CHAPTER 5

The association of low muscle mass with prevalence and incidence of type 2 diabetes in different BMI classes

Dionne Sizoo
Suzanne P. Stam
Loek J.M. de Heide
Marloes Emous
Tim van Zutphen
Peter R. van Dijk
André P. van Beek

Published in
Diabetes Research and Clinical Practice 2023;195:110197

ABSTRACT

Objective: The aim of this study is to investigate whether muscle mass is associated with the prevalence and incidence of type 2 diabetes and whether this association differs within men and women of normal weight, overweight or obesity.

Methods: Adult participants were included from the Lifelines cohort study. Low muscle mass was defined as < -1SD of the gender-stratified creatinine excretion rate (CER). Multivariate logistic regression analysis was used to assess the association between muscle mass and the prevalence and incidence of type 2 diabetes.

Results: Muscle mass was associated with the prevalence of type 2 diabetes both in men and in women (OR 1.51 [95%CI 1.32–1.72]; P<0.001 and OR 1.53 [1.36 – 1.73]; P<0.001). Incident type 2 diabetes was associated with a decreased muscle mass for both men and women (male; OR 1.22 [1.05 – 1.43]; P=0.01 and female; OR 1.36 [1.17 – 1.59]; P<0.001), and remained significant after adjustments in normal weight women (OR 1.77 [1.16 - 2.70]; P=0.008).

Conclusions: Both a low muscle mass and loss of muscle mass are associated with the prevalence and incidence of diabetes in the general population. This association is strongest in people with normal weight, and weakens in people within higher BMI subgroups.

INTRODUCTION

Overweight and obesity are among the largest challenges in modern day medicine. The prevalence has reached such proportions, that despite all effort, approximately 70% of the population in the United States is observed to have overweight or obesity.[1,2] Moreover, the trends are moving upwards, further increasing an already substantial burden on the health care system and leading to increasing health care costs.[3]

Measuring BMI is used worldwide to determine overweight or obesity because of its correlation with body fat mass.[4] However, BMI may overestimate the degree of adiposity in individuals who are overweight but muscular and underestimate it in persons with reduced muscle mass e.g. due to ageing or inactivity. In addition, sex and ethnicity are of influence on adiposity and muscle mass, in which BMI is lacking.[5] Because BMI is unable to discriminate between excess fat mass and reduced muscle mass, it is important to find reliable markers for muscle mass and to determine whether it has added value on top of BMI for prediction of certain metabolic diseases.

This is of particular importance because besides adipose tissue, muscle mass is also metabolically active and is known to play a protective role in the pathogenesis of type 2 diabetes, as muscle mass is a major site of peripheral glucose uptake.[6–8] As a result, reduced muscle mass can be a determinant in the development of type 2 diabetes. It can be hypothesized that the combined presence of a high fat mass and a low muscle mass may even signify a double metabolic burden. The prevalence of low muscle mass is known to be higher in populations with type 2 diabetes, compared to the normoglycemic reference group.[9] Whether the association of muscle mass with type 2 diabetes differs across BMI subgroups in overweight and obese subjects, has not yet been investigated.

A non-invasive method to assess total body muscle mass is the 24h urinary creatinine excretion rate (CER). In general, creatinine is formed at a constant rate from the non-enzymatic conversion of creatine and creatine phosphate in muscle.[10] As the amount of creatinine produced

is dependent on the quantity of muscle mass, the CER is regarded as an accurate and stable marker for muscle mass both in physiological and in wasting conditions[11], and has been validated with both dual-energy x-ray absorptiometry (DXA) and magnetic resonance imaging (MRI).[12,13]

The aim of this study is to investigate whether muscle mass, as determined by 24h urinary CER, is associated with the prevalence and incidence of type 2 diabetes and whether this association differs within men and women of normal weight, overweight or obesity.

RESEARCH DESIGN AND METHODS

Study design and population

This observational study was performed using the data from the Dutch Lifelines cohort study. Lifelines is a multi-disciplinary prospective population-based cohort study examining in a unique three-generation design the health and health-related behaviours of 167.729 persons living in the North of the Netherlands. It employs a broad range of investigative procedures in assessing the biomedical, socio-demographic, behavioural, physical and psychological factors which contribute to the health and disease of the general population, with a special focus on multi-morbidity and complex genetics. The overall design of this cohort study has been described previously.[14,15] Participants were included between 2006 and 2013. At baseline and the first follow-up assessment (after 5 years), participants were invited to the Lifelines center for a health assessment, including questionnaires, anthropometric measurements and collection of blood samples. The Lifelines cohort study is conducted in accordance with to the declaration of Helsinki and approved by the medical ethical committee of University Medical Center Groningen, The Netherlands (reference number: METc 2007/152).[14,15]

A total of 152.500 adults aged 18 – 65 were eligible for the primary analysis of type 2 diabetes prevalence. After exclusion of participants with renal impairment (defined by a eGFR <60 ml/min/1.73m2), kidney disease, missing urinary CER, missing BMI or those with underweight (BMI < 18.5), a total of 127.616 people were included in the analysis on the prevalence of type 2

diabetes (figure 1). For the secondary analysis on the incidence of type 2 diabetes, people with missing urinary CER, renal impairment at the second assessment, or type 2 diabetes at the first assessment were also excluded, resulting in 94.565 people included in this second analysis (figure 1).

