decreased faster for women than for men (18). Furthermore, aging is associated to a loss of bone mass which increases the risk of bone fractures. This effect is reinforced by the relationship between age and loss of muscle mass that has an impact on the general muscle strength of a person. A loss in muscle strength may effect physical ability and stability which can also be related to an increase of bone fractures (20).

Influence of lifestyle factors

Smoking

The effects of cigarette smoking have been widely studied (21). Smoking can be related to many health problems such as cancers, insulin resistance, cardiovascular disorders, dyslipidemia, obesity, and hypertension (22). However, the effect of smoking on body composition and body weight remains inconclusive. It is a common belief among adolescents that smoking promotes thinness and weight control and that smoking cessation induces weight gain (22, 23). Both Akbartabartoori *et al.* and Fang *et al.* observed that smoking was negatively related to BMI (24, 25). This negative correlation could possibly be explained by the effects of nicotine that increase energy expenditure and suppress appetite (23). Kim *et al*., who defined abdominal adiposity as a waist circumference greater than 90 cm, could not found a relation between overall BMI and smoking status. However, he observed that smoking was positively associated to abdominal adiposity. Greater abdominal obesity can be associated with a higher cardiovascular risk and mortality in comparison to overall obesity (26). Efendi *et al.* also found that cigarette consumption was associated with a higher WHR in women smoking more than 150 packs of cigarettes a day (27).

To the contrary, several other studies found a positive correlation between smoking and BMI and body weight (23, 28, 29). De Oliveira Fontes Gasperin *et al.* could not confirm these findings but she observed a relationship between the number of cigarettes smoked per day and both BMI and body weight. Heavy smokers tend to be more overweight or obese and tend to have a higher BMI than light smokers of moderate smokers (28). A possible explanation for these findings may be the association between an unhealthy lifestyle and heavy smoking, since heavy smokers are more likely to engage in less physical activity, to eat less fruit and vegetables and to drink more alcohol than light smokers or non-smokers (23).

Alcohol

Regularly drinking a glass of alcohol is a common phenomenon for most people nowadays. Even though the use of alcohol is often associated with an increase in body weight and a larger waist circumference. Alcohol is very rich in energy since one gram of alcohol equals 7,1 kcal. Nonetheless these are considered as empty calories since alcohol hardly contains any nutrients. As alcohol is often consumed with a meal, this creates an extra calorie intake on top of the meal itself (30, 31). Besides, alcohol positively affects the appetite, which can lead to weight gain in case the extra consumption is not compensated (32). Finally, alcohol is also believed to cause an increased storage of fat since it restrains the fat oxidation (32).

Many people believe alcohol is coherent with higher body weight or a larger waist circumference. Nevertheless, studies on this contradict each other, making it difficult to come to a scientific agreement. On top of this, it is still not clear whether or not the type of alcohol is a relevant factor in this relationship (33).

Multiple studies were able to prove a positive relationship between alcohol and several body composition parameters (30, 32, 34). A positive association could be found between the intake of alcohol and BMI, waist circumference, WHR and body fat percentage. The effect of alcohol on the body fat percentage was mainly observed in the abdominal area. For the influence of different types of alcohol, people drinking wine were mainly found to have a lower BMI than the people drinking beer. While for the other variables (WHR, body fat percentage and waist circumference) no significant differences were observed between different types of alcohol (34).

On the same note, the study of Lean *et al*. was able to prove that the total intake of alcohol is positively correlated to BMI and waist circumference. Hence, people drinking higher amounts of alcohol, on average have a higher BMI and a larger waist circumference. To the contrary,

frequently consuming alcohol in small quantities is associated with a lower BMI and waist circumference, compared to people that do not consume alcohol frequently. Most associations, however, could only prove to be statistically significant after the age of 30. There were no relations found between BMI, waist circumference and different types of alcohol (30).

Not all studies were able to confirm these positive relations between alcohol and body composition parameters. Few studies could even establish an inverse correlation between total alcohol intake and the difference in body weight and waist circumference over a period of five years. Here, higher consumption of alcohol was found to result in a lower body weight and waist circumference (33).

Finally, other studies show that body weight and BMI are not related to alcohol consumption at all, both in men and women. Only in heavy consumers, defined as more than 70g alcohol a day, a lower body weight and BMI was observed. They also found evidence that people with an excessive alcohol consumption did significantly less physical exercise. For men, it was noted that the body fat percentage decreased as the alcohol consumption increased, compared to non-consumers. This effect was not observed with women. All of this suggests a clear difference in body fat percentage and drinking habits between men and women (35).

Coffee

Coffee may have an effect on worldwide health since it is consumed by many people on a daily basis. It contains multiple constituents like caffeine, chlorogenic acid and polyphenols (36, 37). Chlorogenic acid is an antioxidant that is known for its possible contribution to weight loss and blood pressure lowering effects. Additionally, resting energy expenditure and lipolysis can be boosted by caffeine, which might be an explanation as well for the weight reducing effect and the decreasing effect on blood pressure (38).

Even though the effect of coffee on different body composition parameters like fat percentage, BMI and waist circumference have been widely studied, results remain inconsistent. Most of the studies were able to confirm a certain association between the consumption of coffee and body composition parameters. Both Lee *et al*. and Revuelta-Iniesta *et al.* found an inverse correlation between the consumption of coffee and BMI and waist circumference. Drinking coffee should therefore rather have an anti-obesity effect (36, 38). Another study was able to show that drinking coffee was associated to a lower fat percentage (37). Finally, Nordestegaard *et al.* found an association between decreased chances on obesity and the consumption of maximum four cups of coffee per day (39).

However, Bouchard *et al.* could not find an association between the consumption of coffee and BMI or waist circumference. They were able to prove that people adding artificial sweeteners to their coffee, had a higher BMI on average. Coffee-drinkers using sugar or honey as sweeteners, were observed having a lower than average BMI, compared to others who didn't use natural sweeteners. Finally, almost none of the studies provided any type of information on which type of coffee was consumed by the test-population (presence of sweeteners or added cream for example). Nonetheless, this could be an important factor affecting the results of the studies (40).

Physical activity and sedentary behavior

It has been hypothesized that physical activity has an influence on the energy balance. Energy expenditure is increased by physical activity, which makes it an important component in managing body weight. Since weight loss can be achieved by keeping the energy expenditure higher than your energy intake by consuming food, physical activity can be used to influence body weight. On top of this, physical activity can also influence muscle mass. This can be observed by comparing total fat percentages between athletes and their peers who perform less exercise, which on average shows a significant lower fat percentage for the athletes (41).

Almost all studies agree about more physical activity resulting in a healthier body composition. An increase in physical activity is associated with a decline in fat percentage, BMI and waist circumference (42-46). On top of this, it also leads to an increase in lean mass (42).

Westerterp *et al.* suggested that an increase in physical activity would hardly influence the overall body weight for individuals with a normal weight. However, fat mass would decrease and fat free mass would increase significantly. Consequently, a shift from fat mass to lean mass would take place only by increasing the physical activity. To the contrary, for obese individuals, both body weight and body composition measurements will be influenced significantly by performing more physical activity. The size of this change, however, will strongly fluctuate between individual cases. This study also showed that weight loss by training exercise is limited and will eventually reach a plateau phase, making the increase in weight loss come to a stop (41).

Besides physical activity, sedentary behavior is also very important. More and more people are being employed in desk jobs, which might eventually influence weight and body composition. An increased sitting time leads to a lower energy expenditure, since muscles are used less during the day. This might result in an increase of both weight and BMI (47). Energy

expenditure can be increased by swapping sitting time for active movement, which could decrease risk on obesity and could even lead to weight loss (48).

Studies concerning the relationship between sedentary behavior and obesity show mixed results (47). A few studies managed to find a positive correlation between a longer sedentary time and body composition parameters like BMI, waist circumference and body fat percentage (44, 48). Meyers *et al.* also found a slightly positive association, however this observed correlation was found to be rather weak (42). These results are opposed by Pulsford *et al.* who could not confirm any association between sedentary behavior and body composition parameters (49).

Socioeconomic status (SES)

Various studies agree that a lower socioeconomic status (SES) is associated to a higher risk of obesity (50-52) and a worse health outcome in general (50). Lower SES is believed to be associated to an unhealthier lifestyle. This could possibly be explained by limited access to nutritious foods, a higher percentage of smokers, a higher amount of alcohol use and elevated levels of stress (50).

Level of education is a common indicator for socioeconomic status (52). Wardle *et al.* found that a lower level of education was associated to a higher risk of obesity (52). Molarius e*t al.* could also confirm that obesity was more prevalent in people with a lower level of education in comparison to people with a high level of education (51).

Finally, not only level of education but also income and occupational status are being used to assess socioeconomic status. Several studies use different approaches to describe socioeconomic status. This could possibly lead to different outcomes (52).

