Weight Changes in General Practice

An epidemiological study of
- weight changes in the adult general population,
- bias in processing of dietary data, and
- therapeutic weight loss in patients with type 2 diabetes

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ABBREVIATIONS

CPS-I	Cancer Prevention Study – 1
NHIS	National Health Interview Survey
INSUAP	The Insulin Resistance in General Practice study (Insulin resistens i almen praksis)
DCGP	Diabetes Care in General Practice (Danish abbreviation: DIAP)
DANHES	The Danish Health Examination Survey (Danish abbreviation: KRAM)
CVD	Cardiovascular disease
FFQ	Food frequency questionnaire
DSAM	Danish College of General Practitioners
RCT	Randomized controlled trial
Coca	The comparable categories imputation method (from article 2)
BMI	Body Mass Index: weight [kg] / height [m]^2
HbA1c	Glycated hemoglobin A1c

PREFACE

Many taboos surround the topics of weight gain and weight loss. Honestly, I never cared much about weight changes until chance brought me into this field of research, and I had no strong opinions on the matter besides what I learned in medical school. Before I started my PhD I was teaching an epistemology course to medical students. Throughout the medical history paradigms have been rising and falling; now I have seen a paradigm shake in real life - which is quite troublesome. Nonetheless, I have enjoyed my time at the Research Unit for General Practice, and I have thrived well with the values of freedom and trust that this institution is based on. The UPPs group, DSAM, Volkert, Willy and all the other colleges has contributed to making this house a wonderful playground. The general practice perspective and the weekly staff meetings at the Institute for Preventive Medicine have been invaluable for my understanding of epidemiology. All together this has been a mind-blowing experience!

I wish to thank my supervisors; Niels for always taking serious my sometimes airy ideas, Berit for always challenging the ideas and bringing them further out of control, and Jan Erik for insisting on bringing the ideas back to earth (which was well done on an often cracking and scattering Skype connection from Odense). Along with Volkert, the three of you have created an atmosphere of options and confidence - you have been a highly capable group to work with. My respect and thanks goes to Poul Erik Heldgaard who collected the superb INSUAP database, and I am truly grateful that I have been allowed to work with it.

Rasmus Køster-Rasmussen, November 2014

ARTICLES INCLUDED IN THE THESIS:

1. Article 1: Køster-Rasmussen R, Permin CA, Siersma V, Henriksen JE, Heitmann BL, Heldgaard PE, de Fine Olivarius N. Back on track – smoking cessation and weight changes over nine years in a community-based cohort study. Prev Med. 2015; 81: 320-5.
2. Article 2: Køster-Rasmussen R, Siersma V, Halldorsson TI, de Fine Olivarius N, Henriksen JE, Heitmann BL. Missing portion sizes in food frequency questionnaires – alternatives to use of standard portions. Public Health Nutrition 2015; 18 (11): 1914-21.
3. Article 3: Køster-Rasmussen R, Simonsen MK, Siersma V, Henriksen JE, Heitmann BL, de Fine Olivarius N. Intentional weight loss and longevity in overweight patients with type 2 diabetes – a population based inception cohort study. PLOS One 2016, januar. Open access: doi.org/10.1371/journal.pone.0146889

INSTRUCTIONS TO THE READER

This thesis is structured with a background, methods & materials, results, and discussion chapter. The backbone of the thesis is the three articles listed above. Besides additional results from the articles, I have added results from my analyses of dietary intake and comorbidity in relation to weight change. I have also included my preliminary results on the 'normal weight development'.

Those who intend to read the whole thesis; I suggest starting with the history of this PhD thesis on the next page and then the articles. The reader who wants to get an overview I suggest to read the summary, the first couple of sections in the background chapter, the results chapter, and the last sections of the discussion: 'Interpretation of article 3 in relation to the literature in the field', 'Implications for clinical work and research', and the Conclusion. Bon appétit.

THE HISTORY OF THIS PHD THESIS

The three articles included with this thesis may at a first glance seem rather diverse in terms of methods, populations and focus. Rooted at institutions as diverse as the Research Unit for General Practice, Institute for Preventive Medicine, and the PhD School for Molecular Metabolism, the scope of the original PhD project was nutritional epidemiology, obesity, and metabolism in the background population. The objectives were to examine if weight change over 9 years was associated with the intake of fructose or soft drinks sweetened with sugar, and to analyze whether differences in biological markers (e.g. insulin sensitivity or s-urate) and other aspects of nutrition (e.g. total energy intake) could explain these associations. However, after having worked up the nutritional data I realized that there were no such associations in the INSUAP dataset. Rather than publishing these results right away I decided to elaborate on the analysis methods and on the statistical models to be sure that my initial findings were robust.

The INSUAP food frequency questionnaire did not contain portion size questions, and from my struggle with FoodCalc (the computer program that calculates the dietary intake from the food frequency questionnaires) I learned how standard portion sizes were applied to all subjects. I considered this very crude and a possible explanation for my null findings and decided – advised by Berit – to find a better solution for determining portion sizes. This is the background for article 2.

In my work to improve the multivariable model of weight change, I noticed that smoking status – and especially a change in smoking status – was a powerful predictor of future weight and at the same time a likely confounder of the relation between dietary intake and weight change. I wondered if other researchers had adjusted for changes in smoking status during weight monitoring periods, and found that this was not the case. At that time I was also co-authoring an article with Niels and Berit about historical weights in the DCGP population and this opened my eyes for age-related weight changes. I wanted to untangle the dynamics between time, age, and changes in smoking status in order to design my dietary analyses better, but also in order to understand what happens when patients quit smoking and many gain weight. In the clinic I had experienced that weight gain related to smoking cessation was a common dilemma and the postulated magnitude of a post cessation weight gain varied considerably between information material from the National Board of Health and scientific publications. Together with my supervisors and the medical student Caroline Permin we decided to picture the dynamics of smoking status, age and weight change in a graphical model that could maybe find use as a clinical tool for health professionals and patients in better understanding the phenomenon post cessation weight gain - article 1.

At one of the weekly staff meetings at the Institute for Preventive Medicine I heard Mette Kildevæld Simonsen tell about her stay at Harvard and her findings that intentional weight loss did not improve longevity in overweight nurses. I was extremely puzzled by her findings, and did not really believe that they reflected a real causal relationship, but I liked Mette and when Niels asked me to write an invited status article with him for the Danish Medical Bulletin about the effect of lifestyle intervention on mortality in patients with hypertension, we invited Mette along as co-author (110). Intention to lose weight seemed to be a key concept, and learning about Niels diabetes study, DCGP, I realized that the data contained unique information on both intention and weight changes. Since my main subject was weight changes, my supervisors agreed that I could work with these high quality data which resulted in article 3.

Now the three years have passed and I still need to write up the articles on fructose and soft drinks.
I think the model is ready now.

BACKGROUND

INTRODUCTION

Being overweight (BMI≥25) or obese (BMI≥30) is associated with increased mortality (1). During the past 40 years the prevalence of overweight and obesity throughout the world has been increasing and obesity is now regarded as one of the main threats to public health. The causes of obesity are still poorly understood, and the prevailing strategy to handle this epidemic has been to make overweight subjects lose weight combined with attempts to prevent weight gain by diet changes and increased exercise. Both the public opinion and the prevailing medical paradigm consider weight loss healthy for overweight individuals. Scientifically this view is mainly based on the favorable effects of weight loss on intermediate outcomes like blood pressure, blood glucose and blood lipid-profile. These surrogate endpoints are used, as only a few inconclusive randomized clinical trials (RCT) have evaluated the impact of weight loss on mortality or cardiovascular morbidity (CVD) (2-4;55;56). In cohort studies weight loss is generally associated with increased mortality. This apparent paradox has been explained by confounding from wasting disease, e.g. a person with lethal cancer will lose weight and subsequently die, but the death was not caused by the weight loss. Contamination from this kind of pathologic weight loss is believed to disguise the presumed beneficial effects of weight loss on mortality. To get around this, observational studies have tried to differentiate between therapeutic and pathological weight losses, by categorizing a weight loss as intentional or unintentional (5). Thus, instead of analyzing weight loss among all participants, observational studies have in the last 20 years focused primarily on participants reporting that they were trying to lose weight.

In 2009 Mary Harrington et al published a meta-analysis of intentional weight loss in cohort studies, and concluded that among unhealthy obese (in this context BMI≥25-27) subjects, weight loss was associated with a reduced mortality, whereas among healthy overweight or mixed populations, intentional weight loss was associated with increased mortality (6). Intentional weight loss was not associated with mortality in healthy obese or unhealthy overweight subjects. The same year Harrington et al published their meta-analysis, the Danish College of General Practitioners (DSAM) published 'DSAM's Clinical guidelines for detection and treatment of overweight' (7). The meta-analysis was included in the literature base for this guideline, and accordingly it is stated that "intentional weight loss lead to overwhelming health benefits in patients with overweight related risk factors or disease". In December 2013 an independent panel of leading Danish experts in prevention of disease ('Vidensråd for forebyggelse' - estab-

lished by the Danish Medical Association and the Tryg Foundation) published the report 'Should overweight adults lose weight?' (8). A broad spectrum of weight loss literature was evaluated in the report, but the main conclusion was based on Harrington's results: There is no evidence to support that overweight people – healthy or unhealthy - will reduce their mortality by losing weight (8). In the last couple of months, leading Danish researchers in body weight have been communicating this message in the media, but with the twist that body weight and weight loss are poor measures of the metabolically harmful body fat, and that we all may be 'thin-fat' and at increased risk for diabetes, cardiovascular disease, and premature death, regardless of BMI (9;10).

DSAM's clinical guidelines for management of diabetes in general practice suggest 5-10% weight loss for all overweight patients as a first line treatment (11). According to Harrington's meta-analysis individuals with diabetes are 'unhealthy' and subjects with a BMI≥25-27 will improve their life expectancy by losing weight. However, this view was recently challenged when the large scale RCT 'Look AHEAD' failed to demonstrate any effect on cardiovascular outcomes or mortality in patients with type 2 diabetes obtaining and maintaining a substantial weight loss for 10 years (4). Ever since the results came out diabetes experts have been debating how to interpret or explain these results. Interestingly, only few seem to draw the conclusion that weight loss may not be an effective treatment. Thus, despite the straightforwardness of measuring weight and death, studying the relation between weight changes and mortality has turned out to be extremely complex.

In cohort studies multivariable models are used to adjust the effect of weight loss for factors that may cause weight change and affect survival, like for instance smoking: smokers weigh less than never-smokers but live shorter. In contrast, smoking cessation increases weight but prolongs life. Other factors are for instance age, sex, and genotype. Yet, ultimately the weight depends on the energy balance. If the energy intake is different from the energy expenditure the body weight is anticipated to change. Therefore, factors like smoking status, dietary intake, and physical activity are of fundamental interest in these analyses.

OBJECTIVES

The objectives of article 1 was to examine weight changes in the general population in relation to smoking status, and to propose a 'smoking cessation weight change model' for use in clinical work when health providers discuss post cessation weight gain with patients who are smokers.

The objective of article 2 was to compare different methods to include portion sizes in food frequency questionnaires. Which method provides the best estimate of the dietary intake/energy intake?

The objective of article 3 was to estimate the long-term all-cause mortality, cardiovascular mortality and cardiovascular morbidity risk attributable to weight change in a population-based sample of overweight patients with newly diagnosed diabetes, stratified on intention to lose or to maintain weight.

These specific research questions are dealt with in the three articles. The scope of this thesis is broader – it is about weight changes more generally. What determines weight changes in the adult general population? Is it possible that weight loss may not always be healthy? The present clinical guidelines for general practice advise most overweight people, and patients with type 2 diabetes to lose weight. Are the guidelines based on firm evidence?

BODY COMPOSITION AND WEIGHT CHANGES – WHAT DOES CHANGE?

The present evidence suggests that the metabolically harmful effects of overweight derive primarily from the volume of intra-abdominal fat (8;12;13). Fat deposits on the thighs or hips are probably metabolically harmless or even beneficial (13-16). Correspondingly, the volume and distribution of the lean mass is associated with survival (8). Consequently, body weight is a relatively poor way of monitoring the supposedly harmful fat. This also applies for changes in weight. In one individual a weight gain may reflect increased muscle mass and in another increased fat mass. Likewise, a weight loss may reflect a change to a healthy lifestyle in one individual, but severe disease in another. It is well-described that waist circumference, or waist for given BMI better than the crude body weight describe lean body mass and fat mass, compared with body scans (8), and ideally these better measures should be used in studies of weight changes. At least two cohort studies have demonstrated that reductions in fat, quantified as decreases skin fold thickness or waist circumference, were associated with longevity (12;17). However, there is no method to convincingly direct a weight loss to specific skin folds or to the intra-abdominal fat deposits without affecting the seemingly protective fat tissues in other places (18-21). Most people have bathroom scales at home, and body weight change remains the preferred method of self-monitoring in the public and among patients in general practice. Thus, body weight is the measure that is mainly used when doctors and patients communicate about risk related to obesity, but in statistical analyses the use of body weight is causing several challenges in terms of potential bias. This bias should be considered in the design of multivariable analyses, and should also be kept in mind when interpreting the results.