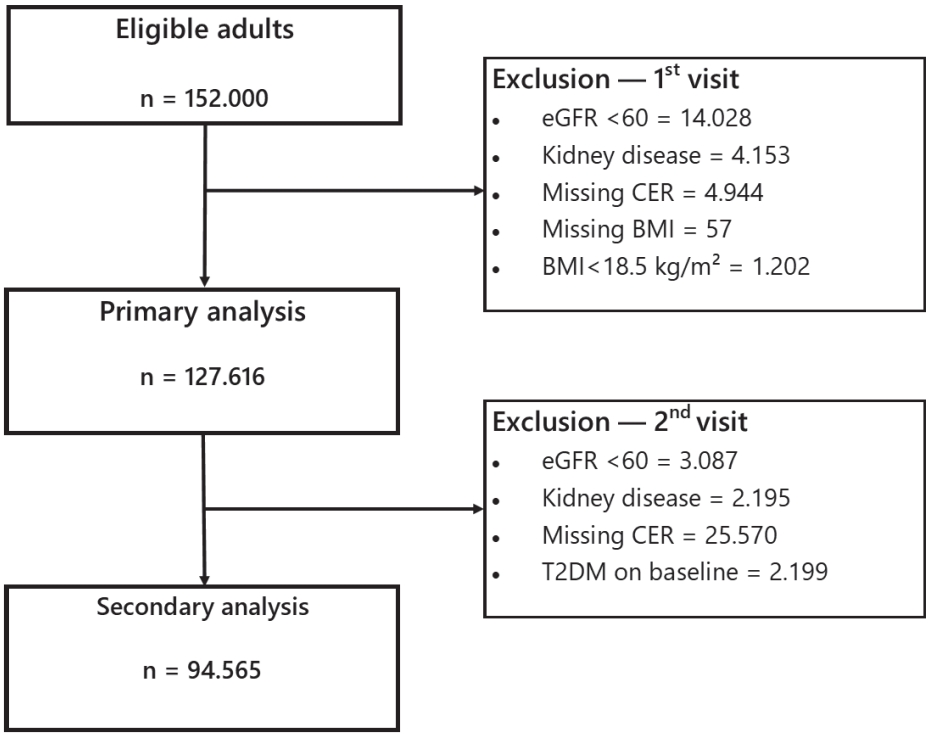

Figure 1 Flowchart of included study population

Baseline data collection and measurements

During the onsite visit, trained research staff measured anthropometric characteristics (weight, height, waist- and hip circumference). For estimation of total muscle mass, the urinary CER was used. This was calculated using the concentration of urinary creatinine (mmol/L) and the total amount of urine (L) collected in 24 hours. The participants received strict guidelines to ensure an appropriate 24-hour urine collection. All participants were instructed to discard a morning void and collect all urine for 24 hours, ending with a morning void exactly 24 hours after the start

of collection. Results were stratified by gender and a low muscle mass was defined as < -1 SD of the mean CER, calculated separately for males and females.[2] A low muscle mass was compared to the reference group defined as ≥ -1 SD of the mean CER. A change in muscle mass was defined as the difference between CER at baseline and CER 5 years later at the second measurement. The WHO BMI cut-off values were used to define normal weight, overweight and obesity in this study (BMI 18.5 - 24.99 kg/m², BMI 25.0 - 29.99 kg/m² and BMI ≥ 30.0 kg/m², respectively).[5]

Age, concomitant diseases, physical activity and dietary intake were assessed by self-administered questionnaires.[14] Physical activity was estimated by moderate-to-vigorous physical activity (MVPA) based on the Short Questionnaire to Assess Health-enhancing physical activity (SQUASH).[16] Dietary intake was assessed by a food frequency questionnaire.[17] To assess protein intake, the protein density was used, which is the amount of protein in gram per 1000 kcal.

Outcome definitions

The prevalence of diabetes mellitus was assessed by a self-administered questionnaire, in which participants were asked to answer the following question “Do you have diabetes mellitus?”. Affirmative answers were considered to be indicative of type 2 diabetes given the fact that in the Dutch population more than 90% of persons with diabetes mellitus, have type 2 diabetes.18 For the analysis of incident type 2 diabetes mellitus, the population at risk was defined as all adults without diabetes mellitus at baseline. New onset diabetes mellitus during the second assessment after five years was assessed by the question “Did the health problems listed below start since the last time you filled in the lifelines questionnaire: Diabetes mellitus”.

Table 1 Baseline characteristics of the male and female population per BMI groups and presence of low muscle mass

Male	BMI 18.5 - 25		BMI 25 - 30		BMI 30+	
	Low CER n=4250	**Reference n=17144**	**Low CER n=2858**	**Reference n=23429**	**Low CER n=461**	**Reference n=7212**
Clinical characteristics						
Age, years	46 (35 - 59)	40 (30 - 49)	51 (43 - 65)	46 (39 - 53)	50 (42 - 63)	47 (40 - 55)
CER, mmol/24h	11.3 ± 1.6	16.6 ± 2.6	11.4 ± 1.6	17.8 ± 3.0	11.1 ± 7.8	19.5 ± 3.8
Hip circumference, cm	92.2 ± 5.4	94.1 ± 5.4	98.7 ± 5.3	100.2 ± 5.2	107.5 ± 6.3	109.0 ± 7.1
Waist circumference, cm	85.5 ± 6.8	86.4 ± 6.3	97.1 ± 6.5	97.4 ± 6.2	111.1 ± 7.8	111.7 ± 8.9[ns]
Comorbidities						
Diabetes Mellitus, n(%)	91 (2.1)	158 (0.9)	141 (4.9)	555 (2.4)	50 (10.9)	471 (6.5)
Hypertension, n(%)	599 (14.1)	1642 (9.6)	810 (28.4)	4883 (20.9)	187 (40.7)	2588 (35.9)[ns]
Lifestyle						
Total energy intake, kcal/day	2208.3 ± 830.8	2404.2 ± 878.4	2056.8 ± 789.2	2242.9 ± 826.1	1959.7 ± 871.6	2189.5 ± 833.0
Total protein intake, g/day	76.0 ± 27.3	83.7 ± 29.1	74.4 ± 27.7	80.8 ± 28.0	73.3 ± 30.9	80.9 ± 28.8
Physical activity, h/week	8.5 (3 - 22)	6.2 (2.5 – 16.1)	10.2 (3.5 – 24.5)	7 (2.5 – 20.8)	9.5 (4 – 22.5)	6.5 (1.9 - 21)