Influence of genetics

Obesity is a multifactorial disease, which results from the interaction between genetics, epigenetics and the environment (53). The genetic and environmental factors also interact with each other. Some individuals are more susceptible to obesity than others within the same environment, which points out the presence of genetic predisposition (14). Genetic factors can explain 57-90% of the variation in BMI among adults (14, 54). In addition, adoption studies showed us that the BMI of a person is more strongly correlated to the biological parents than to the adoptive parents (14).

Twin studies have confirmed the existence of a genetic predisposition for a more energy conserving physique (55). Children of obese parents therefore have an increased chance of developing obesity themselves, both in childhood and in adulthood. This chance is even more elevated when both parents are obese (53, 54).

Obesity can be divided into two forms, namely the syndromic and non-syndromic form. The syndromic variant comprises only a small part of the obese population. The cause of this form could be pleiotropic or due to chromosomal rearrangements. Examples of syndromic obesity are the Prader-Willi syndrome, WAGR syndrome, Fragile X syndrome and Cohen syndrome. The non-syndromic form in turn exists out of two variants, namely the monogenic and polygenic form. The monogenic form is very rare and is the result of a single gene mutation (56). These single gene mutations are responsible for only five percent of the extreme obesity cases (14). The polygenic form is much more complex and is caused by different genes and their mutations (56). It is the most frequent form and recent studies found more than 100 different BMI-associated loci that could possibly be involved in the development of this polygenic non-syndromic obesity (14). A gene that plays a big part in the development of obesity, is the fat mass and obesity associated gene (FTO gene). Solely having a variant of this gene is associated with a higher risk of obesity (54).

Finally, besides the environmental and genetic factors, also the epigenetics could be a causal factor in the development of obesity. Epigenetic modifications are changes in the function of the genes without an impact on the DNA sequence. These modifications can modify the expression of genes, which might cause obesity (56). Environmental factors are also known to induce epigenetic changes, such as methylation or histon modification, which can influence the BMI of a person (54).

1.2 Interrelationship between hormones and body composition

Supplementary to the influence of age and lifestyle factors on body composition, we are also interested the interrelationship between hormones and body composition measurements.

Insulin and glucose

Insulin is a peptide hormone produced by the β cells of the pancreas. It is secreted to regulate blood glucose levels. It will be released when the blood sugar is too high or when there are too many amino acids in the blood circulation. Insulin will promote cellular glucose uptake, facilitate the liver to do glycogenesis, monitor protein metabolism, encourage lipogenesis and it will slow

down lipolysis. In some individuals, insulin resistance can occur. Insulin resistance is a condition in which the cellular reaction on insulin is impaired. This will encourage the pancreas to produce more insulin in an attempt to compensate. Insulin resistance is an important part of metabolic syndrome and is associated to the development of type 2 diabetes (57). In addition, Insulin resistance is often measured by homeostatic model assessment for insulin resistance (HOMA-IR). Greater HOMA-IR values are a marker for higher levels of insulin resistance (58).

Energy is stored in fat tissue in the form of triglycerides. It is released in the form of free fatty acids (FFA) and glycerol. Besides, adipose tissue also produces adipokines such as leptin and adiponectin. In obesity, these adipokines are abnormally released and the FFA levels are raised. These 2 factors are believed to have an effect on insulin resistance (13).

It is a common belief that obesity is linked to diabetes type 2 due to both insulin resistance and insulin deficiency. First of all, obesity is believed to raise plasma FFA levels. This can negatively influence glucose metabolism which can lead to hyperglycemia. Furthermore, this persistent hyperglycemia primarily leads to hyperinsulinemia and ultimately to a decreased insulin sensitivity. Secondly, the excess FFA's will accumulate in the liver and β cells in the form of triglycerides. This can trigger a hepatic and pancreatic islet dysfunction. By decreasing the amount of functioning pancreatic β cells, the insulin levels will decline and the concentration of glucose will increase. This process is known as insulin deficiency (59).

Obesity is defined as an accumulation of adipose tissue in which the adipose cells not only expand (hypertrophy) but also multiply (hyperplasia). These changes differ individually (60). Numerous studies agree that visceral adiposity increases the risk of developing insulin resistance whereas subcutaneous adiposity decreases this risk (13, 57, 59, 60). The different effect of 2 sorts of adiposity can explain why insulin resistance is not found in every obese person (60). Aronne *et al.* stated that insulin resistance and therefore the risk of diabetes type 2 was positively associated to abdominal fat and waist circumference (61). According to Verma *et al*, visceral adiposity releases more FFA's since visceral fat tissue has more lipolytic and metabolic activity. This enforces the process of insulin resistance even more (59). Additionally, other studies show that FFA's from visceral fat will deposit directly into the liver where it can promote gluconeogenesis, VLDL production and insulin resistance (13, 57).

Finally, obesity is related to increased levels of glucose in the blood circulation due to changes in the adiposity distribution and whole-body metabolism. Hyperglycemia is also a risk factor for developing type 2 diabetes, cardiovascular diseases and cancer (62).

Leptin

Leptin is a hormone that is associated with total fat mass and is primarily made by adipose cells. Its main task is the regulation of energy balance by increasing energy expenditure. It also reduces appetite which results in a decrease of fat mass and body weight. Therefore, it can potentially be used to treat overweight and obesity for individuals (63-65)

Leptin has the ability to cross the blood-brain barrier and mostly performs its operation through its receptors in the central nerve system, mainly at the level of the hypothalamus. The hormone exchanges information on energy reserves with the central nerve system. When leptin binds with the leptin receptor, the sympathetic system is activated which induces the release of neuropeptides. These are necessary for the satiety and energy balance (63, 64).

In obese individuals, leptin resistance is often observed. This is a lowered sensitivity to leptin, which reduces the satiety and results in an increased food intake and might lead to weight gain. In addition, the concentration of leptin in obese individuals is often raised, since they have a heightened fat percentage (64). A positive correlation can thus be found between the leptin concentration and BMI (66). There are many hypotheses about the development of leptin resistance. A possible cause might be in the epigenetic modifications that influence the leptin pathway, another explanation might be the failure of leptin to cross the blood-brain barrier or there might be a problem with the intracellular pathways that emerge after the binding of leptin on the leptin receptor (64).

Adiponectin

Adipose tissue secretes different sorts of adipokines, such as the hormone adiponectin (67, 68). Adiponectin is not only secreted by adipose tissue cells, but also by endothelial cells, skeletal myocytes, and cardiac myocytes (69). Adiponectin is important in the regulation of energy balance and is a protective factor for cardiovascular diseases since it increases insulin sensitivity. It also has anti-inflammatory and anti-atherogenic effects (66, 67). The interaction between adiponectin and insulin sensitivity is shown in figure 1.

All studies agree on a negative correlation between obesity and adiponectin levels (66-71). Obese individuals tend to have lower levels of adiponectin than non-obese participants. In addition, an increase in adiponectin could be seen after weight loss (66).

One study could even show an inverse relationship between adiponectin and percentage body fat, BMI, visceral fat and levels of insulin and leptin. This implicates a correlation with both total body fat and abdominal body fat (68).

Finally, the levels of adiponectin are not only typically lowered in obese people, but also in insulin resistant people. Adiponectin stimulates fatty acid oxidation and can therefore increase the insulin sensitivity (13, 69) .

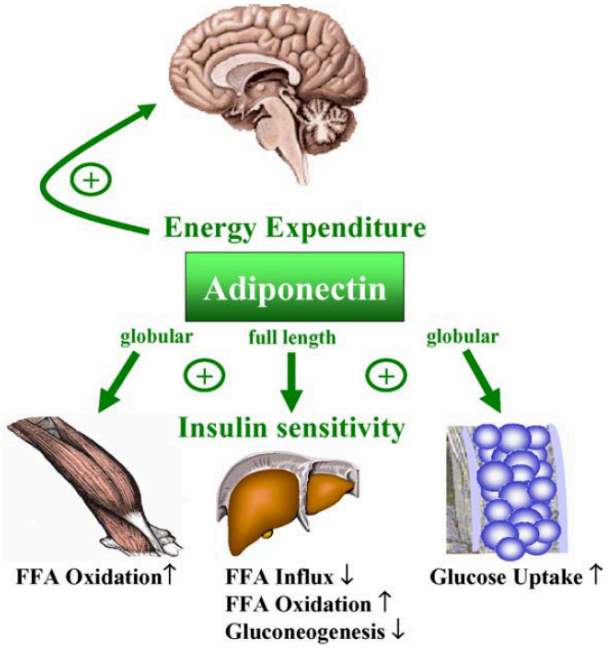

Figure 1: *hypothetical model about the influence of adiponectin on both energy expenditure and insulin sensitivity. Figure from "Adiponectin, obesity, and cardiovascular disease", by Fasshauer M, Paschke R, Stumvoll. Biochimie. 2004;86(11):779-84 (70).*

2. Objectives

The epidemic rise of obesity cannot be ignored and is the most important lifestyle-related risk factor for early death. Knowledge about the exact distribution of body fat and how this changes with aging is important since certain types of adiposity distribution are associated with several comorbidities such as cardiovascular diseases and diabetes type 2. Both body composition and obesity are determined by the interaction between genetic, environmental and lifestyle factors. Especially lifestyle factors have changed a lot over the years. Numerous studies have examined the effect of lifestyle factors on body composition, but the results remain inconclusive.