A SHORT HISTORY OF WEIGHT LOSS THERAPIES

In our cultural hemisphere, those being fat began to be stigmatized for aesthetical reasons in the last half of the nineteenth century. In medical terms obesity was not considered unhealthy unless the ability to move or work was compromised, until statisticians from the Metropolitan Life Insurance Company in the 1920s demonstrated that overweight was associated with increased mortality (22). This gradually affected the view on obesity in the medical society and subsequently in the general population. In the 1950s total fasting was the predominant weight loss strategy in extremely obese subjects (23). This rough method often resulted in electrolyte derangements and death. In the 1960s and 1970s high-protein low-carbohydrate diets, like the Atkins' diet, became popular. Extreme carbohydrate restriction caused increased diuresis because of depleted glycogen stores and had side effects of nausea, hyperurecemia, fatigue and refeeding edema. In the 1970s very low-calorie liquid diets were introduced. In USA 58 deaths were reported to be caused by these diets in 1977-78, which caused the health authorities to temporarily ban the use of these regimens. In the 1980s a new generation of very low-calorie diets was introduced. They were different from the 1960s' low-carb diets as the new very low-calorie diets had a much lower fat content. These commercial products became part of weight loss programs supervised by health professionals (24). Health risks associated with these very low-calorie diets were gallbladder disease and cardiac problems (23). In the 1990s and 2000s the predominant diet strategy was avoidance of dietary fat. However, the narrow focus on fat-free and low-fat diets may not

have decreased the total energy intake. In the recent years the focus has changed to avoiding carbohydrates, like the paleo diet and the comeback of Atkins' diet.

This short history of weight loss diets indicates that weight loss therapy strategies have been driven by fashion rather than by science, and that very aggressive weight loss therapy like total fasting or very low-calorie diets historically have been linked to increased acute mortality and morbidity. Whether intentions and attempts to lose weight in the general population are driven primarily by aesthetic benefits or health benefits is uncertain. Motivating factors does probably depend on sex, age, and the presence of comorbidity (25). Nonetheless, the health care sector has embraced weight loss therapies because of the anticipated preventive effects on numerous diseases.

AGE-RELATED WEIGHT CHANGES AND WEIGHT TRAJECTORIES

The body weight seems to change throughout life. Earlier cohort studies have described how the average weight in adults increases steadily until the age of approximately 55-65 and then plateaus or decreases (26-28). Some cross-sectional studies found that the relation between age and BMI was best described as an 'inverted U', but this model has been criticized for being an effect of increasing weight in younger birth cohorts and a survivor effect among the older participants (with a higher mortality among the obese leaving behind the lean) (26). It is noteworthy that this age-related weight gain and later stagnation or fall coincides with an underlying physiological change of body composition with a gain of fat mass combined with a loss of lean mass or muscle mass (29). Unlike the body weight, waist circumference, and abdominal fat continue to increase throughout life (27). A powerful determinant of body weight at any given time is the recent weight history of an individual (30). This has given rise to the idea about tracking of weight over time - or weight trajectories (31-34). The concept of tracking has been defined as either predicting future measures from earlier values or the consistency of individual's weight development relative to the population mean (31). The trajectory concept is in many ways similar to children's growth percentiles. This rather deterministic view on weight development may be explained by homeostatic mechanisms in the metabolism, mainly determined by genetic predisposition and early life events (35-37). During the last winter of the Second World War pregnant women were starving in Holland (38). The offspring of women who were starving during their first trimester doubled their risk of becoming obese as adults, whereas the offspring from women that were starving during their third semester increased the adult risk of insulin resistance or diabetes with 40%. This 'Dutch hunger study' is one of the cornerstones in the theory of fetal programming. This theory indicates (among other things) that the development of the body weight is not caused by genetic predisposition and lifestyle alone, but also by environmental exposures before birth.

WEIGHT LOSS MAINTENANCE

The body seems to defend its present weight by responding to energy restriction with metabolic changes and reduced physical activity, and to energy surplus with increased energy expenditure (39). Only few weight loss trials have longer follow-up than a few years (23;40-42), and no weight loss program (with acceptably low loss to follow-up) has documented a mean long-term effect of more than 3-4 kg (4;43).

Yet, some individuals do succeed in a sizable weight loss and in maintaining a lower weight afterwards. The participants in the National Weight Control Registry constitutes a highly selected population as the inclusion criteria is a well-documented lifestyle induced weight loss of 10% or more maintained for one year or more (44). The participants are followed up with questionnaires annually and if they regain weight they are excluded from the cohort. These subjects who are able to maintain a major weight loss over long time are characterized by eating a diet low in calories, weighing themselves multiple times a week so that even a tiny increase in weight can be corrected immediately, high control of eating the same amounts in weekends and holydays as on regular week days, and exercising one hour or more every day (44). The strongest predictor for weight regain was 'disinhibition' – moments with temporarily loss of control. Also decreases in physical activity, dietary restraint, and reduced frequency of self-weighing were associated with greater weight regain (45). Thus, a continuous massive self-discipline was needed to maintain a weight loss.

WEIGHT CYCLING

As only few are able to maintain a weight loss over longer time, most often a weight loss results in a subsequent regain. Multiple weight loss and weight gain episodes is referred to as weight cycling. Several cohort studies have examined the association between weight cycling and general mortality. These studies have, like other weight change studies, considerable methodological challenges, but the overall impression from the literature is that weight cycling is associated with increased mortality (46;47). However, the largest cohort study in the field, based on data from the Nurses' Health Study, suggested that repeated intentional weight losses were not predictive of increased mortality (48).

HOW IS WEIGHT CHANGE STUDIED?

Weight changes have been studied in both RCTs and cohort studies. Regarding the long-term consequences of weight loss, DSAM's guidelines rely entirely on observational evidence, as the results from the five weight loss RCTs with mortality or cardiovascular morbidity as outcomes were not available when the guidelines were published (2-4;55;56). Regarding the effect of treatment, the RCT is in general a better study design than the cohort study. However, there are several methodological problems in studying weight changes by the means of clinical trials.

RCTS

The first problem is that weight loss is not an intervention in itself. An intervention with energy restriction or increased energy expenditure may lead to weight loss. If the outcome of interest is survival or surrogate markers like blood pressure or cholesterol, it is a fundamental problem that both diet and physical activity by themselves may affect the exposure (weight loss) and the outcomes. Thus, it is in general not possible to conclude whether a favorable effect of a lifestyle based weight loss therapy is caused by the weight loss per se or by a change in the composition of the diet, an increase in exercise, or by other effects of the intervention. For instance Jaakko Tuomelihto et al demonstrated that 3-year risk of getting diabetes in high-risk individuals was reduced by 58% by an intervention with exercise and healthy diet resulting in weight loss, but was it the diet, the exercise, or the weight loss that caused the beneficial health effect (49)? Another study have demonstrated that diabetes can be prevented by changing the dietary pattern without energy restriction, weight loss, or increased exercise (50), and increased exercise without weight loss or dietary change can reduce HbA1c, and thus prevent - or at least delay - the diagnosis of diabetes (51). It may be argued that weight loss per se is a theoretical entity, as a weight loss is always caused

by something, but in this context a weight loss caused by a reduced intake of one's normal diet is referred to as a weight loss per se.

The second problem is weight cycling. As described above, probably only few individuals are able to maintain a sizable weight loss over many years. When enrolled in a trial many obese participants will have a history of multiple weight loss attempts. Most likely participation in a trial will result in an initial weight loss and subsequently in weight regain (52;53), which may well have other effects on long-term outcomes than the weight loss alone. As a consequence of multiple historical and future weight loss attempts a potential positive long-term effect of a weight-loss in a trial may well be diluted towards null.

The third problem is competing interests. A classical weight loss intervention results in a major weight loss during the first couple of months, then a plateau, and then a more or less steep regression towards the pre-intervention weight (54). Most trials do not report follow-up for longer than some time after the nadir, and the reason for this is rather puzzling. Obviously trials with long follow-up are expensive, and this may be the main reason. Another possible explanation may be that many weight loss trails are sponsored or carried out by companies with commercial interest in a specific weight loss diet. They want to demonstrate that using their product leads to weight loss, and have no interest in showing that the effect is only temporary. Yet another explanation for this could be publication bias; who wants to publish that there was no long-term effect on a clinical parameter of a weight loss that was not maintained?

To my knowledge only five RCTs have reported the relation between successful weight loss interventions and mortality: Look AHEAD, The Finnish Diabetes Prevention Study (55), Da Qing (56), TONE (3) and ADAPT (2). The Finnish Diabetes Prevention Study, Look AHEAD, and Da Qing used broad specter life style interventions with specific diet changes, energy restriction, and exercise in high risk subjects and these studies will be described and evaluated later. ADAPT showed a borderline significant 50% reduction in mortality in the weight loss group, whereas TONE showed a non-significant reduced risk in men and a trend towards an increased risk in women. ADAPT was a reanalysis of a knee-arthritis study, and TONE was a reanalysis of a hypertension trial. The first author on both publications is Kyla Shea. Mortality was not a predefined outcome in the original protocols, and both studies are de facto underpowered to detect differences in mortality with the 8-12 years follow-up data available until now. The spectacular result from ADAPT is based on just 15 deaths in the intervention group and 30 in the control group. However, the ADAPT study was very well-designed for assessing the independent effect of weight loss on mortality, as both the intervention group and the control group participated in the same physical exercise program. The dietary weight loss intervention (which was copied from TONE) used behavioral techniques to "change eating habits in order to lower the caloric intake". Assuming that the participants actually did so, the difference in weight change between the groups was attributable to energy restriction, and not exercise or an especially healthy diet. In this way the 'first problem' described above was to some degree avoided. The seemingly very favorable effects of weight loss in ADAPT may be flawed by an unfortunate randomization with more participants with a history of CVD in the weight-maintenance group. This rather obvious uneven distribution was for unknown reasons not adjusted for in the multivariable model used to estimate the effect of the intervention.

The TONE- study was less well designed for elucidating the independent effect of weight loss on mortality, as the nutritionist intervention in the weight loss group was accompanied by "exercise counselors with experience in lifestyle change techniques" to "increase physical activity". A similar intervention was not offered to the control group. Thus, a potential effect on mortality cannot for sure be ascribed to the weight loss per se, but as well to lifestyle changes induced by the exercise counselors. Accordingly, several RCTs have demonstrated favorable effects of weight loss interventions on intermediate outcomes like blood pressure (57), sleep apnea (58), mobility, life quality, and depression etc. But, whether these beneficial changes were caused by the weight loss per se is uncertain. For instance, physical activity without weight loss also improves blood pressure (59), sleep apnea (60), and may well improve quality of life, mobility, and depression. The clever design of the ADAPT study allows us to regard weight loss as a direct cause of the reductions in symptoms from osteoarthritis in the knee (43).

Of course, the ultimate weight loss intervention is bariatric surgery. The main focus of this thesis is on lifestyle interventions that can be delivered in general practice, and it is outside the scope to make an evaluation of this surgical treatment here. Yet, it should be mentioned that the SOS study found that bariatric surgery was clearly associated with reduced mortality (61). However, the study was not randomized and the most recent Cochrane review more cautiously concluded that Surgery is more effective than conventional management (in inducing weight loss). Certain procedures produce greater weight loss, but data are limited. The evidence on safety is even less clear. Due to limited evidence and poor quality of the trials, caution is required when interpreting comparative safety and effectiveness (between the different methods) (62). The RCT's included were of limited duration, death was infrequent, and therefore the Cochrane review did not provide reliable estimates on long-term mortality. Yet, gastric banding resulted in diabetes remission in 73% vs. 13% in control subjects in one RCT that included only patients with type 2 diabetes (62). In the present DSAM's clinical guidelines for management of diabetes in general practice weight loss surgery is regarded as a last resort in patients with BMI>35 (11). In DSAM's Clinical guidelines for detection and treatment of overweight it is stated that bariatric surgery can be considered in adult individuals with BMI>40 (7).

COHORT STUDIES

Studying weight changes in observational studies is likewise problematic. In general causation cannot be determined with this design in a clinical setting full of unknown confounding factors. However, compared with the RCTs it is an advantage that a large number of participants can be followed over long time at a relatively low cost. Also, cohort studies shed light on weight changes from a different perspective than the RCTs as the actual change can be studied rather than the belonging to an intervention group that may lead to a varying degree of weight loss. This brings about some advantages but also some major methodological obstacles.

The first problem is reverse causality. A presumed beneficial effect on mortality of a therapeutic weight loss will be confounded by weight loss caused by wasting or disease severity. It is a fundamental problem that intentional weight loss cannot convincingly be distinguished from a weight loss caused by disease.

The second problem is that most often the method for a weight loss is unknown or poorly described. For instance, an intentional weight loss may be unhealthy if it is the result of starvation but healthy if it is the result of increased physical activity and a vegetable diet.

The third problem is self-reported weight. Most of the studies included in Harrington's meta-analysis rely on self-reported weight. In general, people underreport their weight. The bias differ between risk groups as for instance obese individuals and subjects with low socioeconomic status tend to underreport more than others (63-65).

