Adult weight change and cardiometabolic disease: studies into underlying pathways

Verkouter, I.

CHAPTER 5

Adult weight change in relation to visceral fat and liver fat at middle age: The Netherlands Epidemiology of Obesity study

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ABSTRACT

Objective: We aimed to investigate the associations between weight change during adulthood and the amount of abdominal subcutaneous fat, visceral fat and liver fat at middle age.

Methods: The Netherlands Epidemiology of Obesity (NEO) study is a population-based cohort of 6 671 middle-aged men and women. We calculated the percentage of weight change during adulthood based on body weight at middle age and recalled body weight at age 20. Abdominal subcutaneous and visceral adipose tissue were assessed by magnetic resonance imaging (MRI), in addition to hepatic triglyceride content by ^{1}H-MR spectroscopy in a random subgroup (maximum of n=2 580). With multivariable linear regression analysis, we examined the associations between categories of adult weight change, body mass index (BMI) at age 20 and measures of abdominal adiposity at middle age, adjusted for age, sex, ethnicity, lifestyle factors, menopausal status, parity, use of medication and total body fat at middle age.

Results: In 2 399 participants (54% women), individuals who gained more than 50% of body weight during adulthood had 1.96 (95% CI: 1.64; 2.33) times more visceral adipose tissue at middle age and 2.39 (95% CI: 1.70, 3.36) times more hepatic triglyceride content than weight maintainers (weight change between -5% and 5%). Associations with abdominal subcutaneous adipose tissue were weaker: participants who gained more than 50% of their body weight had 1.54 (95% CI: 1.38, 1.72) times more abdominal subcutaneous adipose tissue compared with weight maintainers.

Conclusions: In this population-based study, adult weight gain was associated with relatively more visceral adipose tissue and hepatic triglyceride content at middle age than abdominal subcutaneous adipose tissue. Overall, our study suggests that weight maintenance during adulthood plays an important role in limiting excess visceral adipose tissue and hepatic triglyceride content at middle age.

INTRODUCTION

Obesity is a well-established risk factor for development of cardiometabolic diseases such as type 2 diabetes and coronary heart disease (1). Adiposity in early adulthood and weight gain during adulthood have both been associated with a considerable increased risk of major chronic diseases in middle-aged individuals, including type 2 diabetes, cardiovascular disease and obesity-related cancers (2-6). Alternatively, individuals who maintained a body mass index of 18.5 to 25.0 kg/m^2 during adulthood had the lowest risk of these chronic diseases, and all-cause mortality (2, 7).

During adult weight gain, excess adipose tissue is stored in different areas of the body, depending on various factors including genetic variation, sex, age and lifestyle (8-12). Abdominal adiposity is characterized by an increased storage of excess fat in the abdominal subcutaneous and visceral adipose tissue depots. Visceral adipose tissue has a high secretion rate of cytokines such as TNF-α and IL-6, promoting local inflammation and oxidative stress (13). In addition, as a result of the hyperlipolytic state of visceral adipose tissue, non-esterified fatty acids are released into the circulation, subsequently leading to metabolic abnormalities in the liver and increased hepatic glucose production (14-17). These mechanisms contribute to an overflow of lipids that cannot be stored in the subcutaneous adipose tissue, eventually resulting in accumulation of fat in and around the organs, including the visceral area, liver, skeletal muscles, heart and pancreas (18). Previous studies have shown that both visceral adipose tissue and liver fat accumulation are better predictors of the metabolic syndrome than abdominal subcutaneous adipose tissue (19, 20). Additionally, excess visceral adipose tissue and liver fat are important risk factors for type 2 diabetes and cardiovascular disease (19, 21-26).

To date, the importance of adult weight gain in the accumulation of visceral fat and liver fat has not been well described. Only few studies investigated the depots in which body fat is preferentially stored during adult weight gain. A four-year follow-up study in normal-weight premenopausal women (N=65) showed that a gain in body weight was associated with excess visceral adipose tissue after four years (27). In contrast, it was observed that an increase in body fat induced by short-term weight gain during 100 days of overfeeding was not accompanied by accumulation of visceral adipose tissue in a cohort study in men (N=24) (28). Additionally, self-reported adult weight gain was associated with fat deposition in the liver in a large Asian study population (N=21 496) (29).

Because both adult weight gain and excess visceral adipose tissue are strongly associated with insulin resistance and type 2 diabetes (1-6, 21, 22, 30, 31), we hypothesized that individuals with the largest weight gain during adulthood have more visceral adipose tissue and a higher hepatic triglyceride content at middle age. Therefore, the aim of this study was to investigate the associations of adult weight change with visceral adipose tissue and hepatic triglyceride content, irrespective of total body fat at middle age, in a population-based study.