This longitudinal study includes 999 young to middle-aged healthy Flemish men. We want to investigate if lifestyle-related factors are associated with changes in body composition in these adult men. **Therefore, the main objective of this thesis was to investigate to what extent factors in terms of age or lifestyle have an impact on body composition and adiposity**. These lifestyle factors include smoking habits, alcohol consumption, coffee intake, level of education and physical activity. The body composition parameters are considered to be the outcome variables and age and lifestyle factors the co-variables. **Finally, this thesis will also examine the interrelation of body composition with blood hormones as leptin and adiponectin and with HOMA-IR as an index for insulin resistance.**

3. Materials and methods

3.1 Study design and population

This study is part of a population-based study with the aim to investigate the determinants of peak bone mass in healthy men between 25 and 45 years of age. The focus was set on genetic background, body composition, lifestyle factors and sex hormone status. The original study started as a cross-sectional study (SIBLOS). However, a longitudinal component (SIBEX) was added afterwards by requesting the participants to return for follow-up investigations (72, 73).

At the beginning of the cross-sectional study, 24821 men aged 25-45 years were asked if they wanted to participate in the study and if they had a brother within the same age range willing to participate as well. The age difference between the brothers should not exceed 12 years. All of the participants were invited through the population registries of the semi-rural to urban communities around Ghent, Belgium between March 2002 and July 2010. Subsequently, 1114 men were included. All participants were overall healthy and finished questionnaires concerning smoking habits, alcohol consumption, medical history, education, coffee consumption, calcium intake and physical activity. 115 men were excluded from the study on the basis of treatments or diseases affecting sex hormone status, body composition or bone metabolism: cystic fibrosis, hypothyroidism, hypogonadism, malabsorption or eating disorders, alcohol abuse, chronic renal failure, disorders of collagen metabolism or bone development, and auto-immune rheumatoid disease or the current or prolonged use in the past of glucocorticosteroids, androgens, and anti-androgens, vitamin D supplements, insulin, thyroxin, and previous or current use of anti-epileptic drugs. After applying these exclusion criteria 999 men remained in the study cohort for the analysis (72-74).

Between May 2014 and December 2019, all 999 participants of the cross-sectional study were invited for the longitudinal follow-up study (SIBEX). Eventually 709 participants have been re-examined which leads to a follow-up rate of 71.0%. Loss to follow-up was due to death (n=2), no response (n=111) or unwillingness to participate in the follow-up study (n=177). Additionally, 18 participants were excluded after applying the initial exclusion criteria that were used in the SIBLOS study. This results in a study sample of 691 men for the longitudinal analyses (73, 74).

Finally, the study protocol received approval of the ethical committee of the Ghent University Hospital and all participants have given their written consent. Besides, this longitudinal study (SIBEX) was registered on ClinicalTrials.gov (#NCT02997033) (72, 73).

3.2 Anthropometry, body composition and lifestyle factors

Body composition measurements, including subtotal body fat, subtotal lean mass and truncal fat mass, were quantified by using Dual-energy X-ray absorptiometry (DXA) with a Hologic QDR-4500A device (software version 11.2.1; Hologic, Bedford, MA, USA). Subtotal fat percentage was determined by dividing subtotal fat mass by subtotal mass. Subtotal lean percentage was measured by the same principle (72). Measurements of standing height were assessed to the nearest 0,1 cm by using wall-mounted Harpenden stadiometer (Holtain Ltd., Crymuch, UK). Body weight was measured to the nearest 0.1 kg using a calibrated balance scale and all participants dressed in light indoor clothing without shoes. Furthermore, body mass index (BMI) was calculated by body weight (kg) divided by the square of standing height (m^2). Waist circumference (cm) was measured while standing, at the smallest part at the waist, just above the belly button. Hip circumference (cm) was measured at the widest part of the hip. Waist-hip-ratio was calculated by dividing waist circumference by hip circumference. Finally, baseline and follow-up measurements and scans were performed by the same professional technicians (72, 73).

Physical activity was evaluated by using the questionnaire created by Baecke *et al*. Physical activity was quantified by the sum of 3 indices: sport index (sport activities), work index (the activity during work) and free time index (physical activity during leisure time excluding sport). The intensity, frequency and duration of every index was investigated and the indices range between 1 (minimal level) and 5 (maximal level). Therefore, the Baecke total activity index varies between 3 and 15 (75). Coffee consumption was quantified by cups of coffee a week. Smoking was assessed by daily cigarette consumption and the age at which the participant started smoking (73). Alcohol consumption was quantified by questioning the average number of alcoholic drinks in a week. Units of alcohol were calculated using Nubel 6th edition (76). Heavy drinkers were defined as participants drinking more than 21 units of alcohol a week.

3.3 Biochemical measurements

Blood hormone levels (glucose, insulin, leptin and adiponectin) were determined by venous blood samples. After an overnight fasting, venous blood samples were collected between 8:00 and 10:00 in de morning. Serum samples were put aside at -80 ˚C in expectation of bath analysis. To assess serum levels of glucose (hexokinase method; CV ≤ 1.6%), insulin (Roche Diagnostics, Mannheim, Germany; CV ≤ 3,1%), leptin (Linco Research, Inc., St. Louis, MO, USA; CV ≤ 8.3%) and adiponectin (BioVendor LM, Brno, Czech Republic; CV ≤ 8.2%), commercial assays were used (58, 73). The HOMA-IR was calculated by multiplying glucose (mg/dL) and insulin (MU/L) levels and dividing the result by 405. HOMA-IR was used to asses insulin resistance (58).

3.4 Statistical analysis

Descriptive data at baseline and follow-up was represented as adjusted means with a 95% confidence interval for all variables except age at baseline, education at baseline, smoking status and alcohol consumption. The adjusted means were obtained by using a linear mixed model with time in the fixed effects part. The variables age and education at baseline were presented as unadjusted means with their respective standard deviation. Furthermore, the categorical variables smoking and drinking were represented by percentages. Smoking status was divided into non-smoking and smoking. Alcohol consumption was categorized into non-drinking (0 alcohol drinks per week), moderate drinking (>1 alcohol drinks per week) and heavy drinking (>21 alcohol drinks per week) (74).

All statistical analyses were completed using IBM SPSS statistics (version 26, Chicago, IL, USA). To assess cross-sectional and longitudinal associations between the covariates and body composition parameters, linear mixed models were used. Because the study population mainly consists of brother pairs, the familiar interdependence needed to be taken into account. Therefore, linear mixed models with a variance components residual correlation structure for random effects were used (74).

Final statistical models were created using the step-down method. For every dependent variable (BMI, truncal fat, subtotal fat, subtotal lean mass percentage and WHR), a linear mixed model was executed to examine the baseline association between the covariates and the dependents as well as the influence of the covariates on the evolution of the dependent

variable over time. The last part was assessed using interaction terms between de independent variables and the variable time. In order to counter the familiar interdependence within the linear mixed model, a random intercept was generated for the given family number. For the repeated measurements of the follow-up tests of the same participant, unstructured covariance matrix was used (74). In the fixed effects part of the model, age at baseline, time, smoking, drinking, coffee, years of education at baseline, total activity index, HOMA-IR, leptin at baseline and adiponectin at baseline were used. The interaction terms were also added to the fixed effects part. A stepdown method was used to eliminate the not significant interaction terms.

The baseline associations between body composition and potential determinants were obtained from this model. The dependent variables truncal fat, subtotal fat, truncal fat, subtotal lean mass percentage and WHR are all represented as adjusted means for mean age, mean physical activity, mean years of education, means cups of coffee a week, mean HOMA-IR, mean leptin and mean adiponectin unless stated otherwise. However, the longitudinal relationship between body composition and potential determinants was obtained from the same model but with a correction for adiposity at baseline. For the dependent variables truncal fat, subtotal fat, subtotal lean mass percentage and WHR, an additional independent variable was added to the fixed effects part more specifically BMI at baseline. Subtotal fat mass at baseline was added to the fixed effects part of the dependent variable BMI. The dependent variables are all represented as adjusted means for mean BMI at baseline (or subtotal fat mass at baseline), mean age, mean physical activity, mean years of education, means cups of coffee a week, mean HOMA-IR, mean leptin and mean adiponectin unless stated otherwise.

Furthermore, a test for multicollinearity with variance inflator factors (VIF) was executed for each linear mixed model to assess the correlation between the independent variables. A value of VIF between 1 and 2.5 means that the correlation is not strong enough to influence the outcomes. The covariates showing multicollinearity were removed from the model. Finally, validity of the model was checked by analyzing both the normality of the residuals and the homoscedasticity of the residuals and the predicted values. Significance was evaluated with a p-value smaller than 0.05.