REVERSE CAUSALITY

Several methods have been applied to get around confounding from pathological weight loss. Some studies of weight loss and mortality excluded patients with prevalent disease before or incident disease during the weight monitoring period (66;67), excluded all smokers (68), or excluded anyone dying within the first 3-4 years of follow-up (67;69). In a general practice context many exclusions is a problem as it limits the generalizability of the results. Other studies have adjusted for a range of specific baseline conditions like self-reported angina, hypertension, or stroke (70), or stratified the analyses on health status (69). None of the studies referred to in this thesis seem to have considered the role of psychopathology, mental disease or major life events in relation to weight changes. Intention is regarded as an essential method for reducing reverse causality from wasting (5;71). However, despite intention to lose weight, and exclusion of individuals with disease, a planned weight loss can still reflect an underlying pathologic process.

The Charlson comorbidity index provides a weighted score that takes into account the number and the seriousness of comorbid physical diseases and dementia (72). The Charlson score is robustly associated with increasing mortality, but is it associated with weight loss? If so, it may be a valuable covariate to adjust for wasting without excluding participants with disease, especially in a Danish setting where valid and complete information about hospital diagnoses are readily available from national registers (73). To my knowledge no earlier studies of weight loss have used the Charlson score to adjust for confounding from disease severity.

DIETARY INTAKE

The total energy intake is obviously related to weight change, if not compensated for by energy expenditure. The importance of specific diets or foods in relation to weight change is subject to continuous research and attention from the public and the media. An obese person needs a higher energy intake than a lean person to maintain weight (74). On average a person with a high energy intake will have a relatively high intake of most foods and nutrients, and consequently the absolute intake of any food or macronutrient correlates with the present body weight, unless the analysis is adjusted for the total energy intake. Despite the continuous public attention to this field, no strong evidence is connecting normal intake levels of specific foods or macronutrients with weight changes when the total energy intake is taken into account. On the other hand, there is quite some evidence connecting changes in diet composition with changes in weight (75). These results are subject to intense debate between the highest profiled nutritional epidemiologists internationally (75) (See for instance the series of comments to this BMJ paper). In short, the potential impact of food or macronutrient composition on weight change is probably relatively small, and the effects found may be entirely mediated through changes in the total energy intake. This is supported by several meta-analyses and reviews (76-79). Thus, the cornerstone of dietary advice for obtaining a weight loss remains restriction of the total energy intake, while the macronutrient composition seems to be of less clinical importance. Of notice, the 'dietary advice' from the Danish National Board of Health (kostrådene) do not claim to result in weight reduction or weight maintenance, but claims to prevent cardiovascular disease and cancer, which for the most part is relatively well-documented. Thus, at a first glance, the total energy intake appears to be an important parameter to take into account when analyzing weight changes.

NUTRITIONAL EPIDEMIOLOGY METHODS

Nutritional epidemiology studies the relationship between nutrition and health with observational data and multivariable regression models. The dietary intake is measured by either self-reports or biomarkers. With the biomarker 'doubly labeled water' it is possible to accurately measure the total energy intake, and by collecting 24 hours urine samples it is possible to quantify the intake of protein (80). Intake of fat, carbohydrates, or specific foods cannot at present be quantified by biomarkers. The biomarker methods are expensive and are mainly used for validation of self-reported intakes in smaller samples. Self-report is used for assessing dietary intake in population studies. Four methods predominate: 1) the diary (participants carefully weigh and register their food intake in a diary over 4-7 days); 2) the 24hour recall (a dietician systematically interviews the participants regarding their dietary intake within the last 24 hours); 3) the diet history interview (one month recall as assessed by interview); and the food frequency questionnaire (FFQ - participants fill in a questionnaire on how often, in the last months or the last year, they consumed 60 – 300 food items). Newer versions of the classic FFQ are 'semi-quantitative FFQs' in which also questions about portion sizes are included.

In general, all self-report methods underestimate the true energy intake and are subject to differential reporting bias by sex, BMI, and age (80;81). The FFQ is cheaper and covers a longer period than the other methods, and the FFQ is the preferred method in cohort studies. However, there are often many missing values, as it takes long time and much patience to complete a full size FFQ.

Consequently, self-reported dietary intake is subject to considerable measurement error, and often the measurement error is blamed for the many 'null' results in nutritional epidemiology as described above. Whether weight gain is actually caused by increased energy intake cannot be determined with the FFQ instrument, as the measurement error is far too large to accurately quantify the little extra energy needed to gain weight over time. However, the measurement error is not the only methodological problem in this field of epidemiology. A range of general assumptions regarding recipes, portion sizes, added sugar etc. may further contribute to the noise. Many resources have been allocated to improve the measurement method, but surprisingly little effort has been put into improving the processing of dietary data. An area that has not received much attention is how to handle the missing values. Because of the relatively many missing values in a full size FFQ complete case analysis is not an option, and most studies seem to 'fill the blanks' with median values or zeroes although multiple imputation has been demonstrated to be a better technique in epidemiology in general (82).

HARRINGTON'S META-ANALYSIS

Harrington's meta-analysis has become a key reference in the debate concerning weight loss and mortality (6). The meta-analysis included 26 cohort studies of which the weight changes were qualified by a description of intention in 18. Unintentional weight

loss and weight loss not described by intention was clearly associated with increased mortality. Regarding the association between weight loss and mortality this thesis focuses mainly on patients with type 2 diabetes. Thus, the main focus of this review of Harrington's analysis is on studies of 'unhealthy' participants with intentional weight loss.

Four studies analyzed intentional weight-loss in unhealthy participants (68;69;83;84). Two of these included only patients with diabetes (David Williamson et al. Diabetes Care 2000 and Edward Gregg et al. Diabetes Care 2004) (83;84), and the remaining two defined 'unhealthy' as the presence of a health condition including for instance CVD, hypertension, diabetes, or stroke. The first author of these two latter studies was also Williamson, and Williamson was last author on Gregg's paper.

Williamson's three studies of unhealthy individuals are based on data from the Cancer Prevention Study-1 (CPS-1). The more than 1 million participants were recruited by the American Cancer Society volunteer workers throughout USA in 1959-60. The participants completed a questionnaire about health, disease and symptoms of illness (68). After 12 years data on mortality was collected. Regarding weight data the questionnaire was phrased as whether the respondent had had "a change in weight?" Yes or no (without regard to when the change occurred). In case of a change the participants were asked "Loss or gain?", and "About how many pounds?", and "over what period of time?" (Within <1, 1, 2, 3… years). The intention was determined by asking "Did you try to bring about this change?" Yes or no. The authors categorized the exposure as "no change", "unintentional loss", "unintentional gain" "intentional gain", "intentional loss", and "unknown" for missing responses. No data described whether these "no change" participants were intending to lose weight or not. The mortality among participants who reported an "intentional loss" was compared with the "no change" group in multivariable analyses adjusted for covariates including baseline BMI as a continuous variable.

Of notice, it was assumed that a reported weight loss was maintained to the time of the query, and an 'initial BMI' was retrospectively calculated from the present height and weight, and the reported weight loss (Figure 1). In the multivariable analyses the 'initial BMI' value was used for baseline BMI for the intentional weight loss group, whereas the 'present BMI' was used for baseline BMI in the no change group.

The different assessment of baseline BMI in the exposure group (intentional weight loss) and the comparison group (no weight change) may have generated bias by overestimating the baseline BMI in the exposure group. Figure 1 illustrates a hypothetical participant truthfully reporting a weight loss of 10 kg in the last year – but was the weight loss maintained? If any weight was regained since the weight loss, the initial BMI was overestimated. Since BMI is consistently associated with mortality in the background population (1), and since the potential bias pertained solely to the exposure group (intentional weight loss) it may have affected the multivariate analysis of the association between intentional weight loss and mortality in favor of intentional weight loss. This is examined in the results chapter and evaluated further in the discussion chapter.

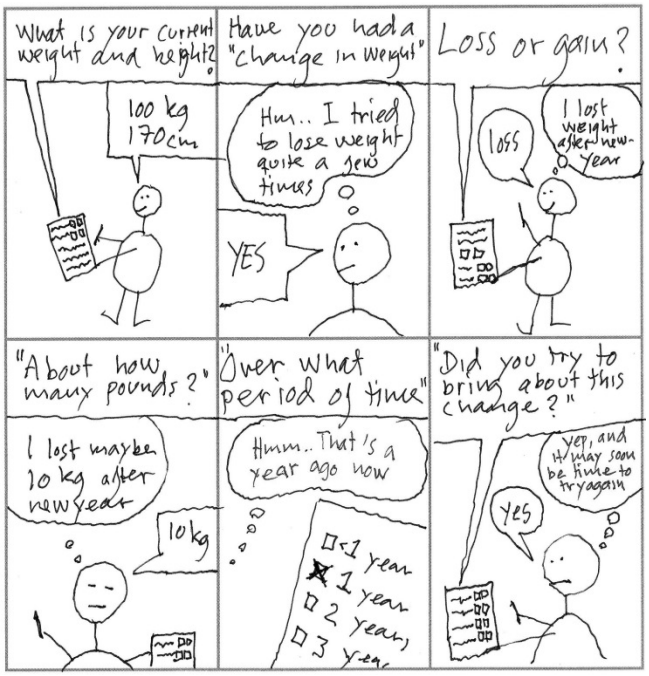

Figure 1. *A hypothetical participant in the CPS-1 filling in the questionnaire in 1959. Did the participant report a weight loss that was maintained?*

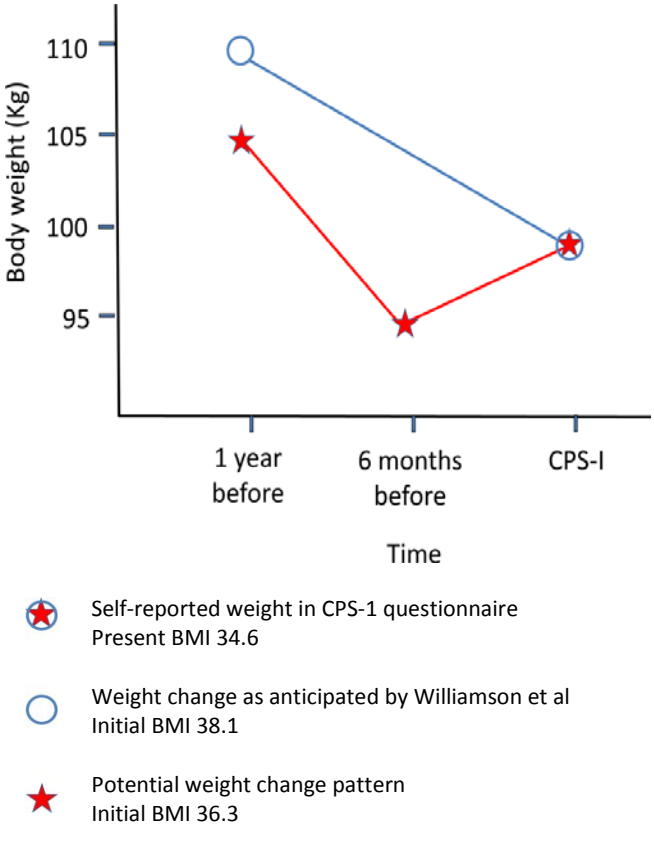

Figure 2. *Two potential scenarios for the weight change reported by the hypothetical participant in CPS-1 in the cartoon in Figure 1. If the participant truthfully reported a 10 kg weight loss, but if the weight loss was not maintained, the historic body weight and*

thereby the 'initial BMI' was overestimated. This measurement error may have resulted in bias in studies with retrospectively estimated baseline BMI.

In 1995 Williamson et al published the first study ever on intentional weight loss (68). From the CPS-1 cohort 43.457 overweight ('initial BMI'≥27) white female never-smokers aged 40-64 years were included. The study found that among the 'unhealthy' women (including 9.5% with self-reported diabetes), intentional weight loss was associated with a 20% reduction in mortality, primarily due to reductions in diabetes and cancer related mortality. In healthy participants the associations between intentional weight loss and mortality were inconsistent. In 1999 the same group published a parallel study including 49.337 overweight ('initial BMI'≥27) white men (smokers and non-smokers) aged 40-64 years from CPS-1 (69). In this study intentional weight loss was not associated with reductions in all-cause mortality among the 'unhealthy' participants, but the diabetes related mortality was reduced 34-36% and cancer mortality with 25%.

The road was now paved for the third article by Williamson et al 2000: Intentional weight loss and mortality among overweight ('initial BMI'≥27) individuals with diabetes (84). From the CPS-1 cohort 4.970 individuals with self-reported diabetes were included (now also smoking women and other ethnicities than whites with diabetes were included). Intentional weight loss was associated with a 25% reduction in total mortality compared to participants with stable weight or unknown weight change.

In 2004 Gregg et al followed up with the paper 'Trying to lose weight, losing weight, and 9-year mortality in 1.401 overweight (BMI>25) U.S. adults with diabetes (83). The data was from the National Health Interview Survey (NHIS). Information on height, weight, potential confounders, and retrospective information on weight change in the last year including intention was obtained in a single baseline telephone interview in 1989. Mortality data was collected after 9 years. Regarding weight data the participants were asked "Have you tried to lose weight in the last year?" (yes/no); "Is your weight now more, less, or about the same as a year ago?" (more/less/about the same); and, "In the past year, about how much have you gained/lost?" (number of pounds). Gregg found that patients with diabetes trying to lose weight had a 23% lower mortality rate than those not trying to lose weight, regardless of whether they actually lost weight or not. In other words, the mere intention of losing weight was associated with a favorable outcome rather than the weight loss in itself. This was true for overweight individuals, but not for obese ('initial BMI'≥30). The study was not adjusted for physical activity.