Female	Low CER n=6427	Reference n=33065	Low CER n=3131	Reference n=22093	Low CER n= 1031	Reference n=11494
Clinical characteristics						
Age, years	49 (40 - 59)	41 (31 - 48)	54 (45 - 63)	46 (38 - 53)	51 (43 - 61)	46 (38 - 52)
CER, mmol/24h	7.4 ± 1.0	11.3 ± 1.9	7.5 ± 1.0	11.7 ± 2.1	7.3 ± 1.1	12.7 ± 2.5
Hip circumference, cm	92.1 ± 6.1	93.6 ± 6.3	102.0 ± 5.9	102.7 ± 6.0	115.2 ± 9.6	116.3 ± 9.5
Waist circumference, cm	78.3 ± 7.3	78.9 ± 7.0	90.2 ± 7.2	90.0 ± 7.2^{ns}	103.9 ± 10.7	104.7 ± 10.4
Comorbidities						
Diabetes Mellitus, n(%)	88 (1.4)	200 (0.6)	138 (4.4)	435 (2.0)	98 (9.5)	693 (6.0)
Hypertension, n(%)	1269 (19.7)	4503 (13.6)	1087 (34.7)	5660 (25.6)	469 (45.5)	4496 (39.1)
Lifestyle						
Total energy intake, kcal/day	1677.3 ± 604.1	1783.8 ± 641.3	1600.9 ± 580.4	1715.6 ± 617.7	1577.8 ± 819.8	1714.9 ± 643.5
Total protein intake, g/day	61.8 ± 21.4	65.3 ± 21.9	61.7 ± 21.2	65.5 ± 21.7	61.3 ± 25.6	66.3 ± 22.3
Physical activity, h/week	6.5 (2.5 - 15)	5 (2.0 - 11.0)	7.7 (3.0 – 16.2)	5.5 (2.0 – 13.5)	5.2 (2.0 – 13.7)	4.7 (1.5 – 12.4)

Data are presented as mean±sd or median (IQR) and total amount (%), Abbreviations: CER, 24h urinary creatinine excretion rate; ns, not significant

Statistical analysis

Continuous data are presented as mean ± standard deviation or median [interquartile range] where appropriate. Categorical data are presented as total amount (n) and percentage (%). The t-test was used for normally distributed variables and the Mann-Whitney U test for skewed distributed variables. In case of more than two groups, a F test (One-way univariate ANOVA) or Kruskal-Wallis test was used, respectively. For qualitative parameters, overall differences were evaluated using a Chi-square test. Logistic regression analysis was used to assess the association between muscle mass and either diabetes mellitus. First, a sensitivity analysis was performed to look at the interaction of both BMI and age, to confirm our hypothesis that especially BMI was an effect modifier. Secondly, the regression analyses were done in the total population, but also stratified by BMI categories and gender. To assess the association of a decrease in muscle mass with new-onset type 2 diabetes, a regression analyses was performed, again for the separate genders and BMI groups. In the logistic regression analysis of the prevalence data, we adjusted for various determinants and confounders of type 2 diabetes, namely age, BMI (continuous; not for subgroup analysis), hip- and waist circumference, protein intake, and physical activity (MVPA). In the logistic regression analysis of the incidence data, we adjusted for CER at baseline, age, BMI (continuous; not for subgroup analysis), hip- and waist circumference, protein intake, and physical activity (MVPA). Data were presented as OR with 95% confidence interval (CI). Statistical analyses were performed using IBM Statistics SPSS version 23.0 (IBM Inc. Chicago, IL, USA). Visual depiction of the associations was done using Microsoft Visio 16.0 (Redmond, WA, USA).

Table 2 Associations of low urinary creatinine excretion rate with the prevalence of diabetes

	Male			**BMI 18.5 - 25**			**BMI 25 - 30**			**BMI 30+**		
	Low CER		**Ref.**	**Low CER**		**Ref.**	**Low CER**		**Ref.**	**Low CER**		**Ref.**
	OR (95%CI)	***P*-value**		**OR (95%CI)**	***P*-value**		**OR (95%CI)**	***P*-value**		**OR (95%CI)**	***P*-value**	
Model 1	1.51 (1.32 - 1.72)	<0.001	1.0	2.35 (1.81 – 3.05)	<0.001	1.0	2.14 (1.77 - 2.59)	<0.001	1.0	1.74 (1.28 - 2.37)	<0.001	1.0
Model 2	1.55 (1.35 - 1.79)	<0.001	1.0									
Model 2a				1.52 (1.16 – 1.98)	0.002	1.0	1.36 (1.12 - 1.65)	0.002	1.0	1.28 (0.93 - 1.76)	0.13	1.0
Model 3	1.51 (1.31 - 1.74)	<0.001	1.0	1.56 (1.18 – 2.05)	0.002	1.0	1.37 (1.13 - 1.67)	0.002	1.0	1.36 (0.99 - 1.88)	0.06	1.0
Model 4	1.51 (1.31 - 1.75)	<0.001	1.0	1.55 (1.17 - 2.04)	0.002	1.0	1.37 (1.13 - 1.67)	0.002	1.0	1.37 (0.99 - 1.89)	0.06	1.0
Model 5	1.52 (1.31 - 1.77)	<0.001	1.0	1.60 (1.20 - 2.13)	0.001	1.0	1.37 (1.11 - 1.68)	0.003	1.0	1.41 (1.00 - 1.98)	0.05	1.0
	Female			**BMI 18.5 – 25**			**BMI 25 - 30**			**BMI 30+**		
Model 1	1.53 (1.36 - 1.73)	<0.001	1.0	2.28 (1.77 - 2.93)	<0.001	1.0	2.30 (1.89 - 2.80)	<0.001	1.0	1.64 (1.31 - 2.05)	<0.001	1.0
Model 2	1.47 (1.29 - 1.68)	<0.001	1.0									
Model 2a				1.43 (1.10 - 1.86)	0.009	1.0	1.45 (1.18 - 1.77)	<0.001	1.0	1.20 (0.95 - 1.50)	0.13	1.0
Model 3	1.47 (1.29 - 1.68)	<0.001	1.0	1.53 (1.17 - 2.00)	0.002	1.0	1.49 (1.22 - 1.83)	<0.001	1.0	1.29 (1.02 - 1.62)	0.03	1.0
Model 4	1.48 (1.29 - 1.69)	<0.001	1.0	1.54 (1.18 - 2.01)	0.002	1.0	1.48 (1.21 - 1.82)	<0.001	1.0	1.29 (1.02 - 1.63)	0.03	1.0
Model 5	1.45 (1.27 - 1.67)	<0.001	1.0	1.53 (1.16 - 2.03)	0.003	1.0	1.47 (1.19 - 1.82)	<0.001	1.0	1.24 (0.98 - 1.59)	0.08	1.0