4. Results

4.1 General Characteristics of the study population

Table 3 : General characteristics of the study participants at baseline (n=999) and follow-up (n=691).

	Baseline (n=999)		*Follow-up (n=691)*		*P-value*
	Mean	*95% CI or SD*	*Mean*	*95% CI or SD*	
Age (y)	34.45	5.54	46.35	5.54	**<0.001**
Height (cm)	179.62	179.12 ;180.11	179.60	179.10 ;180.10	0.498
Weight (kg)	80.90	80.05 ;81.74	84.74	83.78 ;85.70	**<0.001**
Subtotal weight (kg)	75.88	75.06 ;76.70	79.02	78.13 ;79.92	**<0.001**
BMI (kg/m^2)	25.09	24.84 ;25.35	26.28	26.00 ;26.57	**<0.001**
Subtotal fat (kg)	15.25	14.80 ;15.70	17.62	17.11 ;18.14	**<0.001**
Subtotal fat percentage (%)	19.50	19.10 ;20.00.	21.70	21.20 ;22.10	**<0.001**
Truncal fat (kg)	7.98	7.70 ;8.26	9.95	9.62 ;10.28	**<0.001**
Subtotal lean mass (kg)	58.22	57.73 ;58.71	59.11	58.58 ;59.63	**<0.001**
Subtotal lean mass percentage (%)	77.40	77.00 ;77.70	75.40	75.00 ;75.80	**<0.001**
WHR	0.89	0.89 ;0.90	0.88	0.88 ;0.89	**0.013**
Total activity index	8.05	7.97 ;8.13	8.10	8.01 ;8.19	0.206
Education at baseline (y)	14.48	3.02	n.a.	n.a.	n.a.
Non-smokers (%)	76.78	n.a.	88.60	n.a.	n.a.
Smokers (%)	23.22	n.a.	11.40	n.a.	n.a.

Non-drinkers (%-	9.33	n.a.	8.87	n.a.	n.a.
Moderate drinkers (%)	77.63	n.a.	80.67	n.a.	n.a.
Heavy drinkers (%)	13.04	n.a.	10.47	n.a.	n.a.
Coffee (n/week)	15.83	14.77 ;16.89	17.61	16.47 ;18.75	**0.001**
Insulin (ng/mL)	7.65	7.31 ;7.98	10.60	10.1 ;11.1	**<0.001**
Glucose (mg/dL)	84.79	84.04 ;85.53	87.61	86.67 ;88.55	**<0.001**
HOMA-IR	1.63	1.55 ;1.71	2.36	2.22 ;2.49	**<0.001**
Leptin (MI/L)	5.35	5.05 ;5.65	n.a.	n.a.	n.a.
Adiponectin (µg/mL)	8.99	8.71 ;9.28	n.a.	n.a.	n.a.

The variables age and education at baseline are defined by unadjusted means with standard deviation (SD). The variables weight, subtotal weight, height, BMI, subtotal fat, subtotal fat percentage, truncal fat, subtotal lean mass, subtotal lean mass percentage, WHR, physical activity index, coffee, insulin, glucose, HOMA-IR, leptin and adiponectin are defined by adjusted means with a 95% confidence interval (95% CI). Smoking status and alcohol status are defined by percentages. The cut-off p-value is 0.05 and significant p-values are highlighted in bold; n.a. stands for non-applicable.

Table 3 provides an overview of the general characteristics, body composition parameters and hormone levels of the study population at baseline and follow-up. The baseline study population consisted of 999 men with a mean age of 34.5 years. 691 men with a mean age of 46.4 years old were examined at follow-up. At baseline, 0.3 percent of the men were underweight, 54.4 percent had a normal weight, 37.2 percent were overweight and 8.1 percent were classified as obese. The men in the follow-up population were generally heavier; only 0.1 percent of the men were classified as underweight, 39.6 percent had a normal weight, 46.6 percent was overweight and 13.9 percent was obese. Additionally, both the absolute numbers of subtotal fat and subtotal lean mass increased over time. The same could be said for subtotal weight. However, when we compare subtotal fat and subtotal lean mass in function of subtotal weight, mean subtotal fat percentage was 20.1 percent at baseline and raised to an average of 22.3 percent at follow-up. In contrast, the mean subtotal lean mass percentage decreased from 76.7 percent at baseline to 74.8 percent at follow-up. The change in WHR was minimal with 0.89 and 0.88 respectively. Furthermore, a significant difference could be observed between insulin and glucose levels at baseline and at follow-up. Both levels are increased

which results in an increase of HOMA-IR as well. Since HOMA-IR > 2.17 is an indicator for insulin resistance (58) we could say that on average 20 percent of the men have raised insulin resistant at baseline and 36.9 percent at follow-up.

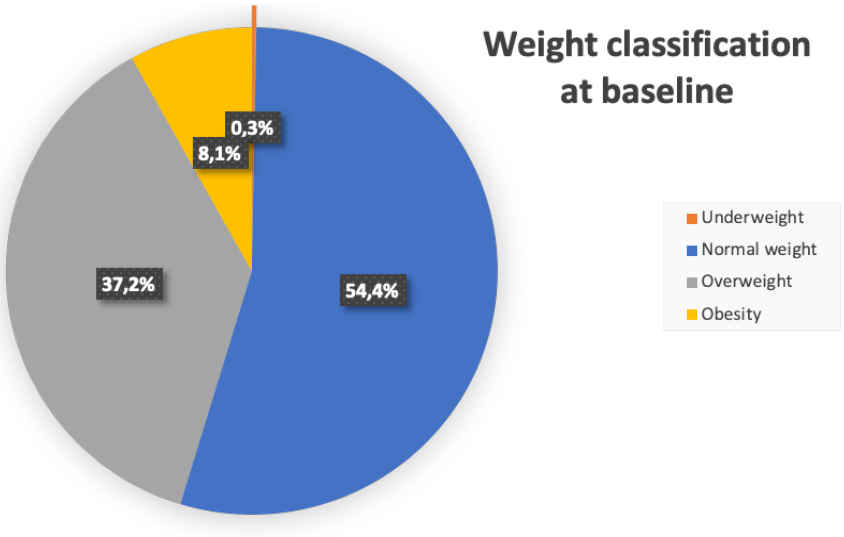

***Figure 2**: weight classification at baseline (n=999) using the WHO weight classification.*

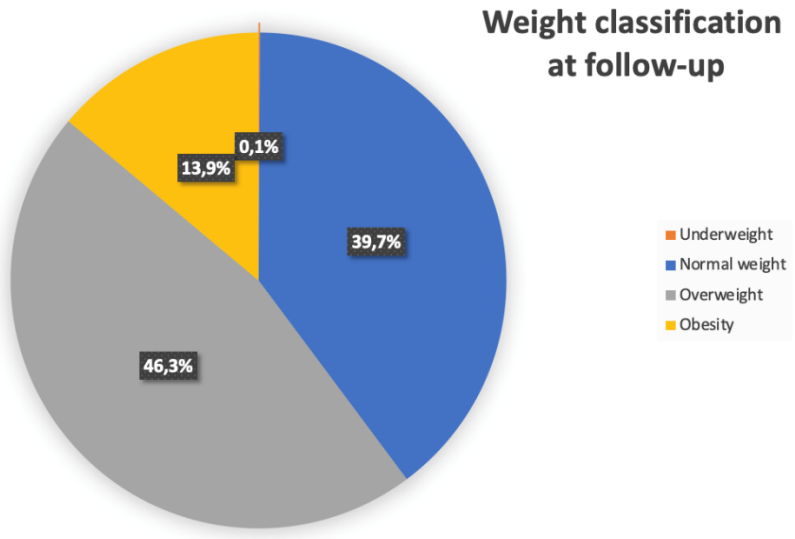

***Figure 3**: weight classification at follow-up (n=691) using the WHO weight classification.*

4.2 Baseline associations between body composition and potential determinants

BMI

Table 4: baseline regression coefficients of BMI and age, lifestyle factors and hormones.

		ß (95% CI)	P-value
Age (y)		8.85e-2 (6.06e-2;1.16e-1)	**<0.001**
Level of education (y)		-1.13e-1 (-1.60e-1;-6.63e-2)	**<0.001**
Smoking	No	-2.33e-1 (-4.88e-1;2.28e-2)	0.074
	Yes	-	-
Drinking	No	-1.56e-2 (-4.57e-1;4.26e-1)	0.945
	Moderate	-9.18e-2 (-3.86e-1;2.03e-1)	0.541
	Heavy	-	-
Total activity index		3.82e-2 (-4.95e-2;1.26e-1)	0.393
Coffee (n/week)		-7.79e-4 (-6.70e-3;5.15e-3)	0.796
HOMA-IR		3.17e-1 (2.21e-1;4.13e-1)	**<0.001**
Leptin (ng/mL)		4.94e-1 (4.59e-1;5.29e-1)	**<0.001**
Adiponectin (µg/mL)		-7.94e-2 (-1.17e-1;-4.22e-2)	**<0.001**

Data was represented as regression coefficients (ß) with a 95% confidence interval (95% CI) for a mean age of 34.55 years at baseline, mean years of education at baseline = 14.54y, mean total activity index = 8.08, mean cups of coffee a week = 16.65, mean HOMA-IR = 1.92, mean leptin at baseline = 5.26 ng/mL and mean adiponectin at baseline = 9.05 µg/mL. Data on interaction terms are not presented. The cut-off p-value is 0.05 and significant p-values are highlighted in bold.