Thus, the present evidence from observational data regarding intentional weight loss among patients with type 2 diabetes derives from just two cohort studies (83;84).

The results regarding intentional weight-loss in healthy obese individuals were based on Williamsons studies with CPS-1 data and two other studies where Williamson was co-author; Gregg et al 2003 (85) and French et al 1999 (70). The data from this latter study derived from Iowa women health study that included a mixed population of normal weight, overweight, obese, healthy, and unhealthy postmenopausal women (the dubious categorization of this study among obese healthy in the meta-analysis will be considered in the discussion chapter). Thus, the evidence regarding intentional weight loss in so called obese subjects ('initial BMI'≥25-30) derived from CPS-1 and HHIS, but results the Iowa women health study was also included in this subgroup.

In healthy overweight or mixed populations the results were based on Sørensen et al 2005 (66), Wannamethee et al 2005 (86), Wedick et al 2002 (87), Williamson et al 1999 (69), and Yari & Goldburt 1999 (88). There was a greater variability in methods and populations in this subgroup compared with the rest of Harrington's meta-analysis.

From all the included studies Harrington et al extracted the difference in mortality rates between subjects with intentional weight loss and subjects with stabile weight and unknown intention, and entered the rate ratios into the meta-analysis.

THE LOOK AHEAD TRIAL

The randomized clinical trial Look Action for HEAlth in Diabetes (Look AHEAD) included 5,145 overweight/obese adults (45-76 years) with type 2 diabetes to examine whether intensive lifestyle intervention designed to achieve weight loss would reduce CVD incidence and CVD mortality. The intervention consisted of caloric restriction, dietary change (<30% energy from fat, >15% from protein, and meal replacement products) and increased physical activity. The control group received 'diabetes support education' as 1-hour group sessions 1-3 times per year. The main results were published in 2013 after 9.6 years of intervention (4). The subjects in the intervention group lost more weight, exercised more, and required fewer medications (antihypertensives, statins, and insulin). The intervention resulted in substantial reductions in all measured risk factors for CVD (except LDL cholesterol), even though the differences between the groups decreased over time. Despite the apparent success of the intervention, the intensive therapy group did not experience a reduction in the composite primary endpoint of CV mortality, non-fatal myocardial infarction, non-fatal stroke, or angina hospitalization compared with the control group. The trial was stopped prematurely, when interim analyses suggested that it was unlikely that longer follow-up would yield a different result. Of notice, there was a tendency towards a reduction in all-cause mortality (HR 0.85; CI 0.69-1.04; p=0.11), indicating that the intervention may have reduced mortality by other means than CVD. Subgroup analyses indicated that the intervention was more favorable in participants without CVD compared with participants with CVD at baseline. The study also demonstrated how partial or total remission of diabetes, defined as a shift to prediabetic or normal glucose levels, persisted in 7% in the intervention group vs 2% in the control group 4 years after inclusion (89). Look AHEAD was not a population based study as the participants were a selection of relatively healthy patients with type 2 diabetes who were motivated to lose weight, able to complete a maximal exercise test, and had HbA1c<11% (mean 7.3) and blood pressure<160/100 at baseline. Accordingly, the authors stated that the results cannot be generalized to all patients with type 2 diabetes (4).

THE PREDIMED TRIAL

In contrast, the Spanish multicenter randomized clinical trial PREDIMED demonstrated a 30% reduction in development of CVD or diabetes over 5 years with adoption of a Mediterranean diet supplemented with nuts or olive oil when compared with a low-fat diet (90). There was no energy restriction, and the diets did not induce weight loss (50). The 3,541 high risk participants (55 to 80 years. 50% with type 2 diabetes) were recruited in a primary care setting.

DIABETES PREVENTION TRIALS

Two large diabetes prevention trials in a western-world setting demonstrated that the diagnosis of diabetes can be prevented by pharmacotherapy or lifestyle modification with exercise, energy

restriction, and dietary composition to induce weight loss in subjects with impaired glucose tolerance: The Finnish Diabetes Prevention Study and The Diabetes Prevention Programme (49;91). However, already after the first year the intervention groups began a regression towards the weight of the control groups, and there was virtually no difference in diabetes incidence after the first couple of years. Overall, the lifestyle interventions resulted in a mean delay of the development of diabetes of 4-5 years. In contrast to findings in other studies, physical activity and dietary composition seemed to be of little importance; weight loss was by far the most important contributor to the diabetes prevention (53;92). Thus, the incidence of diabetes correlated neatly with the weight change. It is a well-established clinical fact that HbA1c can be lowered by weight loss. Hence, it may come as no surprise that diabetes can be prevented by weight loss or pharmacotherapy, since the diagnosis is defined as an HbA1c value over a certain threshold. Several attempts have been made to replicate these studies in community-based settings, but it has not been possible to achieve the same weight reductions or success with the prevention as in the original trials (93). The Diabetes Prevention Project did not report any clinical outcomes in the 10 year follow-up (52), whereas The Finnish Diabetes Prevention Study found no difference in mortality or cardiovascular morbidity between the intervention and control groups (55).

In June 2014 the 23-years follow-up form the Chinese Da Qing Diabetes Prevention Trial was published (56). This cluster-randomized trial enrolled 577 adults with impaired glucose tolerance in 1986 at 33 clinics. Each clinic was randomized to intervention with diet, exercise, exercise + diet, or standard care. Weight loss was recommended to the overweight participants (60%), but the intervention focused mainly on dietary composition (increased intake of vegetables and reduced intake of alcohol and sugar) and exercise (increase in leisure time physical activity). After 6 years the intervention groups had lost 1 kg on average compared with the control group and after 20 years the control group had lost ½ kg more that the intervention groups. After 23 years the incidence of cardiovascular disease was reduced by 40% and all-cause mortality by 30% in the intervention groups compared with the control group. Still 73% in the intervention group and 90% in the control group eventually developed diabetes. The study can be criticized for the low number of randomization units (n=33), and the rather large differences in most baseline characteristics (for instance; individuals in the control group was 2 years older on average than subjects in the intervention groups!) that for mysterious reasons were not adjusted for in the main analysis.

METHODS AND MATERIAL

Each of the three scientific articles of own production included in this thesis is based on a different cohort. The data is observational and a wide range of epidemiological methods have been used including multivariable modeling. The specific methods have been described in detail in each article.

CAUSAL PATHWAYS AND MEDIATION OF EFFECT IN MULTIVARIATE MODELS

The multivariable models used in this thesis are inspired by the theory on causal inference and directed acyclic graphs (DAGs) (94). 'A priori' designs have been used rather than data driven inclusion or exclusion of covariates. The designs are based on existing biological and social knowledge and theory about changes in body weight, applied on the available data. In general, covariates with an anticipated causal effect on both the exposure and the outcome were included; for instance baseline BMI or change in smoking status in article 3 where the exposure was weight change and the outcome was mortality. Covariates that were likely caused by the exposure and that caused the outcome were considered mediators of the effect of the exposure and these were in general not included as covariates; for instance the change in blood pressure in the weight monitoring period in article 3. Adjusting for a mediator like this would remove the part of the effect of weight loss that works through change in blood pressure and thereby make the weight loss appear less favorable. However, in the INSAUP dataset a range of analyses were done which included a variety of potential mediators in order to unveil potential causal pathways.

THE INSUAP COHORT

Study population

The INSUAP (insulin resistance in general practice) cohort study was established by general practitioner PhD Poul Erik Heldgaard. In 1998-2000 he invited all adults aged 20-69 years on the list of his clinic, in the rural village of Ørum in Denmark, to participate in a study on lifestyle and metabolic health. A total of 3108 people (including children) were listed with the practice. Among the 2082 eligible adults 1374 (66%) participated in the baseline study and completed multiple questionnaires, underwent medical examination, had oral glucose tolerance test done and multiple blood samples drawn (Tables 1 and 2). Patients suffering from severe physical or mental illnesses were excluded, as were insulin-treated patients with diabetes.

After nine years (mean 8.6 years; SD 0.6; range 7.5-10.1) in 2007-2008 the participants were send a letter with questionnaires and instructions on how to measure their body weight in underwear; 1122 subjects (85% of the baseline participants still alive) replied (Tables 1 and 2). The patient flow is shown in Figure 3.

The participants gave written informed consent. The investigation was carried out according to the declaration of Helsinki II and was approved by the regional research ethics committee.

Table 1: *INSUAP - clinical data*

	Baseline n=1374	Follow-up n=1122
Weight and height	x	(x) self reported
Blood pressure	x	
Fasting glucose, HbA1c and oral glucose tolerance test	x	
Insulin and blood glucose (t = 0, 30 and 120 min)	x	
Fasting lipid-profile (LDL calculated)	x	
CRP and TSH (+T3 and T4 if TSH > 2.8)	x	

	Baseline n=1374	Follow-up n=1122	Registers All
Food Frequency Questionnaire (FFQ)	x	x	
Baecke questionnaire (Physical activity) (122)	x	x	
Diseases / diagnoses and medication	x	x	x
Weigh history (121)	x		
Tobacco – detailed history	x	x	
Household, education, occupation	x	x	
Death / morbidity / medication			X

Anthropometric measurements
The same investigator (PEH) carried out the physical examination of all 1374 baseline participants. They were weighed in their underwear and the weight was registered to the nearest 100 g (Seca®Electronic 0 –200 kg). Height was measured to the nearest 0.5 cm.

Dietary data
The INSUAP FFQ was identical to the one used in the Danish EPIC cohort (Kost, Kræft og Helbred) except for a few additional questions. There were 228 questions about food frequencies, but no questions about portion sizes. We used the Danish Health Examination Survey (DANHES) as comparable population, and imputed portion sizes from DANHES to INSUAP with 10 imputations with the Coca method as described in APPENDIX 2 chapter 1d. Despite the common nationality, a range of differences may exist between participants in KRAM and INSUAP. Still, we found it reasonable to assume that the internal relation between physiology and portion sizes did not differ substantially between the cohorts.

Missing values in food frequencies, and physical activity were also imputed with the Coca method, but with INSUAP itself as comparable dataset with the method described in APPENDIX 2 chapter 1e and chapter 6. In the multivariable analyses 10 dataset with each their set of imputed missing values were used as described in APPENDIX 2 chapter 5 (82).

Weight history
The baseline questionnaire contained questions about weight history. Body weight 1, 5, and 10 years ago and at age 20 was recorded. The questions (1, 5, and 10 year history) have been validated in a general practice setting (122).

Follow-up
Of the 1374 participants in the baseline study, 1122 answered the follow-up questionnaires in 2007-2008. Of the 252 subjects that did not participate, 52 died in the follow-up period and 9 immigrated to another country. Of the participants in follow-up survey, 6 did not report their new weight.

The Charlson comorbidity index
The Charlson comorbidity index was based on ICD-10 hospital diagnoses (in and out patients) from the Danish National Patient Register (73;95). The Charlson score based on data from this register has been validated to correlate well with mortality (96). In the INSUAP study the score was accumulated during the 9 years of follow-up. Thus, the score was an estimate of the disease burden during the period in which the weight change occurred.

Table 2: *INSUAP questionnaires and register data*

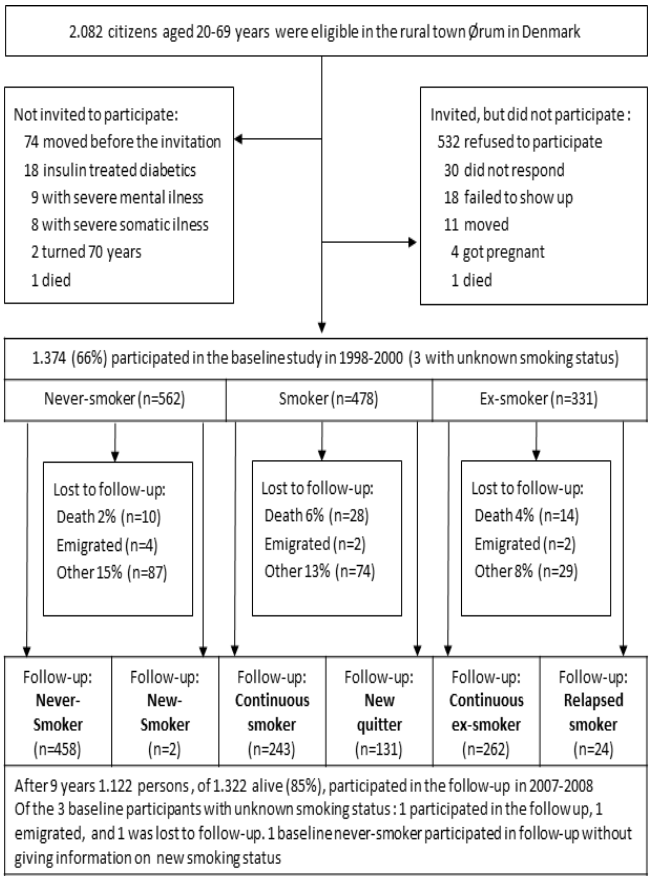

Figure 3. *Patient flow in INSUAP (from article 1)*

Non-attenders baseline
It was not possible to further analyze baseline non-attenders as it was considered unethical to address them, when they had clearly expressed their reluctance to participate in the study. However, information on age and gender was available from Statistics Denmark (Table 3).