Ref.: normal/high CER. Model 1: crude, Model 2: model 1 + adjustment for age and BMI, Model 2a model 1 + (subgroups): adjustment for age, Model 3: model 2 + adjustment for hip- and waist circumference; model 2a (subgroups) + adjustment for hip- and waist circumference, Model 4: model 3 + adjustment for grams of protein per 100 kcal, Model 5: model 4 + adjustment for MVPA. Abbreviations: CER, 24h urinary creatinine excretion rate; BMI, body mass index; OR, odds ratio; MVPA; moderate to vigorous physical activity.

RESULTS

Baseline characteristics

Of the 127.616 (58.3% female) participants present in this analyses, the mean age was 44.5 ± 12.8 years. Mean 24h urinary CER at the first assessment was 16.8 ± 3.7 mmol/24h in men and 11.1 ± 2.5 mmol/24h in women (P<0.001). Baseline characteristics for men and women according to BMI categories and subdivided for muscle mass are shown in table 1. In all BMI categories, both male and female participants with a low muscle mass were older, had a lower total energy and protein intake and were more physically active. Furthermore, the prevalence of type 2 diabetes increased with increasing BMI, and was higher in participants with a low muscle mass in all BMI categories. The prevalence of hypertension also increased with increasing BMI, and was higher in participants with a low muscle mass, except for males with a BMI > 30 kg/m². There was no relevant difference in waist circumference between low muscle mass and the reference group in any of the BMI categories. Furthermore, the CER of the reference group increased with increasing BMI categories.

Effect modification

To test for effect modification by age or BMI, we tested the interaction by age and BMI on the association of CER with type 2 diabetes. There was no effect modification for age (P≥0.05). We observed effect modification by BMI both as continuous variable and BMI categories (P<0.001; P=0.008, respectively).

Table 3 characteristics of the male and female population per BMI groups and presence of a decrease in muscle mass after 5 years

Male	BMI 18.5 - 25		BMI 25 - 30		BMI 30+	
	Decrease in CER	Reference	Decrease in CER	Reference	Decrease in CER	Reference
	n=7407	n=8119	n=9178	n=10277	n=2528	n=3026
Clinial characteristics						
Age, years	44 (34 - 52)	43 (33 - 51)	47 (40 - 57)	47 (40 - 55)	48 (41 - 57)	47 (41 - 55)
baseline CER, mmol/24h	16.6 ± 3.1	14.6 ± 2.7	18.2 ± 3.3	15.9 ± 3.0	20.1 ± 3.7	17.4 ± 3.5
Hip circumference, cm	94.4 ± 4.9	94.4 ± 5.0	100.0 ± 5.0	100.1 ± 5.0	108.7 ± 6.9	108.6 ± 7.0
Waist circumference, cm	86.9 ± 6.3	86.6 ± 6.2	97.7 ± 6.2	97.7 ± 6.2	112.2 ± 8.8	112.2 ± 8.5
Comorbidities						
Diabetes mellitus, n(%)	99 (1.3)	97 (1.2)^{ns}	253 (2.8)	255 (2.5)^{ns}	164 (6.5)	168 (5.6)^{ns}
Hypertension, n(%)	919 (12.4)	883 (10.9)	2072 (22.6)	2197 (21.4)^{ns}	931 (36.9)	1088 (36.0)^{ns}
Lifestyle						
Total energy intake, kcal/day	2194.8 ± 978.6	2252.4 ± 919.6	2109.5 ± 928.8	2126.6 ± 919.2^{ns}	2033.2 ± 899.7	2050.6 ± 933.2^{ns}
Total protein intake, g/day	77.2 ± 32.8	78.8 ± 30.5	76.4 ± 31.7	76.5 ± 31.2^{ns}	75.6 ± 31.3	76.1 ± 32.2^{ns}
Physical activity, h/week	7 (3 - 20)	6.7 (2.8 - 18)	8 (2.8 - 24.3)	7.2 (2.5 - 22)	8 (2.3 - 24)	6.6 (2 - 21.4)