As shown in table 4, a positive correlation was observed between age at baseline and BMI. Level of education was inversely associated with BMI. No associations could be found for smoking, drinking, total activity index and coffee. Furthermore, both HOMA-IR and leptin were positively correlated to BMI while adiponectin was negatively correlated.

Subtotal fat

Table 5: baseline regression coefficients of subtotal fat and age, lifestyle factors and hormones.

		ß (95% CI)	P-value
Age (y)		88.25 (46.81;129.70)	**<0.001**
Level of education (y)		-58.52 (-129.29;12.24)	0.105
Smoking	No	-505.88 (-949.73;-62.03)	**0.026**
	Yes	-	-
Drinking	No	-536.41 (-1269.05;196.23)	0.151
	Moderate	-299.77 (-790.03;190.50)	0.231
	Heavy	-	-
Total activity index		-342.61 (-498.66;-186.57)	**<0.001**
Coffee (n/week)		-1.32 (-11.84;9.21)	0.806
HOMA-IR		437.87 (250.66;625.08)	**<0.001**
Leptin (ng/mL)		1142.13 (1084.4;1199.86)	**<0.001**
Adiponectin (µg/mL)		-71.08 (-127.55;-14.61)	**0.014**

Data was represented as regression coefficients (ß) with a 95% confidence interval (95% CI) for a mean age of 34.55 years at baseline, mean years of education at baseline = 14.54y, mean total activity index = 8.08, mean cups of coffee a week = 16.68, mean HOMA-IR = 1.91, mean leptin at baseline = 5.22 ng/mL and mean adiponectin at baseline = 9.03 µg/mL. Data on interaction terms are not presented. The cut-off p-value is 0.05 and significant p-values are highlighted in bold.

Table 5 illustrates a positive correlation between age at baseline and subtotal fat. Further, total activity index is found to be negatively associated with subtotal fat. A difference could be observed between smokers and non-smokers. Not smoking is associated with a lower amount of subtotal fat in comparison to smokers. No associations were found between level of education, drinking or coffee and subtotal fat. Furthermore, a positive correlation could be observed between HOMA-IR and leptin and subtotal fat while a negative correlation was found with adiponectin.

Truncal fat

Table 6: baseline regression coefficients of truncal fat and age, lifestyle factors and hormones.

		ß (95% CI)	P-value
Age (y)		102.34 (77.26;127.42)	**<0.001**
Level of education (y)		-55.83 (-98.72;-12.94)	**0.011**
Smoking	No	-331.69 (-604.19;-59.18)	**0.017**
	Yes	-	-
Drinking	No	-567.03 (-1019.28;-114.77)	**0.014**
	Moderate	-397.35 (-701.18;-93.52)	**0.010**
	Heavy	-	-
Total activity index		-196.52 (-292.48;-100.57)	**<0.001**
Coffee (n/week)		-5.18 (-11.66;1.3)	0.117
HOMA_IR		449.51 (337.94;561.09)	**<0.001**
Leptin (ng/mL)		640.95 (606.86;675.04)	**<0.001**
Adiponectin (µg/mL)		-89.78 (-124.07;-55.5)	**<0.001**

Data was represented as regression coefficients (ß) with a 95% confidence interval (95% CI) for a mean age of 34.55 years at baseline, mean years of education at baseline = 14.54y, mean total activity index = 8.08, mean cups of coffee a week = 16.68, mean HOMA-IR = 1.91, mean leptin at baseline = 5.22 ng/mL and mean adiponectin at baseline = 9.03 µg/mL. Data on interaction terms are not presented. The cut-off p-value is 0.05 and significant p-values are highlighted in bold.

Table 6 provides an overview of the baseline associations of truncal fat. As can be seen from the table, age at baseline is positively correlated to truncal fat, while level of education and total activity index are inversely correlated to truncal fat. A difference was also seen between smokers and non-smokers. Non-smokers tend to have lower truncal fat than smokers. In comparison to heavy drinkers, non-drinkers and moderate drinkers are associated with lower truncal fat as well. No association was found for coffee. Finally, as for the hormones, a positive correlation was observed between HOMA-IR and leptin and truncal fat while a negative association was found between adiponectin and truncal fat.

Subtotal lean mass percentage

Table 7: Baseline regression coefficients of subtotal lean mass percentage and age, lifestyle factors and hormones.

		ß (95% CI)	P-value
Age (y)		-9,25e-4 (-1,31e-3;-5,43e-4)	**<0,001**
Level of education (y)		4,74e-4 (-1,88e-4;1,14e-3)	0,160
Smoking	No	7,87e-3 (3,64e-3;1,21e-2)	**<0,001**
	Yes	-	-
Drinking	No	4,52e-3 (-2,38e-3;1,14e-2)	0,199
	Moderate	1,43e-3 (-3,21e-3;6,08e-3)	0,545
	Heavy	-	-
Total activity index		5,36e-3 (3,85e-3;6,87e-3)	**<0,001**
Coffee (n/week)		1,53e-5 (-8,46e-5;1,15e-4)	0,764
HOMA-IR		-1,49e-3 (-3,31e-3;3,21e-4)	0,107
Leptin (ng/mL)		-9,43e-3 (-9,98e-3;-8,88e-3)	**<0,001**
Adiponectin (µg/mL)		4,47e-4 (-8,82e-5;9,83e-4)	0,101

Data was represented as regression coefficients (ß) with a 95% confidence interval (95% CI) for a mean age of 34.54 years at baseline, mean years of education at baseline = 14.55y, mean total activity index = 8.08, mean cups of coffee a week = 16.70, mean HOMA-IR = 1.90, mean leptin at baseline = 5.20 ng/mL and mean adiponectin at baseline = 9.05 µg/mL. Data on interaction terms are not presented. The cut-off p-value is 0.05 and significant p-values are highlighted in bold.

As can be seen from the table above, total activity index was positively associated with subtotal lean mass percentage. Besides, not smoking is associated with a higher subtotal lean mass percentage in comparison to smokers. Both age at baseline and levels of leptin were inversely correlated to subtotal lean mass percentage. Finally, no associations were observed for level of education, alcohol consumption, coffee consumption, HOMA-IR and adiponectin.

WHR

Table 8: baseline regression coefficients of WHR and age, lifestyle factors and hormones.

		ß (95% CI)	P-value
Age (y)		2.58e-3 (1.80e-3;3.36e-3)	**<0.001**
Level of education (y)		-2.01e-3 (-3.35e-3;-6.72e-4)	**0.003**
Smoking	No	1.60e-2 (6.28e-3;2.57e-2)	**0.001**
	Yes	-	-
Drinking	No	-3.05e-3 (-2.36e-2;1.75e-2)	0.770
	Moderate	1.43e-2 (3.55e-4;2.82e-2)	**0.044**
	Heavy	-	-
Total activity index		-1.78e-3 (-4.86e-3;1.30e-3)	0.256
Coffee (n/week)		-2.70e-5 (-2.79e-4;2.25e-4)	0.834
HOMA-IR		7.67e-3 (4.80e-3;1.06e-2)	**<0.001**
Leptin (ng/mL)		6.41e-3 (5.20e-3;7.63e-3)	**<0.001**
Adiponectin (µg/mL)		-3.06e-3 (-4.80e-3;-1.32e-3)	**0.001**

Data was represented as regression coefficients (ß) with a 95% confidence interval (95% CI) for a mean age of 34.64 years at baseline, mean years of education at baseline = 14.62y, mean total activity index = 8.06, mean cups of coffee a week = 15.90, mean HOMA-IR = 1.99, mean leptin at baseline = 5.08 ng/mL and mean adiponectin at baseline = 8.83 µg/mL. Data on interaction terms are not presented. The cut-off p-value is 0.05 and significant p-values are highlighted in bold.

Age at baseline seems to be positively associated with WHR while a negative association was observed between level of education and WHR. Additionally, not smokers appear to have a higher WHR than smokers. Alcohol consumption was also associated to WHR. Moderate drinking was associated to a higher WHR in comparison to heavy drinkers while no association could be found for non-drinkers. No associations could be found for total activity index and coffee. Finally, a positive association was observed between both leptin and HOMA-IR and WHR while adiponectin was inversely correlated to WHR.