METHODS

Study design and population

The Netherlands Epidemiology of Obesity (NEO) study is a population-based, prospective cohort study in 6 671 individuals aged 45–65 years, with an oversampling of individuals with a BMI ≥27 kg/m^2. The study design and population are described in detail elsewhere (32).

Men and women living in the greater area of Leiden (Western Netherlands) were invited by letters sent by GPs and municipalities and by local advertisements. They were invited to respond if they were aged between 45 and 65 years and had a self-reported BMI of 27 kg/m^2 or higher. In addition, all inhabitants aged between 45 and 65 years from one municipality (Leiderdorp) were invited to participate irrespective of their BMI, allowing for a reference distribution of BMI.

Participants were invited to a baseline visit at the NEO study centre Leiden University Medical Center after an overnight fast. Prior to this study visit, participants completed a general questionnaire at home to report demographic, lifestyle and clinical information. The participants were asked to bring all medication they were using in the month preceding the study visit, which was recorded by research nurses. At the study centre, participants completed a screening form, asking about anything that might create a health risk or interfere with magnetic resonance imaging (most notably metallic devices, claustrophobia or a body circumference of more than 1.70 meter). Of the participants who were eligible for magnetic resonance imaging (MRI), approximately 35% were randomly selected to undergo MRI.

For the present analysis, we included participants who underwent MRI of the abdomen (n= 2 580), in addition to ^{1}H-magnetic resonance spectroscopy of hepatic triglyceride content. We excluded participants with images of insufficient quality to estimate abdominal subcutaneous or visceral adipose tissue (n=11), with missing recalled weight at age 20 (n=79) or with a BMI at age 20 below 14.0 kg/m^2 (n=2). Additionally, we excluded participants with missing data on total body fat (n=4), ethnicity (n=3), educational level (n=25), smoking (n=2) and physical activity (n=55), resulting in 2 399 participants who were included in the present analysis. Hepatic triglyceride content was available in 1 948 of these, due to technical failures and an insufficient quality of the measurements to estimate liver fat content. The Medical Ethical Committee of the Leiden University Medical Center approved the design of the study. All participants gave their written informed consent.

Data collection

Weight change during adulthood

Recalled body weight at age 20 was based on self-report. The general questionnaire included the question 'How much did you weigh (approximately) when you were 20 years old?' BMI at age 20 was calculated by dividing body weight at age 20 in kilograms by the measured height in meters squared at middle age, with the assumption that height did not majorly change during adulthood. Relative weight change was calculated by subtracting body weight at age 20 from measured body weight at middle age, divided by body weight at age 20, multiplied by 100%.

Abdominal adiposity and liver fat at middle age
Height without shoes was measured with a vertically fixed, calibrated tape measure with precision of 0.1 cm. Body weight and percent body fat were measured by the Tanita bio impedance balance (TBF-310, Tanita International Division, UK) without shoes and 1 kg was subtracted to correct for the weight of clothing. BMI at baseline was calculated by dividing the weight in kilograms by the height in meters squared. Waist circumference was measured between the border of the lower costal margin and the iliac crest with the precision of 0.1 cm.

Abdominal subcutaneous and visceral fat depots were directly assessed by MRI (1.5 Tesla MR imaging, Philips Medical Systems, Best, Netherlands) using a turbo spin echo imaging protocol (300/20; flip angle, 90°; section thickness, 10 mm; section gap, 2mm). At the level of the fifth lumbar vertebra, three transverse 10 mm slices were obtained during one breath-hold. By using in-house-developed software (MASS; Leiden University Medical Center, Leiden, the Netherlands), abdominal subcutaneous and visceral fat areas were quantified by converting the number of pixels to centimetres squared for all three slices, allowing a semi-automated detection of the subcutaneous and visceral adipose tissue area. The mean of abdominal subcutaneous and visceral adipose tissue areas was used in the analysis. Hepatic triglyceride content was quantified using ^{1}H-magnetic resonance spectroscopy of the liver. An 8 ml voxel was positioned in the right lobe of the liver, avoiding gross vascular structures and adipose tissue depots. Sixty-four averages were collected with water suppression (repetition time = 2900 msec; echo time = 23 msec [2900/23]). Without changing any parameters, spectra without water suppression, with a repetition time of 10 seconds and with four averages were obtained as an internal reference. Spectra were not corrected for frequency drift and were analysed while blinded to all study parameters. Spectra were initially included when automatic fitting was successful. When line shapes were distorted by eddy currents or as a result of poor shimming, spectral data were rejected. Hepatic triglyceride content relative to water was calculated as (signal amplitude of triglyceride) / (signal amplitude of water) * 100. Fatty liver was defined as a hepatic triglyceride content of ≥5.56% (33).