Female	Decrease in CER	Reference	Decrease in CER	Reference	Decrease in CER	Reference
	n=13088	n=14554	n=8964	n=10099	n=4536	n=4987
Clinial characteristics						
Age, years	44 (36 - 50)	43 (35 - 51)	47 (40 - 55)	47 (39 -56)[ns]	47 (50 - 54)	46 (39 - 55)
baseline CER, mmol/24h	11.4 ± 2.2	9.9 ± 2.0	12 ± 2.4	10.4 ± 2.1	13 ± 2.7	11.3 ± 2.4
Hip circumference, cm	94.4 ± 5.8	94.5 ± 5.8	103.2 ± 5.7	103.1 ± 5.9	115.9 ± 9.2	115.9 ± 9.2
Waist circumference, cm	78.7 ± 6.9	78.5 ± 6.9	90.3 ± 7.0	90.0 ± 7.1	104.5 ± 10.0	104.2 ± 9.2
Comorbidities						
Diabetes mellitus, n(%)	116 (0.9)	84 (0.6)	218 (2.4)	226 (2.2)[ns]	271 (6.0)	248 (5.0)
Hypertension, n(%)	2150 (16.4)	2206 (15.2)	2481 (27.7)	2681 (26.6)[ns]	1851 (40.8)	1853 (37.2)
Lifestyle						
Total energy intake, kcal/day	1676.7 ± 738.8	1730.5 ± 670.9	1620.8 ± 728.5	1653.5 ± 671.1	1583.6 ± 750.6	1616.9 ± 705.5
Total protein intake, g/day	62.1 ± 25.4	63.8 ± 23.3	62.1 ± 25.7	63.4 ± 23.9	61.9 ± 26.6	63.0 ± 24.8
Physical activity, h/week	5.5 (2.3 - 12.5)	5.5 (2.3 - 12.3)[ns]	6 (2.3 - 15)	6 (2.5 - 14)[ns]	5.2 (2 - 14)	5 (2 - 13)[ns]

Data are presented as mean ± sd or median (IQR) and total amount (%). Abbreviations: CER, 24h urinary creatinine excretion rate; ns, not significant.

Primary analyses – Low muscle mass and the prevalence of diabetes

In crude logistic regression analysis, low muscle mass was associated with the prevalence of type 2 diabetes both in men and women (table 2, model 1). In multivariable analyses, the association of muscle mass with the prevalence of type 2 diabetes was independent of age and BMI in both men and women. When cumulatively adjusted for relevant potential confounders including waist circumference and hip circumference, protein intake, and lastly physical activity, the association did not materially change.

The associations of a low muscle mass with the prevalence of type 2 diabetes were strongest in the normal weight and decreased in overweight and obesity (table 2, model 1). The association remained present independent of age, waist- and hip circumference, protein intake, and physical activity in men with normal weight and overweight, but was borderline significant in men with obesity. In women with normal weight and overweight, the association remained present independent of age, waist- and hip circumference, protein intake, and physical activity. In women with obesity, the association was no longer significant.

Secondary analyses – Loss of muscle mass and the incidence of diabetes

Table 3 shows the baseline characteristics of men and women according to BMI groups and the loss of muscle mass over 5 years. In all BMI categories, both men and women with a decrease in muscle mass had no relevant age, waist- or hip circumference difference. Furthermore, in men, there was no difference in the prevalence of type 2 diabetes between decrease in CER and the reference group. In women, the prevalence of type 2 diabetes was slightly higher in the participants with decreased CER, except for women with overweight.

The loss of muscle mass was associated with incident type 2 diabetes both in men and women (table 4, model 1). After adjustment for baseline CER in model 2, the association remained significant.

In the BMI subgroups, the association was no longer significant in men within either BMI subgroup. In women, the association remained significant in those with normal weight, but no longer in women with overweight or obesity.

DISCUSSION

This study demonstrates that a low muscle mass, measured by CER, is associated with the prevalence of type 2 diabetes in the general population. The association is most evident in the lowest BMI class (18.5 - 25 kg/m^2) and becomes less strong in the higher BMI categories both in men and women. Furthermore, loss of muscle mass during a period of five years was associated with an increased risk of development of type 2 diabetes in women of normal weight.

To the best of our knowledge, this is the first study in a population-based cohort that investigated the association of muscle mass, measured by CER, and type 2 diabetes in adults across different BMI classes. The association was present both in men and women and remained significant after correction for age, BMI (for analysis of total population), hip- and waist circumference, protein intake and physical activity. In the subgroup analysis, this association lost strength with increasing BMI classes, suggesting an opposite role for another factor, likely adipose tissue mass. In support of this, after adjustment for age, hip- and waist circumference, protein intake and physical activity, the association was only significant in men and women with normal and overweight, but not in those with obesity. Our results are in accordance with other studies in literature.

Table 4 Associations of decrease in urinary creatinine excretion rate with the incidence of type 2 diabetes