4.3 Longitudinal determinants of body composition

Determinants of BMI

Figure 4 illustrates the evolution of BMI with BMI significantly increasing over time (ß=2.65; p<0.001). Change in total activity index over time was associated with a less pronounced increase in BMI (ß=-0.26; p<0.001). Likewise, in comparison to heavy drinkers, non-drinkers showed a less pronounced increase in BMI (ß=-0.99; p=0.002). No associations were found for moderate drinkers. Furthermore, an increase of BMI over time was positively associated with a change in HOMA-IR (ß=0.18; p<0.001). A positive association was also observed between level of adiponectin at baseline and the evolution of BMI over time (ß=0.05; p=0.002). Smoking, coffee consumption and level of education were not correlated to longitudinal changes in BMI in our cohort.

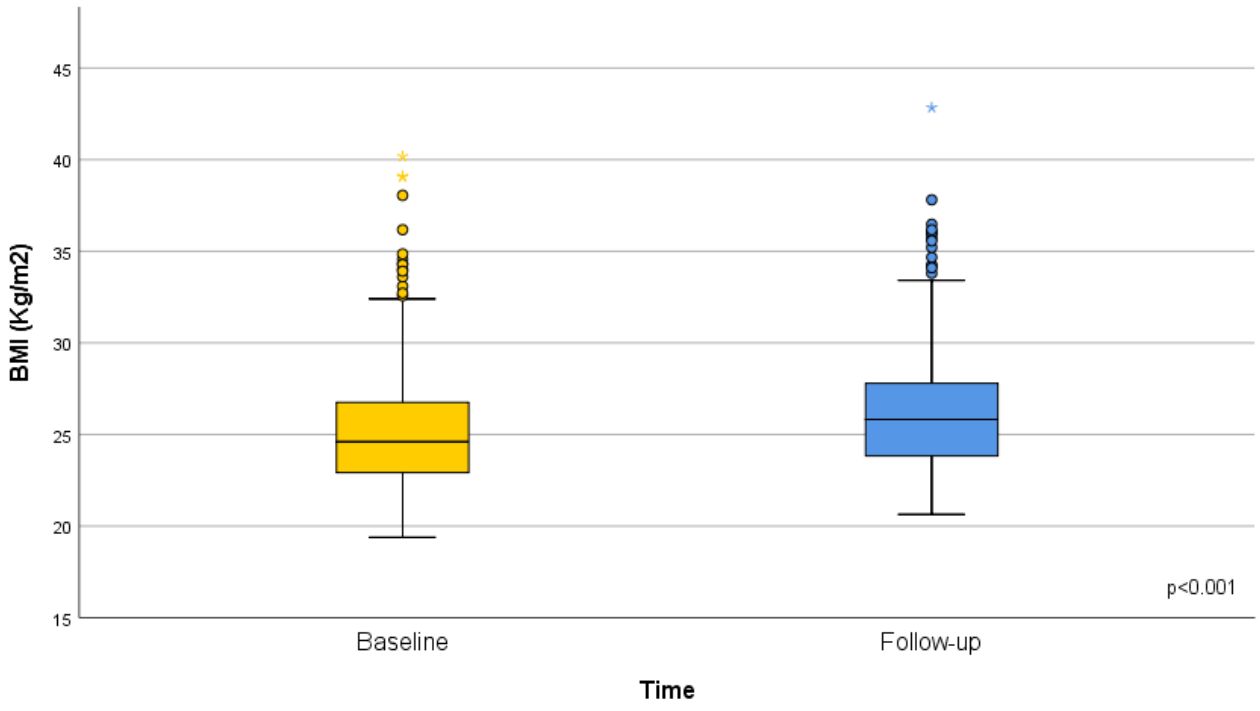

***Figure 4**: Longitudinal changes in BMI (kg/m2) between baseline (SIBLOS) and follow-up (SIBEX) is shown. Data represented are based on adjusted means from the full model.*

Determinants of subtotal fat

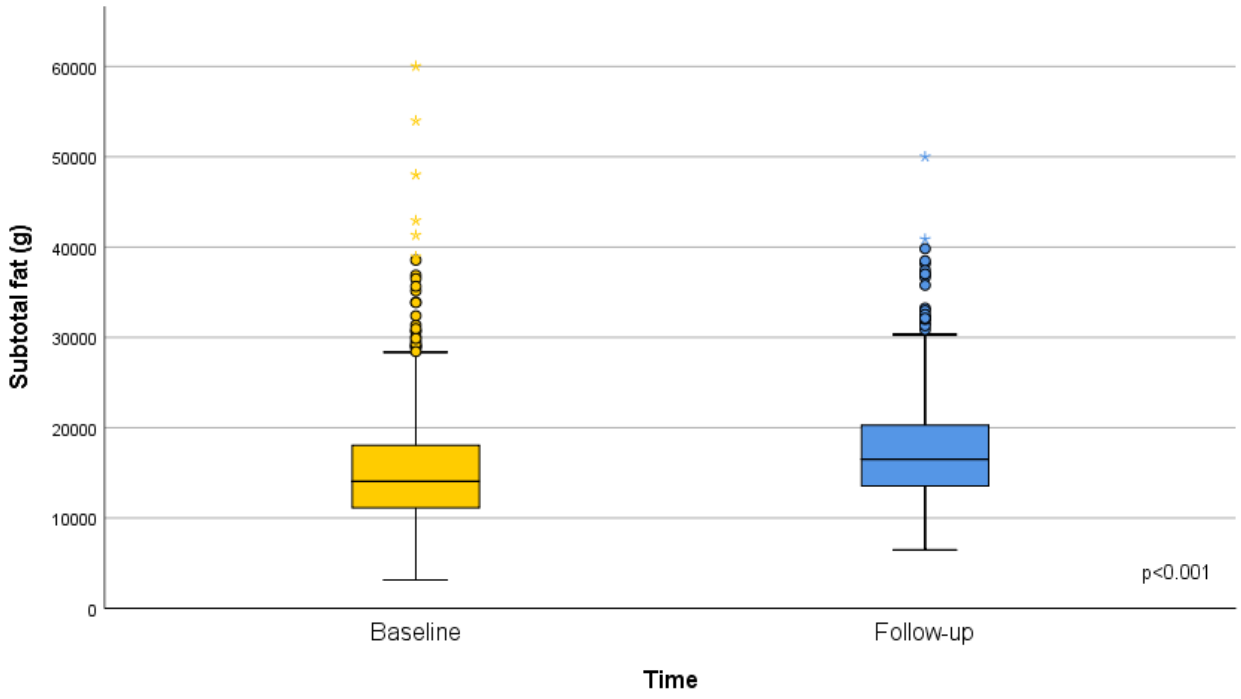

***Figure 5**: Longitudinal changes in subtotal fat (g) between baseline (SIBLOS) and follow-up (SIBEX) is shown. Data represented are based on adjusted means from the full model.*

Figure 5 shows the evolution of subtotal fat mass over time in our population. Subtotal fat mass increases with ageing (ß=3454.28; p=0.004). An increase in total activity index is associated with a less pronounced increase in subtotal fat (ß=-357.36; p=0.005). Additionally, an increase in subtotal fat mass over time is positively correlated to change in HOMA-IR (ß=703.41, p<0.001). Furthermore, a positive correlation was observed between adiponectin at baseline and change in subtotal fat (ß=129.60; p=0.001) while a negative correlation was found for leptin at baseline (ß=-206.72, p<0.001). No associations were found between the change in subtotal fat and smoking, alcohol consumption, coffee consumption or level of education.

Determinants of truncal fat

Figure 6 shows that truncal fat significantly increases with ageing (ß=2425.03; p=0.001). An increase of total activity index over time is associated with a less pronounced increase in truncal fat (ß=-241.58; p=0.002). A positive association between change in truncal fat over time and change in HOMA-IR was observed as well (ß=299.87; p<0.001). A positive

association was also seen between level of adiponectin at baseline and the evolution of truncal fat over time (ß=68.43; p=005). Finally, level of education, smoking, alcohol consumption, coffee consumption and leptin at baseline were not correlated to longitudinal changes in truncal fat in our cohort.