Covariates
On the baseline questionnaire, participants reported ethnicity by self-identification in eight categories which we grouped into white (reference) and other. Level of education was grouped into high versus low education (reference) according to the Dutch education system. Tobacco smoking was reported in three categories: current smoker, former smoker and never smoker. Alcohol consumption was reported in the Food Frequency Questionnaire (FFQ) and expressed as grams of alcohol consumed per day. Participants reported the frequency and duration of their physical activity during leisure time on the Short Questionnaire to Assess Health-enhancing activity (SQUASH), which we expressed in MET-hours per week. Use of antidepressants, antipsychotics, thyroid medication, corticosteroids and/or hormonal treatments in the month preceding the study visit was recorded by research nurses. In women, we grouped the use of contraceptives and hormone replacement therapy into current, past and never (reference) users of oestrogens. Menopausal status was classified as premenopausal, perimenopausal (menopausal during last year) or postmenopausal, according to information on oophorectomy, hysterectomy and self-reported state of menopause in the questionnaire.

Statistical analyses
In the NEO study, individuals with a BMI of 27 kg/m^2 or higher are oversampled. To correctly represent associations for the general population (34), adjustments for this oversampling were made. Adjustment was done by weighting all participants towards the BMI distribution of participants from the Leiderdorp municipality (35), whose BMI distribution was similar to the BMI distribution of the general Dutch population (36). All results were based on weighted analyses. Consequently, the results apply to a population-based study without oversampling of individuals with a BMI≥27 kg/m^2. As a result of the weighting procedure, the numbers of participants per group are presented as proportions.

Baseline characteristics of the study population were expressed as mean (SD), median (25th, 75th percentiles) or as percentage, stratified by categories of weight change. We categorized weight change during adulthood on the basis of the distribution of weight change in the reference population of Leiderdorp: weight change of more than -5%, between -5% and 5% (weight maintenance: reference category), 5% to 25%, 25% to 50% and ≥50%. The majority of participants fell into the weight gain categories. However, according to the distribution of weight change in the Leiderdorp population, who had a BMI distribution similar to the Dutch general population, this is a typical representation of weight change in the Dutch general population (**Figure S1**).

We performed linear regression analyses to examine the associations of adult weight change with waist circumference, abdominal subcutaneous and visceral adipose tissue, and hepatic triglyceride content at middle age, compared with the reference category of weight maintenance during adulthood. Crude models were adjusted for sex and age (model 1). In model 2, we additionally adjusted for BMI at age 20, because the percentage of weight change since age 20 depends on the initial BMI at age 20. Model 3 was additionally adjusted for ethnicity, education, smoking, alcohol consumption, physical activity, menopausal status, use of medication known to affect body weight (antidepressants, antipsychotics, thyroid medication, corticosteroids and hormonal treatments) and parity. Finally, in model 4, we additionally adjusted for total body fat at middle age to investigate to what extent weight change during adulthood was specifically associated with measures of abdominal adiposity instead of merely overall adiposity. Because of the skewed distribution of hepatic triglyceride content and to facilitate interpretation, values of waist circumference, abdominal subcutaneous and visceral adipose tissue and hepatic triglyceride content were all transformed using the natural logarithm. Regression coefficients and corresponding 95% confidence intervals (CI) were back transformed and expressed as ratios, which can be interpreted as relative changes in measure of abdominal adiposity, compared with that measure in the reference category of weight maintenance during adulthood. For example: a ratio of 2 for visceral adipose tissue in individuals who gained 5% to 25% of body weight during adulthood indicates that these individuals have twofold more visceral adipose tissue at middle age than individuals who maintained their body weight during adulthood. Because men and women have different patterns of body fat distribution (8-12), we repeated the analyses stratified by sex.

For the next analyses, we stratified the study population based on BMI at age 20, according to WHO criteria: <18.5 kg/m^2 (underweight), 18.5-25.0 kg/m^2 (normal range, reference), 25.0-30.0 kg/m^2 (overweight) and ≥30.0 kg/m^2 (obese) (37). We used linear regression mo-

dels to examine the associations between BMI at age 20 strata and waist circumference, abdominal subcutaneous and visceral adipose tissue and hepatic triglyceride content at middle age, compared with the reference category. In model 1, we adjusted for sex and age. In model 2, we additionally adjusted for ethnicity, education, smoking, alcohol consumption, physical activity, menopausal status, use of medication known to affect body weight (antidepressants, antipsychotics, thyroid medication, corticosteroids and hormonal treatments) and parity. Finally, in model 3, we adjusted for total body fat at middle age.

Subsequently, we performed joint analysis of the associations between weight change during adulthood and measures of abdominal adiposity within the BMI at age 20 strata, using participants with BMI at age 20 strata between 18.5 and 25.0 kg/m^2 and with weight change ≥-5% to <5% as a reference. The analysis was adjusted for sex, age, ethnicity, education, smoking, alcohol consumption, physical activity, menopausal status, use of medication known to affect body weight (antidepressants, antipsychotics, thyroid medication, corticosteroids and hormonal treatments), parity and total body fat at middle age.