Table 3. *Age distribution of the Danish population and the baseline population in INSUAP*

	Denmark total1		INSUAP total		INSUAP excluded		INSUAP included2	
Age group	Male	Female	Male	Female	Male	Female	Male	Female
20-29	20.8	20.6	18.6	20.8	26.8	32.8	14.2	14.9
30-39	23.7	23.0	26.5*	27.1*	29.0	26.9	25.2	27.2
40-49	21.3	21.1	23.3	22.8	18.8	18.8	25.8	24.7
50-59	21.0	21.1	18.4*	15.6**	13.9	9.3	20.7	18.7
60-69	13.1	14.3	13.2	13.7	11.5	12.2	14.1	14.4

*Data are percentage; * p<0.05; ** p<0.001 (Denmark vs INSUAP total).*
1) Statistics Denmark 1999
2) Age at inclusion

Non-attenders follow-up
Table 4 compares the participants who provided weight data at follow-up with those alive that did not attend. The 200 non-attenders alive were almost 9 years younger on average than the 1116 attenders that provided weight data (Table 4). However, there were no differences in the distribution of sex or education,

baseline BMI, baseline smoking status, or the cumulative Charlson score during the follow-up period.

Table 4. *Attenders and non-attenders in the INSUAP follow-up study 2007-2008*

	Attenders1 (n=1116)	Non-attenders2 (n=200)	P diff
Female sex, %	51	44	0.08
High school, %	24	26	0.47
Smoking at baseline	33	38	0.21
Charlson score3=0, %	80	86	0.06
BMI at baseline>25, %	49	48	0.63
BMI at baseline, mean (SD)	25.7 (4.7)	26.0 (5.3)	0.48
Age at baseline, mean (SD)	45 (12)	37 (11)	< 0.0001

1) Individuals participating in the follow-up study and providing weight data
2) Individuals participating in the baseline study, alive at follow-up study but not participating
3) Charlson score between baseline examination and April 2009

THE DANHES 2007-2008 COHORT

All municipalities in Denmark were invited to apply for participation in DANHES 2007-2008; 44 of 97 municipalities applied, and 12 municipalities were eventually chosen. Adults of 18 years or older in these municipalities (n=538 497) were invited by letter to complete an internet-based questionnaire, and a random subsample of these individuals was invited to participate in a health examination (n=180,103). The FFQ was completed or partially completed by 47 791 individuals (9%). A total of 18 065 subjects (10%) participated in the health examination; and of these 9384 also answered the FFQ. Compared with the total Danish population, women in general, but especially women in the age group 45–64 years, were over-represented, whereas the younger men and eldest women were under-represented. Also, the segment of the population with low income or low level of education, and unmarried individuals were under-represented (97). Information regarding physical activity was self-reported. Nutritional data were collected using an internet-based 267 items FFQ. The clinical examination included (among other measures) height, weight, blood pressure, and resting heart rate. In article 2 the study population consisted of the 3728 subjects with complete information on anthropometry and portion sizes (no missing values). As described in Table 1 in article 2 the participants did not differ substantially from the excluded subjects in terms of sex, BMI, age, or physical activity.

The DANHES was funded by the Ministry of the Interior and Health and the Tryg Foundation. The survey was carried out by the National Institute of Public Health, University of Southern Denmark.

THE DCGP COHORT

Study population

The Diabetes Care in General Practice (DCGP or DIAP – Diabetesomsorg I Almen Praksis) study was a pragmatic, open, controlled trial with cluster randomization to structured personal care or routine care (98). Altogether, the practices of 474 general practitioners throughout Denmark were randomized, and 1381 consecutive patients newly diagnosed with diabetes (99.1 % were of Western European descent) were included in 1989-92. The intervention included regular follow-up visits and individualized goal setting. The doctors in the intervention group were supported by clinical guidelines, feedback, and continuing medical education. It was suggested to the intervention doctors that they recommend increased physical exercise and simple diet rules: to increase the intake of complex carbohydrate to at least 50% of the diet, and in particular to increase the intake of water soluble fiber, reduce fat intake to a maximum of 30%, reduce alcohol intake, and eat 5-6 meals a day. After 6 years of intervention (as well as before the intervention) there was no statistically significant difference in body weight between the two randomization arms (98).

In the present thesis the 761 patients in the well-monitored intervention arm of the DCGP study formed an inception cohort. Less than 4% of the eligible patients declined to participate, but 8% were excluded due to severe somatic or psychiatric disease. The patient flow is described in Figure 4. Thus, article 3 is a cohort study, overlaid the original trial. In this context the 6 year intervention period is referred to as the monitoring period.

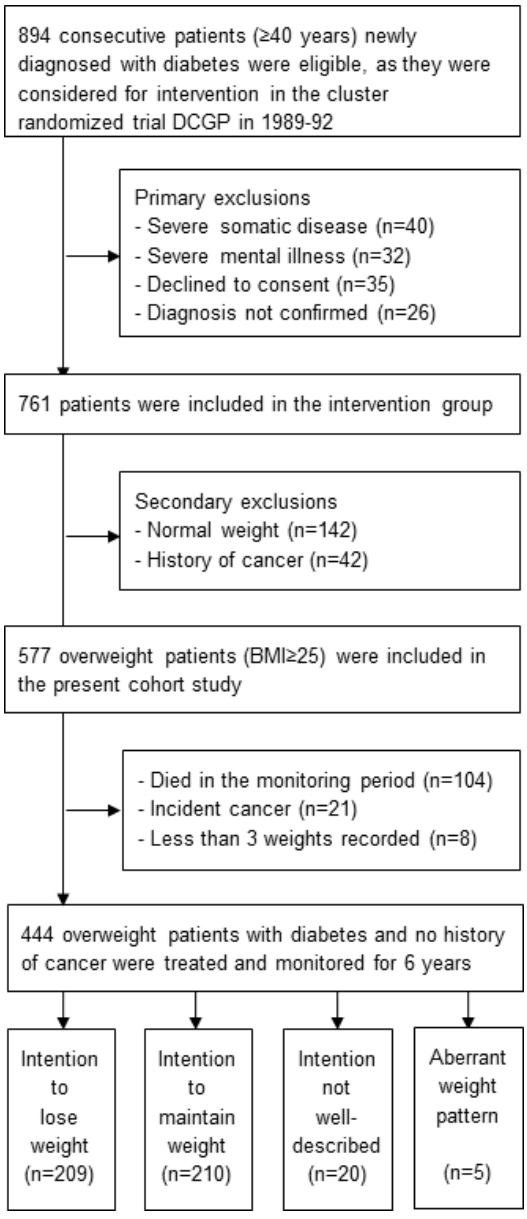

Figure 4. *Patient flow (from article 3)*

Monitoring of weight and intention

Every third month in the monitoring period, the patients were invited to control visits at their general practitioner. Among other measures, body weight and a prospective goal for intended weight change in the next 3 months were recorded at each visit. The median number of weight registrations was 13 per patient. The median time between consultations was 106 days.

The control group

The patients in the control arm were not included in the cohort studied in this thesis because they were not monitored with weight and intention.

Examinations and assays

Immediately after the diabetes diagnosis, the general practitioner did a structured clinical examination of each patient (98). The time from the day of diagnosis until measurement of the body weight was ≤30 days in 79.1% of the patients and ≤60 days in 90.5%. In questionnaires filled in at diabetes diagnosis the patients gave information about education, smoking habits, leisure time physical activity, and former or present cancer. After 6 years the patients gave information on smoking habits and physical activity in the follow up questionnaire. Fasting blood samples were analyzed at Odense University Hospital. Urinary albumin concentration was measured in freshly voided morning urine at Aarhus University Hospital. Microalbuminuria was defined as >15 mg albumin/L (not adjusted for urinary creatinine. Including proteinuria >200 mg/L).

RESULTS

In this first part of the results chapter analyses of weight changes in the INSUAP population are presented.

AGE

Age and weight change

Age was the predominant predictor of future weight. On average, the body weight increased in subjects 20 to 60 years old, whereas it decreased in subjects older than 60 years at baseline. Figure 5 demonstrates the crude correlation between age at baseline and mean weight change over 9 years in the INSUAP cohort. Over 9 years weight gain rates were large in young adults (6 kg in 9 years in participants 20-25 years at baseline) and incrementally smaller in older adults. Subjects more than 60 years at baseline lost weight on average.

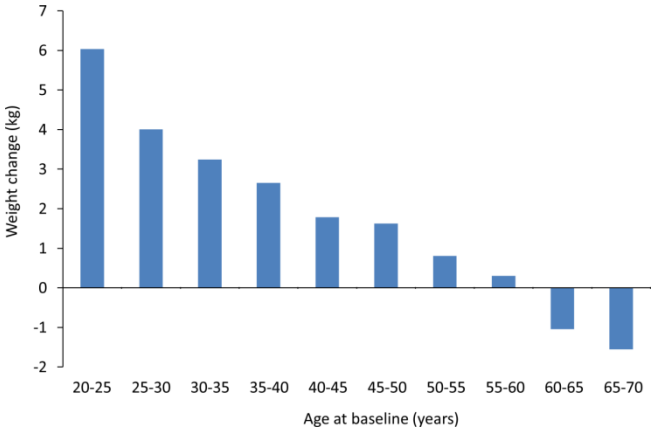

Figure 5. *Weight change over 9 years by age category in the INSUAP cohort – all participants*

To test whether the age related differences in weight change rate was explained by a survival effect, the weight of the dead participants were imputed with the Coca method as described in APPENDIX 2 chapter 1e. However, including statistically neutral values for the hypothetical weight change of the dead participants did not change the pattern (Figure 6 panel A). Depicting the age related weight change as change in BMI did not alter the pattern neither (Figure 6 panel B).

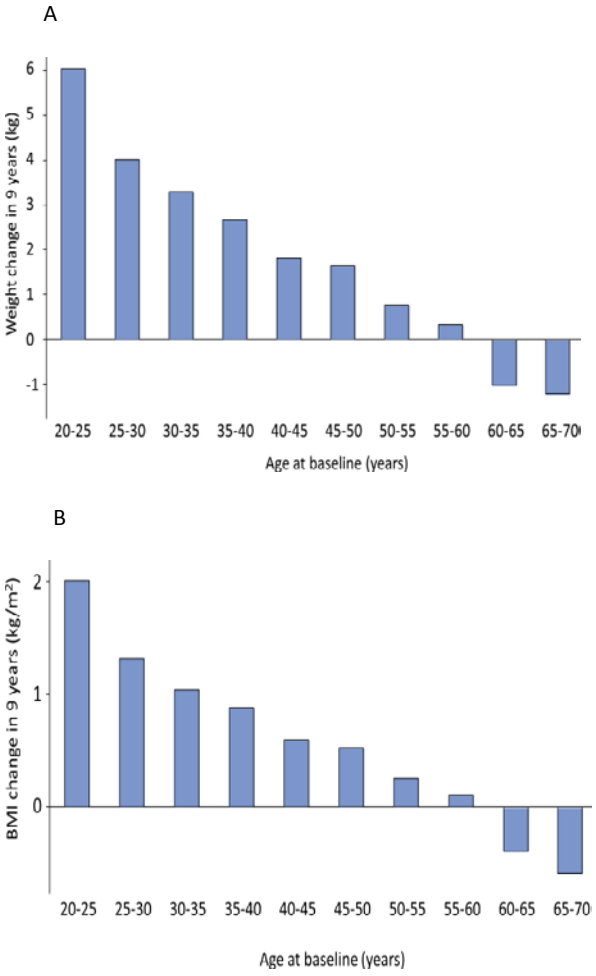

Figure 6. *Panel A displays weight change over 9 years by age category in the INSUAP cohort including the weight of dead participants imputed to adjust for a potential survivor effect. Panel B displays change in BMI over 9 years by age category in the INSUAP cohort.*

Figure 7 holds a series of stratified versions of Figure 5. In female subjects the weight change was negative 10 years earlier than in men. Normal weight subjects was gaining weight despite age (with a decreasing pace in older subjects), whereas participants who were obese at baseline lost weight on average if they were older than 40 years. Neither education level nor the Charlson comorbidity index did affect the age weight change patterne much. The almost linear decrease in weight gain rate with subjects of increasing age was common for all the stratified graphs.