	Male			**BMI 18.5 - 25**			**BMI 25 - 30**			**BMI 30+**		
	Decrease in CER		**Ref.**	**Decrease in CER**		**Ref.**	**Decrease in CER**		**Ref.**	**Decrease in CER**		**Ref.**
	OR (95%CI)	***P*-value**		**OR (95%CI)**	***P*-value**		**OR (95%CI)**	***P*-value**		**OR (95%CI)**	***P*-value**	
Model 1	1.22 (1.05 - 1.43)	0.01	1.0	1.05 (0.67 - 1.64)	0.84	1.0	1.21 (0.96 - 1.51)	0.10	1.0	1.14 (0.89 - 1.47)	0.31	1.0
Model 2	1.22 (1.03 - 1.44)	0.02	1.0	1.41 (0.88 - 2.25)	0.16	1.0	1.52 (1.20 - 1.94)	0.001	1.0	1.27 (0.97 - 1.67)	0.08	1.0
Model 3	1.27 (1.07 - 1.51)	0.007	1.0									
Model 3a				1.11 (0.69 - 1.81)	0.66	1.0	1.30 (1.01 - 1.65)	0.04	1.0	1.10 (0.83 - 1.45)	0.50	1.0
Model 4	1.26 (1.06 - 1.50)	0.009	1.0	1.12 (0.69 - 1.81)	0.66	1.0	1.28 (1.00 - 1.63)	0.05	1.0	1.11 (0.84 - 1.47)	0.46	1.0
Model 5	1.25 (1.06 - 1.49)	0.01	1.0	1.11 (0.68 - 1.80)	0.68	1.0	1.27 (1.00 - 1.63)	0.05	1.0	1.10 (0.83 - 1.46)	0.50	1.0
Model 6	1.25 (1.04 - 1.50)	0.02	1.0	1.10 (0.67 - 1.83)	0.71	1.0	1.28 (0.99 - 1.66)	0.06	1.0	1.10 (0.82 - 1.48)	0.52	1.0
	Female			**BMI 18.5 – 25**			**BMI 25 – 30**			**BMI 30+**		
Model 1	1.36 (1.17 - 1.59)	<0.001	1.0	1.59 (1.10 - 2.29)	0.01	1.0	1.13 (0.87 - 1.48)	0.36	1.0	1.27 (1.01 - 1.60)	0.04	1.0
Model 2	1.34 (1.14 - 1.58)	0.001	1.0	1.88 (1.28 - 2.77)	0.001	1.0	1.32 (0.99 – 1.75)	0.06	1.0	1.42 (1.11 - 1.81)	0.005	1.0
Model 3	1.31 (1.11 - 1.55)	0.002	1.0									
Model 3a				1.67 (1.13 - 2.47)	0.01	1.0	1.14 (0.85 – 1.51)	0.38	1.0	1.25 (0.98 - 1.60)	0.08	1.0
Model 4	1.31 (1.11 - 1.55)	0.002	1.0	1.67 (1.13 - 2.47)	0.01	1.0	1.12 (0.84 – 1.50)	0.43	1.0	1.28 (1.00 - 1.64)	0.05	1.0
Model 5	1.32 (1.11 - 1.57)	0.002	1.0	1.69 (1.13 – 2.47)	0.009	1.0	1.12 (0.84 – 1.50)	0.43	1.0	1.28 (1.00 - 1.65)	0.05	1.0
Model 6	1.25 (1.05 - 1.50)	0.01	1.0	1.77 (1.16 – 2.70)	0.008	1.0	1.02 (0.75 – 1.37)	0.91	1.0	1.23 (0.95 - 1.58)	0.12	1.0

Ref.: No decrease in CER. Model 1: crude, Model 2: model 1 + adjustment for 24h urinary creatinine excretion at baseline, Model 3: model 2 + adjustment for age and body mass index, Model 3a (subgroups): model 2 + adjustment for age, Model 4: model 3 + adjustment for waist- and hip circumference; model 3a + adjustment for waist- and hip circumference, Model 5; model 4 + adjustment for grams of protein per 1000 kcal, Model 6: model 5 + adjustment for physical activity

The study of Kim et al. found that type 2 diabetes is associated with a decreased skeletal muscle index in Korean patients, measured with dual-energy x-ray absorptiometry. Furthermore, they also found that type 2 diabetes was independently associated with sarcopenia, defined by skeletal muscle index <-2 SD of a young reference population.[19] Moreover, a systematic review and meta-analysis of Anagnostis et al. showed that people with type 2 diabetes had an increased risk of sarcopenia.[20] These two studies did not look into differences of the association within different BMI classes.

We also investigated the association between a loss of muscle mass and the incidence of type 2 diabetes over 5 years. Our results show that loss of muscle mass is associated with the incidence of type 2 diabetes in both men and women. This association remained present after correction for baseline muscle mass, age, BMI, waist- and hip circumference, protein intake and physical activity. These results are comparable to the study of Maliszewska et al., who showed, using multi-frequency bioelectrical impedance analysis (BIA), that a reduction in muscle mass over five years is a risk factor for type 2 diabetes in adults.[21] To find out in which subgroup this association is strongest, we performed a subgroup analysis. Unfortunately, the association between decreased muscle mass and incident type 2 diabetes was only significant for women with normal weight. The lack of significance in the subgroup analyses might be due to the low numbers of new diabetes cases in the 5 year follow-up (see table 5). It is important to note that various methods have been used to measure the muscle mass in the different studies. Despite the differences in methods to measure muscle mass, all indicate an association between muscle mass and diabetes. Our study reinforces this association and shows the loss of strength of this association in higher BMI classes for both genders. Additionally, we used a widely available tool for measuring muscle mass with cut-off values that can be applied directly in clinical practice (men: 13.04; women: 8.57 mmol/24h).

Table 5 Number of cases (%) of incident diabetes per BMI category and decrease in CER after 5 years.

	BMI 18.5 - 25		BMI 25 - 30		BMI 30+	
	Decrease in CER	**No decrease in CER**	**Decrease in CER**	**No decrease in CER**	**Decrease in CER**	**No decrease in CER**
Male	11 (0.7)	93 (0.8)	49 (2.2)	259 (1.7)	39 (5.5)	188 (4.5)
Female	17 (0.6)	103 (0.5)	24 (1.1)	210 (1.4)	55 (3.9)	235 (3.3)

Data presented as number of cases and percentage. Abbreviations: CER - Creatinine Excretion Rate.