We repeated all analyses after excluding participants who reported weight loss, while creating a new reference category of 0-5% relative weight change. We performed a sensitivity analysis to correct for potential measurement error of adult weight gain, in which the regression analysis was corrected for the correlation between recalled past weight and measured past weight reported in a previous study (r=0.87) (42). Additionally, we repeated all analyses after exclusion of participants who reported alcohol consumption of more than 4 units per day.

Analyses were performed with STATA Statistical Software version 12.1 (Statacorp, College Station, Texas, USA). Figures were constructed with GraphPad Prism version 7.02 (GraphPad Software Inc, La Jolla California, USA)

RESULTS

Characteristics of the study population

A total of 2 399 participants (54% women) were analysed in the present study, of whom 1948 had measurements of hepatic triglyceride content. Mean (SD) age of the study population was 56(6) years, mean BMI was 25.9(4.0) kg/m^2, and mean percentage of adult weight gain was 19.5(16.5) %. Characteristics of the study population stratified by the five weight change categories are presented in **Table 1**.

More women than men gained more than 25% of body weight during adulthood than participants who gained less than 25% of body weight or remained at a stable body weight. In addition, participants who gained more than 25% of body weight more often had a low education. Waist circumference, abdominal subcutaneous and visceral adipose tissue and hepatic triglyceride content were higher in participants who gained more than 25% of body weight than in the categories of less than 25% weight gain.

Table 1. Characteristics of participants of the Netherlands Epidemiology of Obesity (NEO) study with measurements of abdominal subcutaneous and visceral adipose tissue by magnetic resonance imaging, stratified by adult weight change (N=2 399)

	Weight change categories				
	<-5%	**≥-5% to <5%**	**≥5 to <25%**	**≥25% to <50%**	**≥50%**
Proportion of population (%)	4.5	11.0	54.1	25.4	5.0
Sex (% men)	17	18	51	48	36
Age (years)	54 (4)	57 (4)	55 (5)	56 (7)	56 (8)
Ethnicity (% white)	100	95	96	96	91
Education (% high)	48	50	51	38	34
Weight at age 20					
Recalled weight at age 20 (kg)	71.3 (8.6)	67.3 (7.6)	66.5 (9.3)	64.5 (14.1)	58.8 (14.7)
BMI at age 20 (kg/m^2)	24.6 (2.2)	22.8 (1.9)	21.8 (1.9)	21.3 (3.4)	20.0 (4.0)
Change in weight (%)	-7.6 (-32.2; -5.2)	1.7 (-5.0; 4.8)	14.8 (5; 24.9)	32.6 (25; 49.8)	58.8 (50; 130.4)
Smoking (% current)	18	18	14	14	11
Alcohol consumption (g/day)	7 (1-21)	10 (2-15)	11 (3-22)	8 (2-22)	7 (1-21)
Physical activity (MET-h/week)	27 (11-58)	38 (25-56)	30 (16-53)	27 (15-45)	24 (11-41)
Use of medication[1] (% yes)	13	8	17	10	19
In women:					
Postmenopausal (% yes)	41	80	51	65	69
Use of sex hormones (% current)	11	2	11	8	7
Number of liveborn children	2 (0-2)	2 (1-2)	2 (2-3)	2 (2-3)	2 (1-3)
Body weight (kg)	64.0 (6.9)	67.9 (7.9)	76.4 (11.1)	86.5 (18.6)	95.0 (23.6)
BMI (kg/m^2)	22.1 (1.6)	23.0 (2.0)	25.0 (2.4)	28.5 (4.7)	32.3 (6.7)
Waist circumference (cm, M/W)	90 (6)/76 (6)	88 (6)/77 (6)	96 (7)/ 83 (7)	104 (12)/94 (14)	111(15)/104(17)
Total body fat (%, M/W)	18 (3)/30 (4)	20 (3)/31 (4)	24(4) / 35 (4)	28(7) / 41 (6)	32(9) / 45 (9)

	Weight change categories				
	<-5%	**≥-5% to <5%**	**≥5 to <25%**	**≥25% to <50%**	**≥50%**
Abdominal subcutaneous adipose tissue (cm^2, M/W)	125 (125-159) /165 (117-223)	140 (99-182)/177 (130-226)	187(154- 222) /230 (184-281)	241(199-298)/309 (258-376)	300(254-273)/ 403 (331-485)
Visceral adipose tissue (cm^2, M/W)	50 (44-66)/21 (14-37)	50 (32-79)/36 (24-53)	97 (75-131)/51 (36-72)	135 (103-178)/89 (63-114)	170 (137-132)/125 (94-159)
Hepatic triglyceride content[2] (%, M/W)	2.2 (0.9-2.6)/0.9(0.7-1.6)	1.8 (1.0-3.8)/1.2 (0.8-1.8)	3.5 (1.9-7.1)/1.6 (1.1-3.6)	6.0 (3.5-14.1)/3.4 (1.6-8.4)	11.1 (3.8-20.3)/8.1 (3.7-19.0)
Fatty liver[2] (%, HTGC>5.56%)	6	8	25	44	66