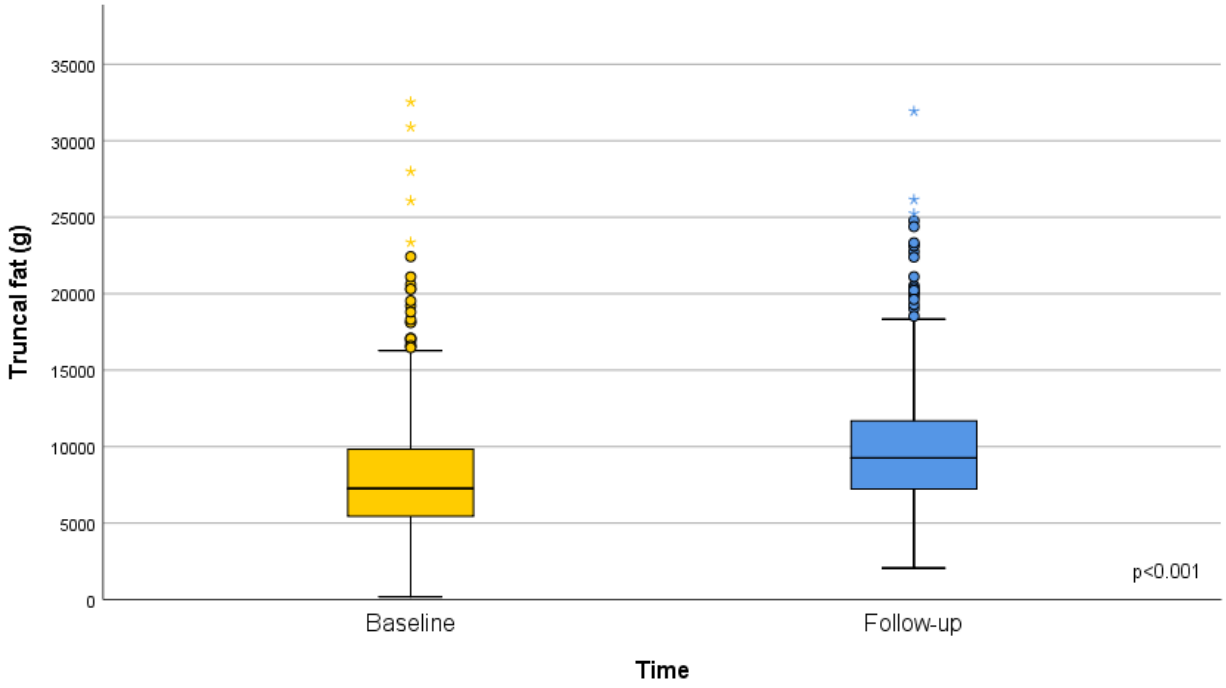

***Figure 6**: Longitudinal changes in truncal fat (g) between baseline (SIBLOS) and follow-up (SIBEX) is shown. Data represented are based on adjusted means from the full model.*

Determinants of subtotal lean mass percentage

From the graph below we can see that subtotal lean mass percentage decreases with ageing (ß=-0.026; p=0.016). An increase in total activity over time results in a more pronounced increase of subtotal lean mass percentage (ß=0.002; p=0.040). Additionally, an increase in subtotal lean mass percentage over time is negatively associated with change in HOMA-IR (ß=-0.005; p<0.001). A positive association was also observed between level of leptin at baseline and the evolution of subtotal lean mass over time (ß=0.002; p<0.001) while a negative correlation was found between level of adiponectin at baseline and change in subtotal lean mass percentage (ß=-0.001; p<0.001). No associations were found for level of education, smoking, alcohol consumption and coffee consumption.

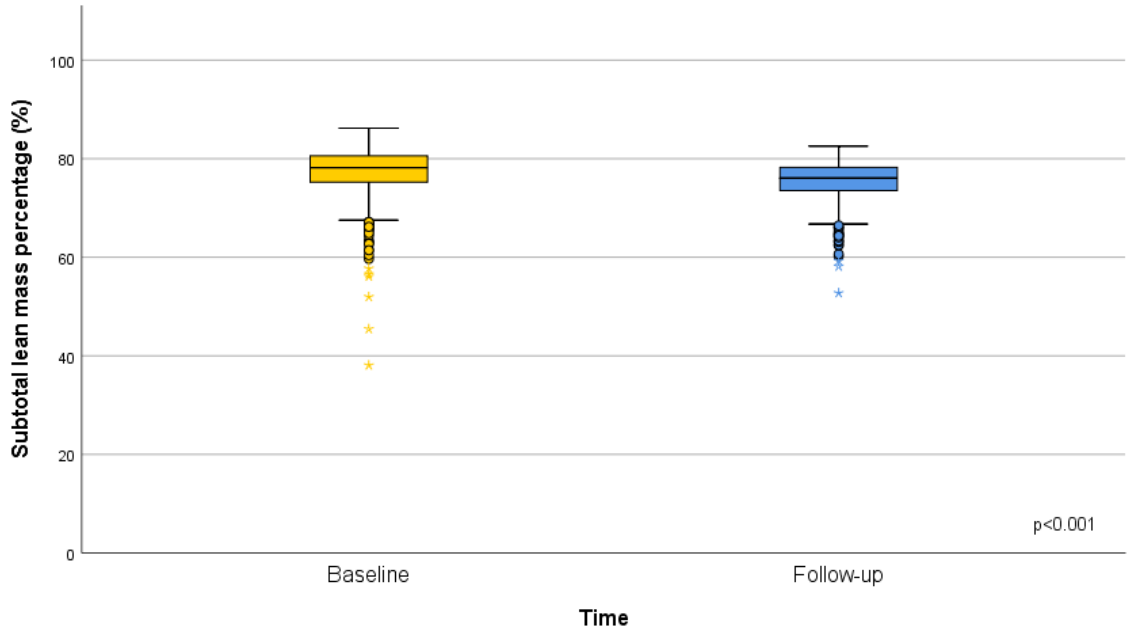

***Figure 8**: Longitudinal changes in subtotal lean mass percentage between baseline (SIBLOS) and follow-up (SIBEX) is shown. Data represented are based on adjusted means from the full model.*

Determinants of WHR

In our full model, WHR did not change in our population over time. In comparison to heavy drinkers, moderate drinkers showed a less pronounced increase in WHR (ß=-0.024; p=0.015). No associations were found for non-drinkers. Additionally, a positive association was observed between level of adiponectin at baseline and change of WHR over time (ß=0.002; p=0.028). Level of education, physical activity index, coffee consumption, smoking status, HOMA-IR and leptin at baseline were not associated to a change in WHR over time.

5. Discussion

5.1 General

In this present study, we investigated to what extent factors in terms of age or lifestyle have an impact on body composition and adiposity as well as the interrelationship of body composition with blood hormones (leptin and adiponectin) and HOMA-IR as an indicator for insulin resistance. Our main findings include that age, smoking habits, alcohol consumption, physical activity, level of education and level of hormones at baseline are associated to certain body composition parameters. No associations were found for coffee. Furthermore, we have observed that alcohol consumption, physical activity, HOMA-IR, leptin at baseline and adiponectin at baseline have an influence on the evolution of several body composition parameters over time. Coffee intake, smoking status and level of education did not have a significant impact on the change of body composition over time.

Age

Our data shows that age at baseline was positively associated to BMI, subtotal fat, truncal fat and WHIR. Age at baseline was also associated with a lower WHR. Longitudinally, BMI, subtotal fat and truncal fat increase with ageing. Most studies agree on BMI and subtotal fat increasing with ageing. Some of them even found that both BMI and fat mass increase with age until a certain age at which a small decline starts (15-20). We could not confirm this decline since only healthy young men were included in our study. The mean age was 35.5 years old at baseline and 45.4 years old at follow-up. Change in body composition and obesity in older men was thus not investigated.

Furthermore, several studies found an inverse relationship between subtotal lean mass and time (16, 18, 19). Our results were in line with these outcomes. In our cohort, a decreasing trend for subtotal lean mass percentage was seen.

Finally, Bemben *et al*. observed an increase in abdominal circumference as the subjects get older until the age of 70 (19, 20). Vermeulen *et al*. also noted that the abdominal region was the body region most influenced by the increase in fat mass (19, 20). However, in this study, WHR did not change significantly over time. Nonetheless, an increase for truncal fat could be

seen. It can thus be suggested that WHR is possibly a less precise body composition parameter for abdominal adiposity than truncal fat.

Smoking

In this present study, a difference was observed between smokers and non-smokers for most of the body composition parameters. This study however cannot confirm the common belief that smoking promotes thinness and weight control (22, 23). Smoking was positively associated with both subtotal fat and truncal fat. Meanwhile, smoking was correlated to a lower WHR and lower percentage subtotal lean mass while no associations were found for BMI. In accordance to De Oliveira Fontes Gasperin *et al*., we could not find an association between smoking status and BMI. However, De Oliveira Fontes Gasperin *et al.* observed an association between numbers of cigarettes smoked a day and BMI (28). Since numbers of cigarettes smoked a day were not included in our cohort, we could not verify this finding. Additionally, in our cohort, smoking did not modulate the change of BMI, subtotal fat, truncal fat, subtotal lean mass percentage and WHR over time.

Alcohol consumption

Multiple studies were able to demonstrate a positive relationship between alcohol consumption and several body composition parameters (30, 32, 34). The study of Wannamethee *et al.* found a positive association between increasing alcohol intake and BMI, WHR, waist circumference and body fat percentage. Heavy drinking was correlated to a higher waist circumference, BMI, WHR and body fat percentage than moderate drinkers or non-drinkers (34). Lean *et al.* also observed a positive association between both BMI and waist circumference and heavy drinking in comparison to moderate drinking (30).The results of this study show that in our cohort alcohol consumption was not associated to BMI, subtotal fat or subtotal lean percentage at baseline. However, both non-drinking and moderate drinking were associated to a lower amount of truncal fat in comparison to heavy drinking. Besides, we found an association between WHR and drinking status; moderate drinking was associated to a higher WHR in comparison to heavy drinkers and no association was found for non-drinkers.