The association between muscle mass and type 2 diabetes can be reciprocal.[22] First, considering the association of decreasing muscle mass and type 2 diabetes, a decrease in muscle mass can induce hyperglycemia, through altering glucose availability, as skeletal muscle is the primary site for postprandial glucose uptake and metabolism. Muscle resistance to insulin mediated glucose uptake is an important contributor to type 2 diabetes.[23,24] Second, type 2 diabetes can increase the loss of muscle mass in time.[25] Loss of muscle mass (and strength) in hyperglycaemic states likely occurs due to increased protein degradation and decreased protein synthesis.[24] Furthermore, accumulation of advanced glycation end products in muscle and increased presence of inflammatory cytokines can increase loss of muscle mass.[26,27] Finally, low muscle mass can enhance insulin resistance, which leads to a vicious cycle of muscle loss and insulin resistance.[28]

Our results demonstrate that low muscle mass is indeed associated with diabetes, yet interestingly this association loses strength with increasing BMI classes. This might be due to the fact that obesity (i.e. excess adipose mass) itself is one of the strongest risk factors for diabetes.[29] Hypertrophy of the adipocytes is a hallmark of adipose tissue expansion in obesity. This hypertrophic adipose tissue is known to correlate with pathologic vascularization, hypoxia, fibrosis and macrophage-mediated inflammation. However, people with obesity are in general quite muscular and prevalence of low muscle mass in our study was much lower compared than people with normal weight, suggesting that the quantitative effect of low muscle mass in people with obesity is much smaller than in the normal BMI class. Vice versa, the pathogenic effect of excess adipose tissue in the normal

weight individuals is lower than in those with obesity, potentially providing a more important role for low muscle mass as a contributing factor for prevalent and incident type 2 diabetes in people with normal weight.

The strengths of this study include a large sample size together with the longitudinal design in the general population of the northern Netherlands. Furthermore, the use of the cut-off values for CER is easy to use and can be directly implemented (men: 13.04; women: 8.57 mmol/24h). Using loss of muscle mass in time instead of just baseline measures in relation to incidence provides a more realistic picture of natural behavior over time. Additionally, the use of BMI subgroups is reinforced by the outcome of the sensitivity analysis, in which we found that BMI is indeed an effect modifier in the association between muscle mass and diabetes. This study is not without its limitations. First, due to the nature of the cohort in this study, there are only data available from two time points (1st and 2nd assessment). The exact time of the diagnosis of type 2 diabetes was not reported, rendering survival analysis impossible. However, considering the low event rate compared with the short follow-up, logistic regression should provide similar estimates. Second, diabetes is self-reported by participants of the Lifelines cohort and therefore does not comply with the American Diabetes Association (ADA) criteria. Self-reporting could lead to misclassification and therefore, a larger measurement error and some underestimations of associations. The prevalence in the Lifelines cohort (3%) is lower than the general Dutch population from general practitioner registries (5.8%).[30] This could be due to selection bias towards healthy individuals participating in this study. Additionally our population only includes people up to an age of 65 years, whereas the Dutch diabetes prevalence steeply increases after the age of 65.[30] When age is taken into account in the data from GP registries it is likely that the prevalence therefore will approach 3%. Third, using 24-hour urinary CER to measure muscle mass can also have some limitations. Although there was a strict protocol for the collection of 24-hour urine collection, there could be some collection errors.

In conclusion, low muscle mass, as measured by CER, is associated with the prevalence of diabetes and loss of muscle mass is associated with incidence of diabetes in the general population. This association is strongest in people with normal weight, and weakens in people in higher BMI categories.

REFERENCES

1. Benjamin EJ, Blaha MJ, Chiuve SE, et al. Heart Disease and Stroke Statistics-2017 Update: A Report From the American Heart Association. *Circulation*. 2017;135(10):e146-e603. doi:10.1161/CIR.0000000000000485
2. NCD Risk Factor Collaboration (NCD-RisC). Worldwide trends in body-mass index, underweight, overweight, and obesity from 1975 to 2016: a pooled analysis of 2416 population-based measurement studies in 128·9 million children, adolescents, and adults. *Lancet*. 2017;390(10113):2627-2642. doi:10.1016/S0140-6736(17)32129-3
3. CDC. Obesity is a Common, Serious, and Costly Disease. Centers for Disease Control and Prevention. Published November 12, 2021. Accessed February 21, 2022. https://www.cdc.gov/obesity/data/adult.html
4. Gallagher D, Visser M, Sepúlveda D, Pierson RN, Harris T, Heymsfield SB. How useful is body mass index for comparison of body fatness across age, sex, and ethnic groups? *Am J Epidemiol*. 1996;143(3):228-239. doi:10.1093/oxfordjournals.aje.a008733
5. Obesity: preventing and managing the global epidemic. Report of a WHO consultation. *World Health Organ Tech Rep Ser*. 2000;894:i-xii, 1-253.
6. Weisman A, Fazli GS, Johns A, Booth GL. Evolving Trends in the Epidemiology, Risk Factors, and Prevention of Type 2 Diabetes: A Review. *Can J Cardiol*. 2018;34(5):552-564. doi:10.1016/j.cjca.2018.03.002
7. Galicia-Garcia U, Benito-Vicente A, Jebari S, et al. Pathophysiology of Type 2 Diabetes Mellitus. *Int J Mol Sci*. 2020;21(17):E6275. doi:10.3390/ijms21176275
8. DeFronzo RA, Jacot E, Jequier E, Maeder E, Wahren J, Felber JP. The Effect of Insulin on the Disposal of Intravenous Glucose: Results from Indirect Calorimetry and Hepatic and Femoral Venous Catheterization. *Diabetes*. 1981;30(12):1000-1007. doi:10.2337/diab.30.12.1000
9. Izzo A, Massimino E, Riccardi G, Della Pepa G. A Narrative Review on Sarcopenia in Type 2 Diabetes Mellitus: Prevalence and Associated Factors. *Nutrients*. 2021;13(1):183. doi:10.3390/nu13010183
10. Heymsfield SB, Olafson RP, Kutner MH, Nixon DW. A radiographic method of quantifying protein-calorie undernutrition. *Am J Clin Nutr*. 1979;32(3):693-702. doi:10.1093/ajcn/32.3.693
11. Oterdoom LH, Gansevoort RT, Schouten JP, de Jong PE, Gans ROB, Bakker SJL. Urinary creatinine excretion, an indirect measure of muscle mass, is an independent predictor of cardiovascular disease and mortality in the general population. *Atherosclerosis*. 2009;207(2):534-540. doi:10.1016/j.atherosclerosis.2009.05.010
12. Proctor DN, O'Brien PC, Atkinson EJ, Nair KS. Comparison of techniques to estimate total body skeletal muscle mass in people of different age groups. *American Journal of Physiology-Endocrinology and Metabolism*. 1999;277(3):E489-E495. doi:10.1152/ajpendo.1999.277.3.E489
13. Welle S, Thornton C, Totterman S, Forbes G. Utility of creatinine excretion in body-composition studies of healthy men and women older than 60 y. *The American Journal of Clinical Nutrition*. 1996;63(2):151-156. doi:10.1093/ajcn/63.2.151