[1]Use of medication includes thyroid hormone, antithyroid preparations, antipsychotic and antidepressant use and systemic corticosteroids. [2]n=1 948. Abbreviations: BMI, body mass index; MET, metabolic equivalent of task; M, men; W, women. Data are presented as mean (SD), median (25^{th}-75^{th} percentile/range) or percentage. Results were based on analyses weighted towards the BMI distribution of the general population (N=2 399)

Adult weight gain and measures of abdominal adiposity at middle age

A gain in body weight was associated with more abdominal adiposity at middle age (**Table 2**). After adjustment for potential confounding factors including total body fat, participants who gained more than 50% of their body weight showed 1.96 (95% CI: 1.64; 2.33) times more visceral adipose tissue and 2.39 (95% CI: 1.70, 3.36) times more hepatic triglyceride content than weight maintainers. Participants who gained more than 50% of their body weight had 1.54 (95% CI: 1.38, 1.72) times more abdominal subcutaneous adipose tissue and their waist circumference was 1.18 (95% CI: 1.15, 1.22) times higher compared with the reference category. Results were similar for men and women (men: **Table S1**, women: **Table S2**). After excluding all participants who reported a loss of body weight, the results remained similar (**Table S3**). **Table S4** shows the association between adult weight change and measures of insulin resistance, uncorrected and corrected for the measurement error in recalled body weight. Results of both analyses are similar. Furthermore, results on hepatic triglyceride content remained similar when we excluded 216 participants with alcohol consumption of more than four units per day (results not shown).

Table 2. Ratios with 95% confidence intervals in measures of abdominal adiposity by categories of weight change during adulthood, compared with weight maintenance (N=2 399)

	Weight change categories								
	<-5%		≥-5% to <5%	≥5% to <25%		≥25% to <50%		≥50%	
	Ratio	95% CI	Reference	Ratio	95% CI	Ratio	95% CI	Ratio	95% CI
Waist circumference	0.99	0.95; 1.03	1	1.08	1.06; 1.10	1.20	1.18; 1.23	1.31	1.28; 1.35
Model 2	0.93	0.90; 0.96	1	1.12	1.10; 1.13	1.26	1.24; 1.28	1.42	1.40; 1.45
Model 3	0.93	0.90; 0.96	1	1.11	1.10; 1.13	1.25	1.23; 1.28	1.41	1.39; 1.44
Model 4	0.98	0.94; 1.01	1	1.05	1.04; 1.07	1.12	1.09; 1.14	1.18	1.15; 1.22
Abdominal subcutaneous adipose tissue	0.98	0.84; 1.13	1	1.34	1.23; 1.47	1.83	1.67; 2.00	2.32	2.11; 2.56
Model 2	0.82	0.73; 0.92	1	1.48	1.37; 1.60	2.10	1.95; 2.27	2.94	2.71; 3.19
Model 3	0.80	0.72; 0.90	1	1.45	1.35; 1.57	2.07	1.93; 2.22	2.88	2.66; 3.11
Model 4	0.96	0.87; 1.06	1	1.20	1.11; 1.30	1.37	1.25; 1.49	1.54	1.38; 1.72
Visceral adipose tissue	0.83	0.64; 1.06	1	1.73	1.50; 1.99	2.64	2.30; 3.03	3.60	3.11; 4.16
Model 2	0.70	0.55; 0.88	1	1.90	1.67; 2.16	3.01	2.65; 3.42	4.53	3.97; 5.18
Model 3	0.67	0.54; 0.83	1	1.86	1.65; 2.11	2.93	2.59; 3.32	4.36	3.83; 4.97
Model 4	0.85	0.68; 1.05	1	1.46	1.29; 1.65	1.72	1.50; 1.98	1.96	1.64; 2.33
Hepatic triglyceride content[1]	1.00	0.73; 1.38	1	1.70	1.42; 2.04	2.92	2.42; 3.54	5.41	4.31; 6.78
Model 2	0.81	0.59; 1.12	1	1.88	1.58; 2.25	3.33	2.77; 3.99	6.96	5.59; 8.68
Model 3	0.84	0.61; 1.15	1	1.85	1.55; 2.22	3.24	2.70; 3.89	6.69	5.38; 8.32
Model 4	1.09	0.78; 1.53	1	1.34	1.11; 1.63	1.62	1.27; 2.07	2.39	1.70; 3.36

[1]n=1 948. Results were based on analyses weighted towards the BMI distribution of the general population (N=2 399), and were derived from beta coefficients with 95% confidence intervals from linear regression analyses and expressed as ratios of outcome measures compared with weight maintenance during adulthood. Abbreviations: CI, confidence interval. Adjusted for sex and age; 2: additionally adjusted for BMI at age 20; 3: Adjusted for 2 + ethnicity, education, smoking, alcohol consumption, physical activity, menopause status, use of antidepressants, antipsychotics, thyroid, corticosteroids or hormonal use and parity; 4: Adjusted for 3 + total body fat

BMI at age 20 and measures of abdominal adiposity at middle age

We observed a higher BMI and total body fat, larger waist circumference, more abdominal subcutaneous and visceral adipose tissue and a higher hepatic triglyceride content at middle age in participants who were overweight or obese at age 20 (**Table S5**).