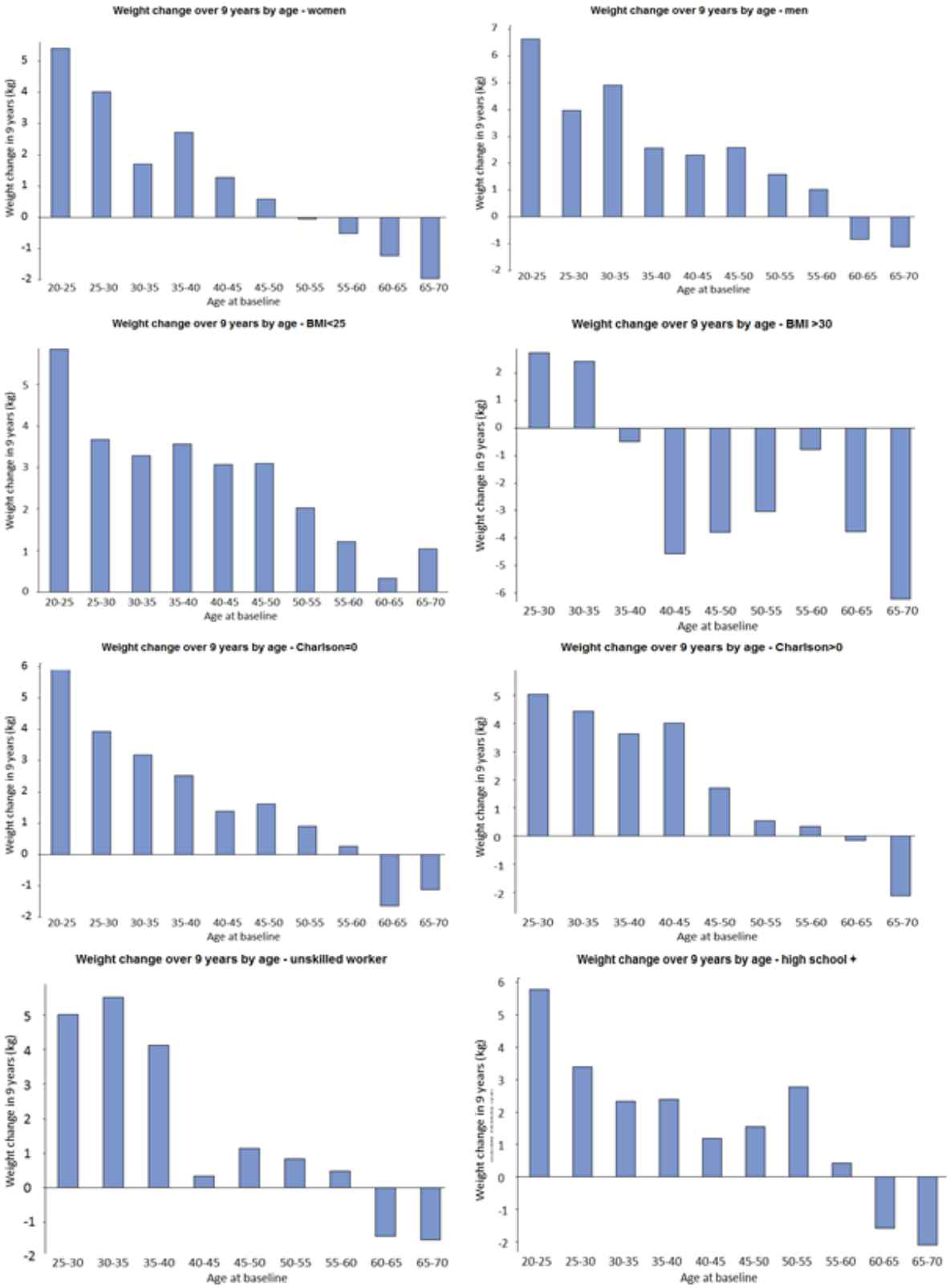

Figure 7 *(page 13). Stratified univariate analyses of weight change by age category in the INSUAP cohort. Note: different scales on the Y-axes.*

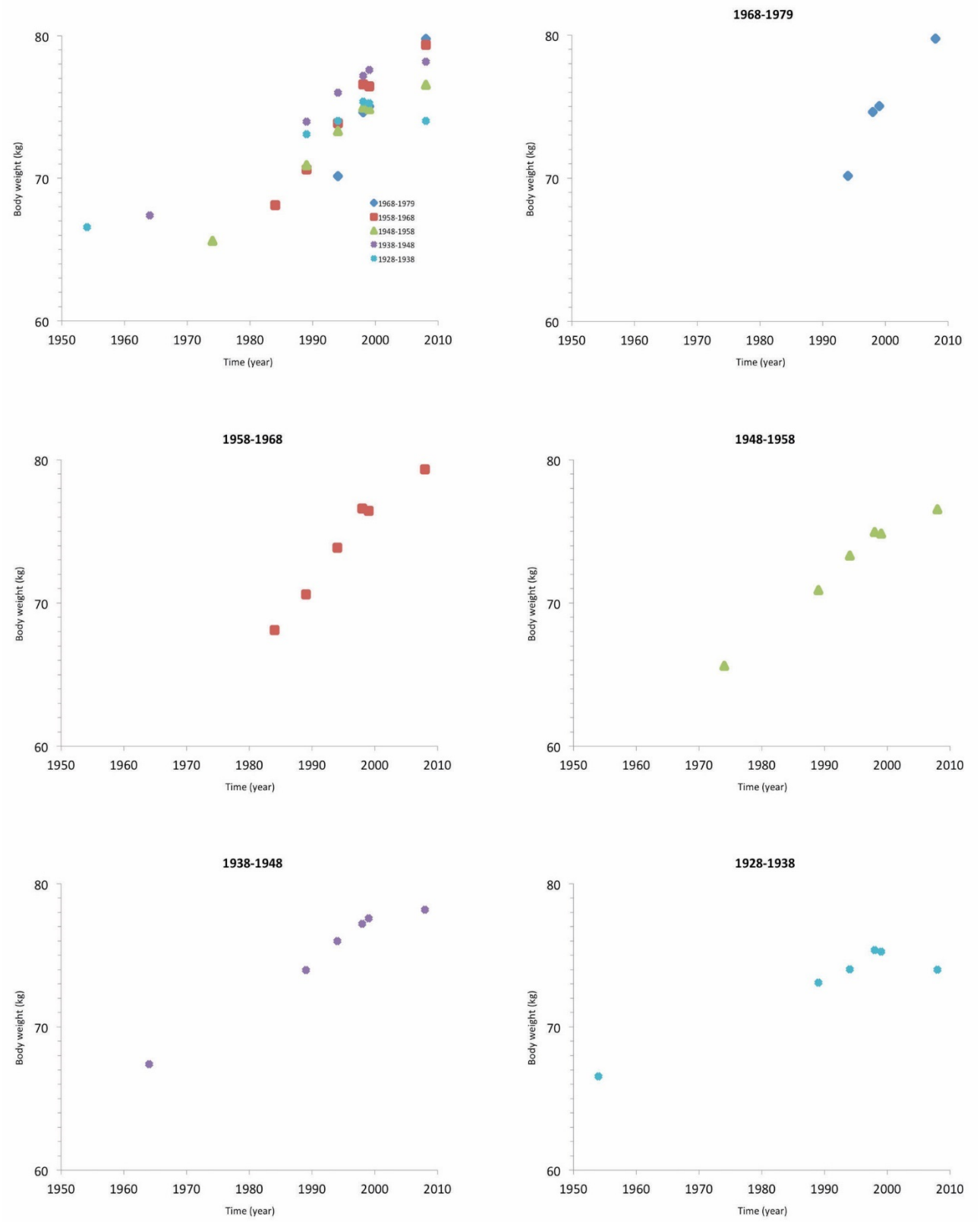

Figure 8 *(page 14). Weight history in birth cohorts in the INSUAP population. The data points are self-reported weight at age 20 years, weight 10, 5, and 1 year before the baseline study, age at the baseline study (measured), and age at follow-up. The data points are set at the mean age in the birth cohort.*

Weight history

The self-reported weight history in combination with the baseline and follow-up assessments of body weight made it possible to estimate the longitudinal weight development in each individual. Figure 8 illustrates how the body weight increased throughout adulthood in all birth cohorts from 1928-1979 in the INSUAP population. The weight development appears to be almost linear but steeper in the younger birth cohorts compared with the older. This pattern indicate that the large divergence in weight gain rates observed between age categories in Figure 5-7 is partly explained by a birth cohort effect. In the older birth cohorts (the two lower panels in Figure 8) the average weight gain from 20 years to around 60 years was approximately 10 kg. All of the younger birth cohorts also gained around 10 kg since they were 20 years old but they had not reached the age of 60 yet.

Age and baseline BMI

In a cross-sectional analysis the baseline BMI was increasing with increasing age (Figure 9). The crude correlation between age and baseline BMI was 0.06 BMI point/year of age (CI 0.04-0.08; p<0.0001). The magnitude of this correlation was virtually unchanged by adjustment for sex, education, smoking status, and physical activity.

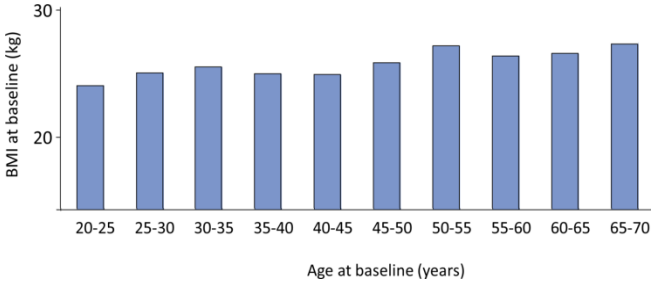

Figure 9. *Baseline BMI by age category in the INSUAP cohort*

SEX, SOCIOECONOMICS, AND BASELINE BMI

The multivariable analysis of weight change revealed that women gained 1.4 kg (CI 0.7-2.1) less than men on average during the 9-year period. Also the socioeconomic differences were associated with weight changes as unskilled workers gained 1.3 kg / 9y (CI 0.2-2.4) more than participants with a high school degree. For skilled workers the equivalent relative weight gain was 0.9 kg / 9y (CI 0-1.8). A higher baseline BMI was very robustly associated with a weight loss of [0.4 kg / 9y] / BMI unit (CI 0.3-0.5). Different versions of the model design did not influence these estimates much (in the presented model the covariates were age, education, sex, BMI, smoking status, Charlson score in 4 categories, and physical activity).

SMOKING STATUS

Smoking status – especially cessation but also being a smoker – was independently associated with weight change in the INSUAP cohort. Article 1 describes weight gain after smoking cessation in relation to the normal age-related weight development in smokers and never-smokers. The 'smoking cessation weight change model' from article 1 is reprinted here as Figure 10. The complexity of weight changes related to smoking status and age are illustrated. Panel A demonstrates how smokers had a lower baseline weight than comparable never-smokers, and that the never-smokers' age-related weight gain trajectory was steeper than the smokers'. After a post cessation weight gain the quitters regressed towards the 'normal' weight of comparable never-smokers. Panel B highlights the average age-related weight loss in the population aged 50-70 years.

Model check

The variance in the different smoking categories was analyzed. The standard deviation (SD) of weight changes in smokers, quitters, and ex-smokers was similar (Table 5).

Table 5. *Analysis of variance in weight change over 9 years in the smoking categories*

	Never-smokers	Quitters	Cont smokers	Cont ex-smokers	Relapsed
SD	5.9	7.0	5.8	6.0	5.6

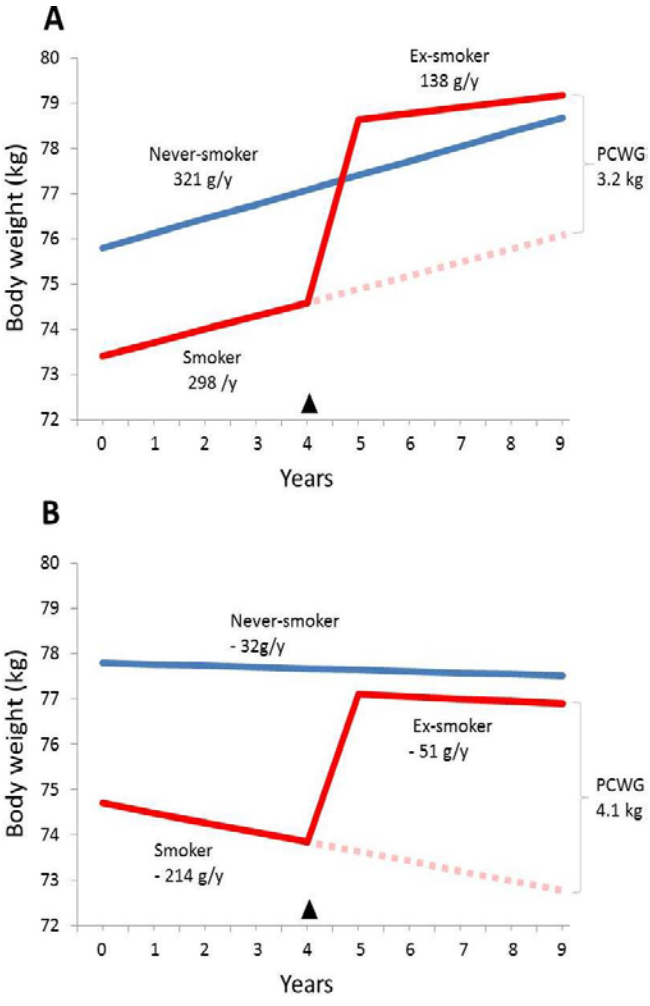

Figure 10. *'Smoking cessation weight change model'. Panel A illustrates the mean weight trajectories by smoking status for subjects*

aged 20-50 years at baseline, and panel B illustrates this in subjects aged 50-70 years at baseline. The triangles denote smoking cessation. (from article 1)

DIETARY DATA

Intake of fructose and soft drinks in relation to weight change

Originally the objective of the PhD project was to analyze the intake of fructose and sugar sweetened beverages in relation to weight changes. However, the intake of these foods was not associated with weight change. This is demonstrated in the 'Standard' analyses in Table 6. To improve the quality of the dietary data, portion sizes and missing frequencies (Appendix 2.6) were imputed with the coca method and multiple imputation. Still, the intake of fructose and soft drinks were not associated with weight change after 9 years.