14. Scholtens S, Smidt N, Swertz MA, et al. Cohort Profile: LifeLines, a three-generation cohort study and biobank. *Int J Epidemiol*. 2015;44(4):1172-1180. doi:10.1093/ije/dyu229
15. Sijtsma A, Rienks J, van der Harst P, Navis G, Rosmalen JGM, Dotinga A. Cohort Profile Update: Lifelines, a three-generation cohort study and biobank. *Int J Epidemiol*. Published online December 13, 2021:dyab257. doi:10.1093/ije/dyab257
16. Wendel-Vos GCW, Schuit AJ, Saris WHM, Kromhout D. Reproducibility and relative validity of the short questionnaire to assess health-enhancing physical activity. *Journal of Clinical Epidemiology*. 2003;56(12):1163-1169. doi:10.1016/S0895-4356(03)00220-8
17. Streppel MT, de Vries JHM, Meijboom S, et al. Relative validity of the food frequency questionnaire used to assess dietary intake in the Leiden Longevity Study. *Nutr J*. 2013;12:75. doi:10.1186/1475-2891-12-75
18. Diabetes mellitus in Nederland. Prevalentie en incidentie: heden, verleden en toekomst. | Nivel. Accessed March 31, 2022. https://www.nivel.nl/nl/publicatie/diabetes-mellitus-nederland-prevalentie-en-incidentie-heden-verleden-en-toekomst
19. Kim TN, Park MS, Yang SJ, et al. Prevalence and determinant factors of sarcopenia in patients with type 2 diabetes: the Korean Sarcopenic Obesity Study (KSOS). *Diabetes Care*. 2010;33(7):1497-1499. doi:10.2337/dc09-2310
20. Anagnostis P, Gkekas NK, Achilla C, et al. Type 2 Diabetes Mellitus is Associated with Increased Risk of Sarcopenia: A Systematic Review and Meta-analysis. *Calcif Tissue Int*. 2020;107(5):453-463. doi:10.1007/s00223-020-00742-y
21. Maliszewska K, Adamska-Patruno E, Goscik J, et al. The Role of Muscle Decline in Type 2 Diabetes Development: A 5-Year Prospective Observational Cohort Study. *Nutrients*. 2019;11(4):834. doi:10.3390/nu11040834
22. Hesselink MKC, Schrauwen-Hinderling V, Schrauwen P. Skeletal muscle mitochondria as a target to prevent or treat type 2 diabetes mellitus. *Nat Rev Endocrinol*. 2016;12(11):633-645. doi:10.1038/nrendo.2016.104
23. Shu J, Matarese A, Santulli G. Diabetes, body fat, skeletal muscle, and hypertension: The ominous chiasmus? *J Clin Hypertens (Greenwich)*. 2018;21(2):239-242. doi:10.1111/jch.13453
24. Mesinovic J, Zengin A, De Courten B, Ebeling PR, Scott D. Sarcopenia and type 2 diabetes mellitus: a bidirectional relationship. *Diabetes Metab Syndr Obes*. 2019;12:1057-1072. doi:10.2147/DMSO.S186600
25. Park SW, Goodpaster BH, Lee JS, et al. Excessive loss of skeletal muscle mass in older adults with type 2 diabetes. *Diabetes Care*. 2009;32(11):1993-1997. doi:10.2337/dc09-0264
26. Pereira S, Marliss EB, Morais JA, Chevalier S, Gougeon R. Insulin Resistance of Protein Metabolism in Type 2 Diabetes. *Diabetes*. 2008;57(1):56-63. doi:10.2337/db07-0887
27. Tabara Y, Ikezoe T, Yamanaka M, et al. Advanced Glycation End Product Accumulation Is Associated With Low Skeletal Muscle Mass, Weak Muscle Strength, and Reduced Bone Density: The Nagahama Study. *The Journals of Gerontology: Series A*. 2019;74(9):1446-1453. doi:10.1093/gerona/gly233

28. Al-Ozairi E, Alsaeed D, Alroudhan D, et al. Skeletal Muscle and Metabolic Health: How Do We Increase Muscle Mass and Function in People with Type 2 Diabetes? *The Journal of Clinical Endocrinology & Metabolism*. 2021;106(2):309-317. doi:10.1210/clinem/dgaa835
29. Bellou V, Belbasis L, Tzoulaki I, Evangelou E. Risk factors for type 2 diabetes mellitus: An exposure-wide umbrella review of meta-analyses. *PLoS One*. 2018;13(3):e0194127. doi:10.1371/journal.pone.0194127
30. Diabetes mellitus | Volksgezondheid en Zorg. Accessed November 23, 2022. https://www.vzinfo.nl/diabetes-mellitus#node-aantal-nieuwe-gevallen-van-diabetes