Compared with the reference group (18.5-25.0 kg/m^2) and adjusted for potential confounding factors, a higher BMI at age 20 was associated with a relatively higher waist circumference, abdominal subcutaneous and visceral adipose tissue and hepatic triglyceride content (**Table 3**). However, after additional adjustment for total body fat at middle age, we observed that a higher BMI at age 20 was associated with a relatively lower visceral adipose tissue and lower hepatic triglyceride content at middle age compared with the reference category. Obesity (BMI ≥30 kg/m^2) at age 20 was associated with 0.78 (95% CI: 0.67, 0.92) times less visceral adipose tissue and 0.60 (95% CI: 0.39, 0.93) times less hepatic triglyceride content at middle age. BMI >30.0 kg/m^2 at age 20 remained associated with a relatively higher waist circumference (1.05, 95% CI: 1.03, 1.07) and abdominal subcutaneous adipose tissue (1.11, 95% CI: 1.03, 1.20) at middle age. The results on hepatic triglyceride content remained similar after additional exclusion of 216 participants who consumed more than four units of alcohol per day (results not shown).

Table 3. Ratios with 95% confidence intervals in measures of abdominal adiposity by categories of BMI at age 20, compared with BMI 18.5 – 25.0 kg/m^2 at age 20 (N=2 399)

	BMI at age 20 categories (kg/m^2)						
	<18.5		**18.5 – 24.9**	**25.0 – 29.9**		**≥30.0**	
Proportion	8.1%		82.3%	8.7%		0.9%	
	Ratio	**95% CI**	**Reference**	**Ratio**	**95% CI**	**Ratio**	**95% CI**
Waist circumference	0.96	0.94; 0.98	1	1.11	1.09; 1.14	1.23	1.16; 1.30
Model 2	0.96	0.94; 0.98	1	1.11	1.08; 1.13	1.20	1.14; 1.27
Model 3	0.99	0.97; 1.00	1	1.02	1.01; 1.04	1.05	1.03; 1.07
Abdominal subcutaneous adipose tissue	0.92	0.85; 0.99	1	1.39	1.30; 1.49	1.81	1.50; 2.19
Model 2	0.91	0.84; 0.98	1	1.37	1.29; 1.47	1.71	1.42; 2.06
Model 3	1.00	0.95; 1.05	1	1.07	1.03; 1.10	1.11	1.03; 1.20
Visceral adipose tissue	1.02	0.91; 1.15	1	1.29	1.16; 1.43	1.49	1.09; 2.05
Model 2	1.00	0.90; 1.11	1	1.26	1.14; 1.40	1.40	1.04; 1.88
Model 3	1.14	1.03; 1.25	1	0.90	0.85; 0.96	0.78	0.67; 0.92
Hepatic triglyceride content[1]	0.98	0.73; 1.31	1	1.35	1.12; 1.63	1.26	0.66; 2.39
Model 2	0.95	0.72; 1.25	1	1.33	1.11; 1.59	1.19	0.65; 2.18
Model 3	1.10	0.86; 1.42	1	0.90	0.75; 1.07	0.60	0.39; 0.93

[1]n=1 948. Results were based on analyses weighted towards the BMI distribution of the general population (N=2 399), and were derived from beta coefficients with 95% confidence intervals from linear regression analyses and expressed as ratios of outcome measures compared with BMI 18.5 – 25.0 kg/m^2 at age 20. Abbreviations: BMI, body mass index; CI, confidence interval. Adjusted for sex and age; 2: additionally adjusted for ethnicity, education, smoking, alcohol consumption, physical activity, menopause status, use of medication (antidepressants, antipsychotics, thyroid, corticosteroids, hormonal use) and parity; 3: Adjusted for 2 + total body fat.