Table 6. *Daily intake of energy, fructose and soft drinks and subsequent 9 year weight change in the INSUAP population, estimated with standard portion sizes and portion sizes imputed with multiple imputation.*

	Univariate			Energy adjusted			Full model		
	Est (kg/9y)	CI low	High	Est (kg/9y)	CI low	High	Est (kg/9y)	CI low	high
Free fuctose (MJ)									
Standard	-1.8	-4. 8	1.2	*-3.2	-6.3	-0.1	-0.4	-3.3	2.6
MI	-2.5	-5.3	0.4	*-3.8	-6.7	-0.8	-0.2	-3.1	2.7
Total fructose (MJ)									
Standard	1.2	-0. 2	2.6	0.1	-1.5	1.7	0.3	-1.3	1.8
MI	0.2	-1.5	2.0	-1.2	-3.2	0.7	0.0	-1.9	1.9
Sugar sweetened carbonated soft drinks (serving)									
Standard	0.29	-0.29	0.88	0.09	-0.52	0.69	-0.45	-1.00	0.11
MI	0.30	-0.30	0.89	0.10	-0.51	0.70	-0.44	-0.99	0.12
All sugar sweetened bewerages (serving)									
Standard	*0.22	0.01	0.44	0.12	-0.12	0.35	0.07	-0.14	0.28
MI	*0.24	0.02	0.45	0.13	-0.10	0.37	0.09	-0.12	0.30
Total energy (MJ)									
Standard	*0.17	0.07	0.27	-	-	-	0.02	-0.08	0.12
MI	*0.16	0.05	0.28	-	-	-	0.00	-0.11	0.11

*Est is the estimated weight change over 9 years attributable to a daily intake of 1 MJ or one serving of the food in the table. Standard are analyses with the dietary intake estimated with standard portion sizes, and MI are analyses with dietary intake estimated with multiple imputation of portion sizes and missing frequencies (Coca method). Full model: the multivariate model is a general linear model adjusted for the total energy intake, age, sex, baseline BMI, education, change in smoking status, and level of physical activity at baseline. *P<0.05.*

Standard portions vs. multiple imputation techniques to estimate individual portion sizes

In article 2 the dietary intake computed with standard portion sizes, or with portion sizes determined by the 'multinomial logistic regression', Coca, or the 'K nearest neighbors' methods were compared to a reference dietary intake, which was computed with the originally self-reported portion sizes in the DANHES cohort. It was demonstrated how the use of standard portion was inferior to the use of stochastic models conditioning on the available information on physiology. The new stochastic method, 'the comparable categories method' or Coca, was invented. Along with the multinomial logistic regression model, the Coca method induced less bias than the other methods tested.

Intake of fructose and soft drinks in relation to weight change in INSUAP - standard portion sizes vs. portion sizes estimated with multiple imputation

Examples of the differences in estimated weight change attributable to the dietary intake calculated with standard portion sizes (Standard) vs. multiple imputation (MI) is presented in Table 6. The difference between Standard and MI was most pronounced in the univariate and energy adjusted analyses of fructose intake. In the multi variable analyses there not much of a difference. Of notice, multiple imputation of portion sizes affected the estimated intake of fructose and the total energy intake but not the intake of soft drinks (since these were reported as servings with a fixed size - the tiny differences in the univariate analyses derive from imputation of missing frequencies). There was no difference between the two methods in the univariate analyses of energy intake and weight change.

Intake of fat, protein, carbohydrates, and alcohol in relation to weight change

The estimated intake of fat, carbohydrates, or alcohol was not independently associated with weight change (Table 7). The intake of protein was associated with 1.570 kg weight gain (CI 0.311-2.829) over 9 years for each MJ protein consumed per day.

Table 7. *Multivariable analysis of the daily intake of fat, protein, carbohydrates, and alcohol in relation to 9 year weight change in the INSUAP population.*

	Weight change (kg/9 years)	95% low	CI High	P
Dietary intake (MJ/day)				
Fat	-0.397	-0.952	0.158	0.16
Protein	1.570	0.311	2.829	0.01
Carbohydrates	-0.116	-0.637	0.404	0.66
Alkohol	0.118	-0.678	0.914	0.77

Weight change: The estimated weight change over 9 years attributable to a daily intake of 1 MJ of the macronutrient at baseline. The dietary intake is estimated with multiple imputation of portion sizes and missing frequencies (Coca method). The multivariable model is a general linear model adjusted for the total energy intake, age, sex, baseline BMI, education, change in smoking status, and level of physical activity at baseline.

Table 8. *Multivariable analysis of the daily level of physical activity in relation to 9 year weight change in the INSUAP population.*

	Weight change (kg/9 years)	95% low	CI High	P
Physical activity (METs/day)				
Total	-0.185	-0.459	0.088	0.18
Workindex	0.959	-0.023	1.213	0.06
Leisureindex	-0.202	-0.902	0.497	0.57
Sportindex	-0.391	-0.974	0.191	0.19

Weight change: The estimated weight change over 9 years attributable to 1 MET/day. Physical activity was assessed with Baeckes questionnaire (122). Total=workindex+leisureindex+ sportindex. The multivariable model is a general linear model adjusted for the total energy intake, age, sex, baseline BMI, education, change in smoking status. Missing values of physical activity were estimated with multiple imputation (Coca method).

PHYSICAL ACTIVITY

The total level of physical activity, as measured with the Beacke questionnaire (122), was not associated with weight change over 9 years in multivariable analysis (Table 8). However, there was a trend towards weight gain in participants with a high level of physical activity at work.

BIOCHEMICAL MEDIATORS

Baseline insulin sensitivity (HOMA, HOMA2, Matsuda, fasting insulin, insulin AUC), triglyceride level, TSH, or CRP was not independently associated with weight changes in multivariate analyses adjusted for age, baseline BMI and sex. Inclusion of these potential biochemical mediators in the multivariable models did not change the parameter estimates of dietary intake in relation to weight change substantially (results not shown).

THE CHARLSON COMORBIDITY INDEX AND WEIGHT CHANGES

Weight change associated comorbidity and multimorbidity was explored. The Charlson comorbidity index correlates with mortality, but does it correlate with weight changes? If so the Charlson score could be a new tool for adjusting cohort studies of weight change for potential confounding from wasting.

In the INSUAP cohort incident morbidity over 9 years measured with the Charlson comorbidity index (as a continuous covariate) was not associated with weight loss (Table 9). In table 10 the Charlson score was analyzed as a categorical covariate. A Charlson score of 1 or 2 was correlated with weight gain compared with healthy participants, and there was a trend towards weight loss in patients with a score of 3 or more. Among participants with a Charlson score >0 (n=291) there was a tendency (P=0.09) towards an association between the Charlson score as a continuous covariate and weight loss (Table 11). Thus, among 'unhealthy' subjects the continuous Charlson score co-varied with weight loss, whereas it co-varied with weight change as a categorical covariate in the unselected population.

Table 9. *Charlson score as continuous variable – association with weight change over 9 years – all participants*

	Estimate Kg /point / 9 years	p
Univariate	- 0.492	0.08
Adjusted for age	- 0.118	0.44
Full model1	- 0.068	0.64

1) Multiple regression linear model with incremental adjustment for covariates. The full model included age, sex, baseline BMI, education and change in smoking.

Table 10. *Charlson score in four categories – association with weight change over 9 years – all participants*

Charlson Categori	n	Full adjustment Kg / 9 years	p
A (0)	1083	ref	ref
B (1,2)	222	1.247	0.01
C (3-8)	57	- 889	0.39
D (9-12)	12	- 2255	0.30

Table 11. *Charlson score as continuous variable – association with weight change over 9 years – only participants with disease (charlson>0)*

	Estimate Kg /point / 9 years	P
Univariate	- 0.565	0.007
Adjusted for age	- 0.386	0.06
Full model1	- 0.317	0.09

1) Multiple regression linear model. Adjusted for age, sex, baseline BMI, education and change in smoking.

LONG-TERM OUTCOMES ASSOCIATED WITH WEIGHT CHANGE IN PATIENTS WITH TYPE 2 DIABETES

Article 3 revealed that in the DCGP cohort intentional therapeutic weight loss among overweight patients with type 2 diabetes was not associated with reduced cardiovascular morbidity, cardiovascular mortality, or all-cause mortality after 13 years of follow-up.

In a multivariate analysis the β-coefficient of a regression line through all registered weights in each patient (Figure 11) was correlated with the incidence of the outcomes within the group of patients with intention to lose weight. Figure 12a demonstrates the main result from article 3: The patients who, despite intention, maintained their weight had the lowest risk for all-cause mortality. The confidence interval did not overlap a HR of 1.0 between 0.5 and 3 kg of weight loss per year, indicating an increased risk in this interval. Among patients with intention to maintain weight this tendency was more pronounced (Figure 12b). There was an increased risk of all-cause mortality attributable to intentional weight loss among patients with a BMI>30 (Table 12), microvascular disease (Table 13), or microalbuminuria (1.85, CI 1.06-3.24. data not shown in tables) at diagnosis. There was an insignificant tendency towards a protective effect of weight loss in patients with BMI of 25-30 (Table 11). Tables 14 and 15 hold results from the sensitivity analyses. Adjustment for laboratory test results or a narrowed definition of intention to lose weight did not change the associations much. Neither among active patients (Figure 12d) nor among patients without cardiovascular disease at diagnosis (12e) there was any association between weight loss and mortality.

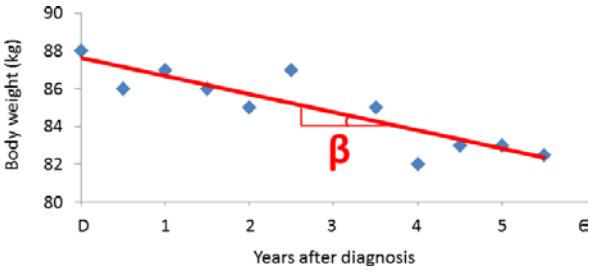

Figure 11. *Example of weight monitoring in one patient. The median number of weight registrations was 13 per patient. The median time between consultations was 106 days. For each patient the weight change was modelled with a regression line through all measured weights in the 6-year monitoring period. The exposure of interest is the slope (the β coefficient) of this regression line. This slope denotes the average yearly weight change (kg/year).*

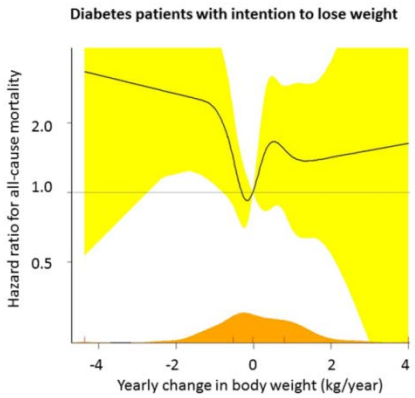

Figure 12a. *Weight change in patients with intention to lose weight and subsequent HR for all-cause mortality (the main analysis from article 3). The spline function illustrates the association between the average yearly weight change in the 6 years monitoring period after the diabetes diagnosis, and the subsequent 13 years' hazard ratio (HR) for all-cause or cardiovascular mortality. The cox model is adjusted for age, gender, education, BMI at diagnosis, smoking change, physical activity change, medication change, and the Charlson comorbidity score in the 6 year monitoring period. The y-axis is logarithmic. Black line: cubic spline estimate, 6 data driven nodes. Yellow: 95% confidence intervals. Orange: the distribution of the patient material. Red lines: median, inter-quartile range and min/max.*

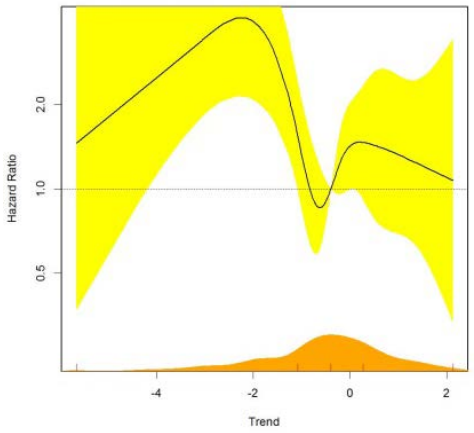

Figure 12b. *Weight change in patients with intention to maintain weight and HR for all-cause mortality.*