Furthermore, we have examined to what extent alcohol consumption is associated with the evolution of body composition parameters over time. In our study, non-drinkers showed a less pronounced increase in BMI in comparison to heavy drinkers. Besides, moderate drinkers showed a less pronounced increase in WHR in comparison to heavy drinkers. No associations

were found between drinking status and change in subtotal fat, truncal fat and subtotal lean mass percentage over time.

Finally, the disproportion between the three groups should be pointed out. Only 9.3 percent of the study population were non-drinkers, 77.6 percent were moderate drinkers and 13.1 percent were heavy drinkers. Our results could possibly be confounded by this irregularity. Additionally, the lifestyle factors were assessed by completing questionnaires. Alcohol consumption was quantified by questioning the average number of alcoholic drinks in a week. This could lead to recall bias since participants do not always answer accurately or honestly.

Coffee

On the overall cohort, no associations were found between coffee and any of the body composition parameters. Besides, no correlation was observed between change in coffee intake and the change of body composition parameters over time. These findings are contrary to previous studies. Most studies found an inverse correlation between coffee consumption and BMI, waist circumference or fat percentage (36-38). However, the study of Sarria *et al.* only included 52 participants and the study of Revuelta-Iniesta *et al.* only had 20 participants. Besides, none of the studies used DXA to assess body fat mass or body fat percentage. In accordance to Bouchard *et al*., we could not find an association between the consumption of coffee and body composition parameters (40).

Physical activity

The majority of the studies found an association between physical activity and a more favorable body composition (42-45). An increase in physical activity is associated with a decline in fat mass, BMI and waist circumference (42, 44, 45). In contrast to previous findings, this present study could not find an association between physical activity and both BMI and WHR at baseline. However, in accordance to preceding studies, physical activity was associated with a lower amount of both subtotal fat and truncal fat and associated with a higher percentage of subtotal lean mass at baseline. These findings may suggest that subtotal fat, truncal fat and subtotal lean mass percentage are more precise indicators for body composition than WHR and BMI in healthy men.

Furthermore, we investigated the relationship between change in physical activity over time and change in body composition parameters. First of all, an increase of physical activity was associated with a less pronounced increase of BMI, subtotal fat and truncal fat. Secondly, the

change in total activity index was correlated to a more pronounced increase in subtotal lean mass percentage over time. Finally, no association was found between change in physical activity and the evolution of WHR over time.

Sedentary behavior was not investigated in our study. It could be interesting to take this variable into account as well in future research since the amount of sedentary jobs is increasing (47). Physical activity in our study was determined by total activity index, created by Braecke *et al*. The total activity index was calculated by taking into account 3 indices: sport during leisure time (sport index), physical activity during work (work index) and physical activity during leisure time (leisure-time index) excluding sport (75). Thus, no difference was made between these indices in our analysis and therefore it was not possible to evaluate which one was the most influential.

Socioeconomic status (SES)

Multiple studies agree that a lower socioeconomic status, especially level of education, is associated to a higher risk of obesity (50-52). We could confirm the correlation between a lower level of education at baseline and a higher BMI. However, over time, level of education did not modulate the change of BMI. Not much research was done yet on the association of SES and body composition parameters. In the current study, an inverse association was observed between level of education and both truncal fat and WHR at baseline. No associations were found between level of education and both subtotal fat of subtotal lean mass. Over time, education at baseline did not modulate the change of body composition parameters.

In the present study, level of education was used as a marker for socioeconomic status. However, various methods can be used to define SES. Usually, income, level of education or occupational status or a combination between two or three of them are used. Different outcomes can be obtained, depending on which method is used (52).

Hormones and HOMA-IR

According to Aronne *et al.,* insulin resistance was associated to a higher level of abdominal fat and an increased waist circumference (61). Similarly to Aronne *et al.*, HOMA-IR was positively associated to BMI, WHR, subtotal fat and truncal fat in this study. A higher HOMA-IR was associated to a lower subtotal lean mass percentage as well. Our longitudinal results further support the baseline associations. An increase in BMI, subtotal fat and truncal fat over time was positively associated to an increase in HOMA-IR. Change of subtotal lean mass

percentage however was negatively associated to change in HOMA-IR. No association was seen between the evolution of WHR over time and change in HOMA-IR. These results further support the idea of adiposity, especially truncal adiposity, being associated to HOMA-IR and therefore to insulin resistance and metabolic health. Nonetheless, no distinction could be made between visceral and subcutaneous fat.

Secondly, Yang *et al*. stated that level of leptin changes in proportion to adiposity (65). Our baseline results seem to be consistent to previous studies. A positive association was found between leptin and BMI, subtotal fat, truncal fat and WHR. Moreover, a higher level of leptin at baseline was associated to a lower percentage of subtotal lean mass. In contrast to the baseline associations, we observed a positive association between levels of leptin at baseline and change in subtotal lean mass percentage. Leptin at baseline was also negatively associated with change in subtotal fat. No associations were found between leptin at baseline and longitudinal changes in truncal fat and WHR in our cohort.

Finally, our results are consistent with other studies who found a negative correlation between obesity and adiponectin levels (66-71). Ryan *et al*. even observed that the level of adiponectin was inversely associated to the percentage of body fat, BMI, visceral fat and subcutaneous abdominal fat (68). In our cohort, adiponectin at baseline was found to be negatively associated with all body composition parameters except from subtotal lean mass percentage. A higher level of adiponectin at baseline was associated to a higher percentage of subtotal lean mass. Since whole body DXA cannot make a difference between visceral and subcutaneous fat, nothing can be said about these variables. Furthermore, the longitudinal findings are in contrast with the baseline associations. A higher level of adiponectin at baseline was positively associated to the evolution of BMI, subtotal fat, truncal fat and WHR over time. No association between level of adiponectin at baseline and change in subtotal lean mass percentage was found. The longitudinal associations of leptin and adiponectin are not in line with the baseline associations. However, for the longitudinal associations, we tried to adjust for baseline adiposity by entering BMI at baseline in the mixed models of subtotal fat, truncal fat, subtotal lean mass percentage and WHR. For the dependent variable BMI, we adjusted by entering subtotal fat at baseline. The inconsistency between baseline and longitudinal associations could possibly be explained by the limitations of BMI and subtotal fat as markers for adiposity.

5.2 Strengths and limitations

A major strength of this study is the large sample size. This study consisted of 999 healthy men at baseline (24-46 years old) and 691 at follow-up (32-60 years old). Most participants were Caucasian males from around Ghent, Belgium and no women were included. The results should be interpreted with caution when generalizing to the entire world population since racial and gender differences might have an influence as well.

Furthermore, all participants were volunteers and on average highly educated. Eventually 709 participants have been re-examined which leads to a follow-up rate of 71.0%. The majority of the people who did a re-examination were rather health-conscious people. Thus, a possible health seeking bias need to be taken into account. Next, lifestyle factors were evaluated by using questionnaires. Due to possibly inaccurate or incorrect answers, a recall bias needs to be considered as well.

No data about dietary habits or sedentary behavior was investigated. It could be interesting to take these variables into account as well since the amount of sedentary jobs is increasing (47). Dietary habits are also believed to have an important impact on obesity and body composition. Moreover, only at baseline information was available for levels of adiponectin and leptin. Further research with longitudinal data is required to evaluate the relationship between change in leptin or adiponectin and change in body composition parameters.

Finally, DXA was used to assess body composition measurements. DXA is a simple, precise and objective technique to perform whole-body scans. It is used worldwide to measure both bone and soft-tissue mass but also regional body composition (10, 11). The accuracy of DXA is similar to whole-body CT but the effective radiation dose the subject is exposed to, is smaller (10). However, DXA cannot distinguish between visceral and subcutaneous fat. For this, CT, MR of DXA-VAT is required (12). Since visceral fat percentage is associated with an increased insulin resistance, which is a risk factor for diabetes type 2 and cardiovascular diseases (4), further research is required to examine whether lifestyle-related factors have an influence on rather visceral or subcutaneous fat.

5.3 Conclusion

In conclusion, the main findings of this study include that alcohol consumption, physical activity, HOMA-IR, leptin at baseline and adiponectin at baseline are associated with the evolution of several body composition parameters over time. Especially level of adiponectin at baseline, ageing, change in HOMA-IR and change in physical activity have an association with the evolution of almost all body composition parameters over time. Alcohol intake was only correlated to the change of BMI and WHR over time and not with the other body composition parameters. The level of leptin at baseline was only associated with the change of subtotal fat and subtotal lean mass percentage over time. Finally, level of education, coffee intake and smoking status were not correlated to longitudinal changes in body composition parameters in our cohort. We can thus conclude that these variables do not have a significant impact on evolution of body composition and adiposity.

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