Relative contributions of weight change and BMI at age 20

Within each category of BMI at age 20, we observed that a higher gain in body weight was associated with a relatively higher waist circumference, abdominal subcutaneous and visceral adipose tissue and hepatic triglyceride content, compared with the reference category (≥-5% - <5% weight change and BMI 18.5-25.0 kg/m^2 at age 20, **Figure 1**). The strongest associations were observed for visceral adipose tissue, for individuals with BMI at age 20 of 25-30 kg/m^2 who gained more than 50% of weight (2.17, 95% CI: 1.51, 3.13) and individuals with BMI at age 20 of more than 30 kg/m^2 who gained 25-50% of weight (1.98, 95% CI: 1.41, 2.80). In addition, strong associations were observed for hepatic triglyceride content for individuals with a BMI at age 20 of 25-30 kg/m^2 who gained more than 50% of weight (3.11, 95% CI: 1.57, 6.18).

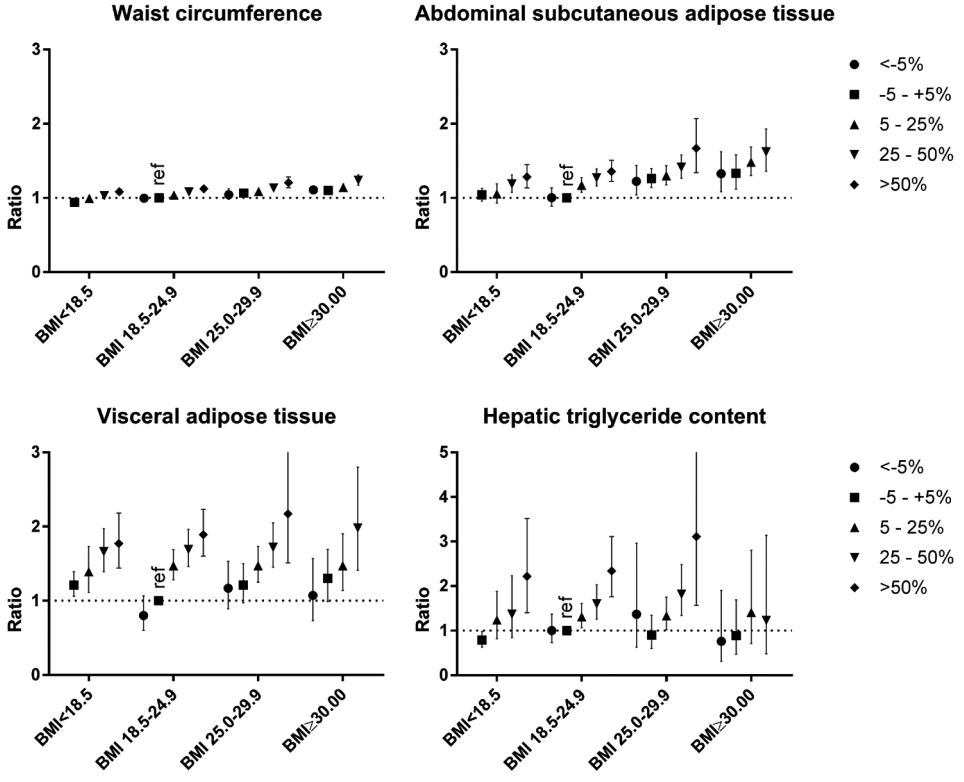

Figure 1. Weight gain is associated with a relatively higher amount of abdominal adiposity within each BMI category at age 20. Results were based on analyses weighted towards the BMI distribution of the general population (N=2 399, HTGC; n=1 848), and were derived from beta coefficients with 95% confidence intervals from linear regression analyses and expressed as ratios of outcome measures compared with weight maintenance during adulthood. Linear regression models were adjusted for sex, age, ethnicity, smoking, alcohol consumption, physical activity, menopause status, use of medication (antidepressants, antipsychotics, thyroid, corticosteroids, hormonal use), parity and total body fat.

DISCUSSION

The aim of our study was to investigate the association between adult weight change and measures of abdominal adiposity (waist circumference, abdominal subcutaneous adipose tissue and visceral adipose tissue) and hepatic triglyceride content at middle age, irrespective of total body fat. In this study, we consistently observed that weight gain was associated with a relatively higher amount of abdominal adiposity within each BMI category at age 20, compared with BMI 18.5-25.0 kg/m^2 at age 20 and weight maintenance during adulthood as a reference. Adult weight gain was more strongly associated with a relatively higher amount of visceral adipose tissue and hepatic triglyceride content than of waist circumference and abdominal subcutaneous adipose tissue. This result underlines the importance of measuring visceral adipose tissue and hepatic triglyceride content, since measuring waist circumference alone seems to underestimate the consequences on abdominal adiposity after adult weight gain. Additionally, our results suggest that adult weight gain is associated with relatively more storage of excess fat in the visceral area and the liver, compared with storage in the subcutaneous adipose tissue depot. In contrast, after adjustment for total body fat at middle age, we observed that overweight and obesity at age 20 were associated with relatively less visceral fat and liver fat at middle age compared with normal weight at age 20. Notwithstanding that on an absolute level all measures of abdominal adiposity at middle age were highest in those with overweight or obesity at age 20, these results may suggest that individuals who were already overweight or obese at age 20 have less visceral adipose tissue and hepatic triglyceride content relatively to their amount of total body fat at middle age compared with individuals who had a normal weight at age 20. In our study, we included a small group that lost more than 5% of body weight since age 20. However, because this group is heterogeneous, with weight loss ranging from 5% to more than 30% and it was not reported whether their weight loss was intentional or unintentional (e.g. due to wasting as a result of underlying disease), we are not able to determine the associations of intentional weight loss with measures of abdominal adiposity.

Two short-term overfeeding studies, one in normal weight, male twin pairs aged 21±2 years and the other in normal weight men and women aged 30±6 years, demonstrated that weight gain was associated with increases in both abdominal subcutaneous and visceral adipose tissue (28, 38). A prospective cohort study in 65 lean premenopausal women, aged 22 to 47 years, observed that weight gain was associated with an increased accumulation of visceral adipose tissue relative to total body fat (27), in line with the results of our study. In an Asian cohort of 21 496 participants (29), it was observed that the prevalence of non-alcoholic fatty liver disease increased proportionally with a larger weight gain since age 20. The association of weight gain with the prevalence of non-alcoholic fatty liver disease was even stronger in individuals with a normal weight, in accordance with our study where individuals who were overweight or obese at age 20 had relatively less hepatic triglyceride content at middle age.

The biological mechanism underlying these observations may be the limited capacity of subcutaneous adipose tissue to expand and store lipids. It was previously shown that the number of adipocytes remained constant in both lean and overweight individuals after age 20 (39). This indicates that during adult weight gain, the size of adipocytes is increasing in order to store excess lipids, but not adipocyte number. However, when adipocytes in the

subcutaneous adipose tissue increase in size, their ability to store lipids decreases. As a result, excess lipids will 'spill over' and will be stored in the visceral compartment or deposited at ectopic sites, such as the liver, heart, muscles and pancreas (25). Here, the excess visceral fat and ectopic fat may exert their detrimental effects by secreting cytokines and fatty acids and thereby inducing an inflammatory state (13). However, individuals who were overweight at age 20, may have higher adipocyte numbers and therefore enhanced capacity to store excess lipids subcutaneously, in agreement with our findings.

Additionally, it has been shown that metabolically abnormal, obese postmenopausal women (obesity according to their BMI and with an impaired insulin sensitivity) had twice as much visceral adipose tissue than metabolically normal, obese postmenopausal women (obesity according to their BMI, but with high insulin sensitivity) (40). Strikingly, they observed an association between early onset of obesity during adolescence and a more favourable metabolic profile, e.g. higher insulin sensitivity. The positive association between duration of obesity and variation in insulin sensitivity was reported earlier in a case-control study in 42 non-diabetic obese subjects (41).

Strengths of our study are the large population size and the availability of measures of abdominal subcutaneous and visceral adipose tissue by MRI and of hepatic triglyceride content by ^{1}H-MRS, providing more accurate measures of abdominal adiposity than waist circumference, and information on a wide range of potential confounding factors. This enabled us to investigate the specific associations of weight gain and BMI at age 20 with waist circumference, abdominal subcutaneous and visceral adipose tissue and hepatic triglyceride content after adjusting for total body fat at middle age.

This study also has some limitations that need to be considered. First, we calculated BMI at age 20 using recalled weight at age 20. Therefore, weight at age 20 might be misclassified. However, previous studies have shown that recalled weight is strongly correlated with measured weight (42, 43) and our sensitivity analyses correcting for the measurement error in recalled body weight gave similar results. Second, one of the contraindications of undergoing MRI was a waist circumference over 1.70 meter. Therefore, we might have missed a small number of severely obese individuals in our analyses. Third, because study participants were selected on having a self-reported BMI of 27 kg/m^2 or higher, weight gain patterns might be different in this overweight population. However, the distribution of weight gain in the study population was similar to that of the reference population of Leiderdorp. Finally, because our study population included predominantly white men and women, the results of our study need to be confirmed in other ethnic groups.

In conclusion, our study indicated that adult weight gain is associated with more abdominal adiposity, in particular with more visceral adipose tissue and hepatic triglyceride content, within all BMI categories at age 20. This implies that weight maintenance during adulthood plays an important role in limiting excess visceral adipose tissue and liver fat accumulation and its detrimental effects on cardiometabolic health. Future prospective studies need to investigate to what extent excess visceral adipose tissue and hepatic triglyceride content mediate the associations between adult weight gain and risk of cardiometabolic diseases.

CONFLICT OF INTEREST STATEMENT

All authors declare to have no conflict of interest.

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SUPPLEMENTARY MATERIAL

Supplementary information is available at International Journal of Obesity's website: https://www.nature.com/articles/s41366-018-0163-5#Sec17