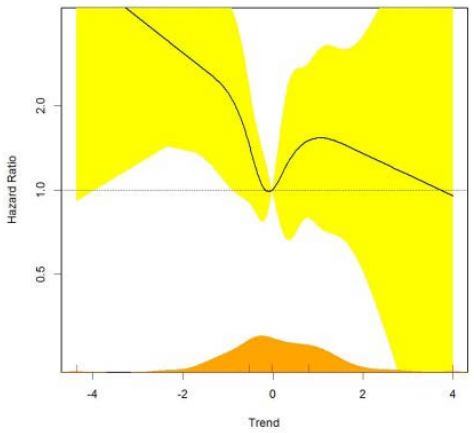

Figure 12c. *Weight change in patients with intention to lose weight and HR for all-cause mortality (adjusted for CVD at baseline in 3 categories [none, micro, macro] instead of the Charlson comorbidity score).*

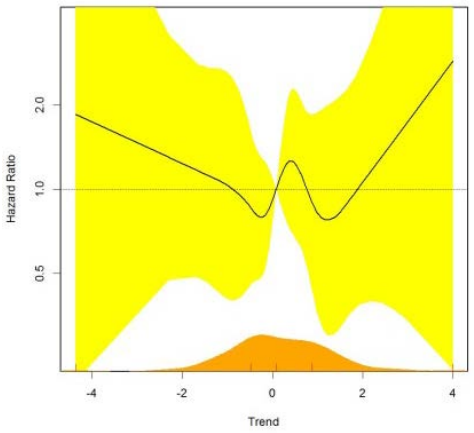

Figure 12d. *Weight change in active patients with intention to lose weight and HR for all-cause mortality.*

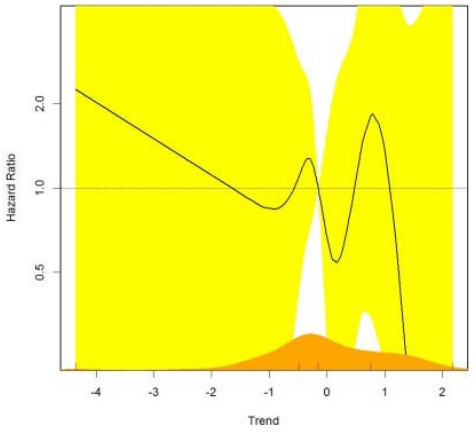

Figure 12e. *Weight change in patients without CVD at diagnosis with intention to lose weight and HR for all-cause mortality.*

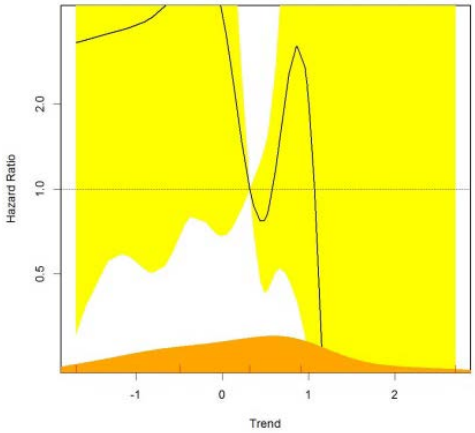

Figure 12f. *Weight change in patients with microvascular disease at diagnosis with intention to lose weight and HR for all-cause mortality.*

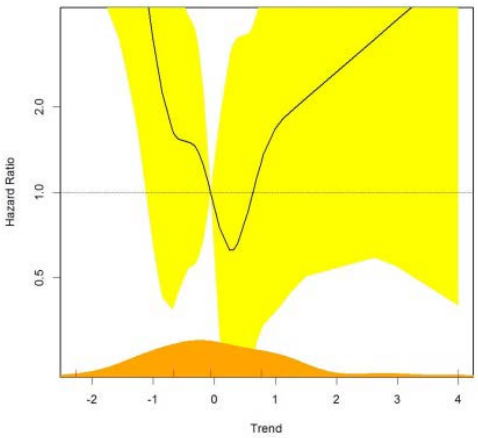

Figure 12g. *Weight change in patients with macrovascular disease at diagnosis with intention to lose weight and HR for all-cause mortality.*

Intention to lose weight

Post hoc we analyzed the mortality risk between the group with intention to lose weight and the group with intention to maintain weight. Otherwise, the same confounders were used as in the main analysis. Intention to lose weight was not associated with mortality in the multivariable analysis (HR 1.16; CI 0.85-1.59; P=0.34). In this analysis weight loss was - independent of intentions - significantly associated with increased mortality (HR 1.20; 1.06 - 1.35; P=0.004).

DCGP proportional hazards assumption

The hazard has to be parallel and incrementally increased (or decreased) in covariates. The proportional hazard criteria were fulfilled with the included covariates. Asymmetry could arise if the risk of weight loss was greater right after the monitoring period. The two year period ('First 2 years' in Tables 12-15) serves as a protection from that the hazards may not be proportional in this period. However, among patients with intention to lose weight there was no difference in hazard between the first two years and the remaining 11 years follow-up (Table 2 in article 3).

DATA SIMULATION STUDY

In the background chapter it was explained how different assessment of baseline BMI in the exposure group (intentional weight loss) and the comparison group (no weight change) may well have generated bias by overestimating the baseline BMI in the exposure group. High BMI is a well-known predictor of increased mortality (1), and is often included as a continuous covariate in the multivariable analyses of mortality.

The potential bias (the overestimated baseline BMI) pertained solely to the exposure groups' baseline BMI, and did not affect the size of the exposure (since it was categorical). The objective of this simulation study was to demonstrate the direction of the resulting bias in a multivariable analysis if baseline BMI was overestimated in the weight loss category only.

Methods

Logistic regression with simulated data (the R code is in Appendix 1). The multivariable analysis includes two variables: the exposure and one covariate

- Exposure: weight loss vs. constant weight (categorical +/-).

Table 12. *Sensitivity analyses of mortality and morbidity risk attributable to one kg of weight loss per year in patients with 'intention to lose weight.'*

	All patients1 BMI<30		All patients1 BMI>30		BMI<30 and 'intention to lose weight'		BMI>30 and 'intention to lose weight'	
	HR (95%CI)	p	HR (95%CI)	P	HR (95%CI)	p	HR (95%CI)	p
All-cause mortality	[102/167]		[115/212]		[36/63]		[56/128]	
First 2 years2	1.53 (0.90-2.60)	0.12	1.47 (1.08-2.00)	0.01	0.60 (0.13-2.90)	0.53	1.49 (0.84-2.61)	0.17
After 2 years3	0.93 (0.69-1.23)	0.59	1.17 (1.01-1.36)	0.04	0.67 (0.38-1.18)	0.17	1.33 (0.96-1.84)	0.09
Difference4		0.08		0.18		0.90		0.72
Full follow up period5	1.01 (0.77-1.32)	0.94	1.22 (1.06-1.40)	<0.01	0.66 (0.38-1.14)	0.14	1.36 (1.01-1.84)	<0.05
Cardiovascular mortality	[60/166]		[76/212]		[19/63]		[30/128]	
First 2 years	1.68 (0.91-3.10)	0.10	1.46 (1.03-2.03)	0.02	0.80 (0.13-5.01)	0.81	1.38 (0.66-2.65)	0.44
After 2 years	1.04 (0.71-1.51)	0.33	1.05 (0.85-1.29)	0.68	0.91 (0.38-2.18)	0.83	1.00 (0.62-1.61)	0.99
Difference		0.16		0.08		0.90		0.49
Full follow up period	0.96 (0.67-1.38)	0.83	1.10 (0.92-1.32)	0.28	0.89 (0.40-1.98)	0.77	1.07 (0.69-1.66)	0.75
Cardiovascular morbidity	[58/135]		[74/175]		[25/55]		[30/106]	
First 2 years	0.91 (0.45-1.87)	0.80	1.40 (0.94-2.10)	0.10	1.81 (0.41-8.05)	0.43	1.71 (0.39-7.61)	0.48
After 2 years	0.76 (0.53-1.10)	0.15	1.01 (0.81-1.26)	0.92	1.11 (0.54-2.27)	0.77	1.02 (0.65-1.60)	0.92
Difference		0.64		0.15		0.54		0.51
Full follow up period3	0.78 (0.55-1.11)	0.17	1.08 (0.89-1.32)	0.44	1.19 (0.60-2.35)	0.62	1.06 (0.68-1.64)	0.81

Values are [number of events/ numbers of observations used] and hazard ratios, HR (95% confidence intervals) from multivariate Cox regression analysis of the association between weight loss in kg/year (modeled as the slope of a regression line through all the measured weights for each patient) and the outcomes. The multivariate analyses are stratified on BMI, or on BMI and intention to lose or to maintain weight, and the covariates are: age, gender, education, BMI at diagnosis, smoking change, physical activity change, medication change, and the Charlson comorbidity score in the monitoring period. 1) All patients despite intention to change weight. 2) HR for mortality in the first 2 years period of follow up. 3) HR for mortality in the remaining 11 years period of follow up ('After 2 years'). 4) Difference in HR between the first 2 years and the remaining 11 years of follow-up. 5) The total follow up period was 13 years.

- Covariate: baseline BMI (continuous).

Outcome: mortality (categorical +/-).

Data properties:

- Mean baseline BMI=25 (SD=4) in both categories (weight loss and constant weight).
- One unit increase in BMI increases the odds of mortality with 20%.
- Mortality risk for BMI 25 is 10%.
- Weight loss (+/-) is not associated with mortality (OR=1).

Table 13. *Sensitivity analyses of mortality and morbidity risk attributable to one kg of weight loss per year in patients with 'intention to lose weight.'*

	No CVD at diagnosis		Microvascular complications at diagnosis		Macrovascular complications at diagnosis	
	HR (95%CI)	p	HR (95%CI)	p	HR (95%CI)	p
All-cause mortality	[28/78]		[29/59]		[31/47]	
First 2 years1	1.33 (0.45-3.92)	0.61	1.79 (0.43-7.50)	0.43	1.65 (0.60-4.54)	0.33
After 2 years2	1.01 (0.61-1.68)	0.98	2.16 (1.18-3.94)	0.01	1.19 (0.77-1.86)	0.43
Difference3		0.62		0.80		0.54
Full follow up period4	1.04 (0.63-1.70)	0.89	2.11 (1.19-3.74)	0.01	1.25 (0.82-1.91)	0.31
Cardiovascular mortality	[10/78]		[14/59]		[24/47]	
First 2 years	1.32 (0.41-4.27)	0.65	1.46 (0.29-7.38)	0.64	1.44 (0.36-5.78)	0.61
After 2 years	1.04 (0.45-2.42)	0.93	4.18 (1.10-15.9)	0.04	1.00 (0.60-1.66)	0.98
Difference		0.68		0.31		0.61
Full follow up period	1.09 (0.49-2.45)	0.83	2.82 (1.01-7.91)	0.048	1.02 (0.62-1.70)	0.93
Cardiovascular morbidity	[21/74]		[17/53]		[15/27]	
First 2 years	1.95 (0.51-7.48)	0.33	0.91 (0.20-4.21)	0.91	1.05 (0.04-30.2)	0.98
After 2 years	0.83 (0.40-1.72)	0.62	1.20 (0.55-2.61)	0.65	0.63 (0.14-2.75)	0.53
Difference		0.21		0.75		
Full follow up period	0.92 (0.46-1.84)	0.81	1.14 (0.55-2.34)	0.73	0.63 (0.14-2.80)	0.54

Values are [number of events/ numbers of observations used] and hazard ratios, HR (95% confidence intervals) from multivariate Cox regression analysis of the association between weight loss in kg/year (modeled as the slope of a regression line through all the measured weights for each patient) and the outcomes. The covariates are: age, gender, education, BMI at diagnosis, smoking change, physical activity change, medication change, and the Charlson comorbidity score in the monitoring period. 1) HR for mortality in the first 2 years period of follow up. 2) HR for mortality in the remaining 11 years period of follow up ('After 2 years'). 3) Difference in HR between the first 2 years and the remaining 11 years of follow-up. 4) The total follow up period was 13 years.

Table 14. *Sensitivity analyses of mortality risk attributable to one kg of weight loss per year – in patients with intention to lose weight.*

	Additionally adjusted for mean HbA1c1		Additionally adjusted for triclycerides2		Additionally adjusted for systolic and diastolic blood pressure2	
	HR (95%CI)	p	HR (95%CI)	p	HR (95%CI)	p
All-cause mortality						
First 2 years3	1.42 (0.63-3.20)	0.40	1.26 (0.77-2.07)	0.37	1.33 (0.81-2.19)	0.26
After 2 years4	1.02 (0.73-1.41)	0.93	1.21 (0.95-1.55)	0.13	1.21 (0.95-1.54)	0.13
Difference5		0.46		0.89		0.72
Full follow up period6	1.21 (0.97-1.50)	0.093	1.22 (0.97-1.53)	0.085	1.23 (0.98-1.54)	0.075
Cardiovascular mortality						
First 2 years	1.35 (0.77-2.38)	0.29	1.38 (0.77-2.47)	0.28	1.44 (0.82-2.54)	0.21
After 2 years	1.22 (0.86-1.72)	0.27	1.29 (0.91-1.84)	0.15	1.27 (0.89-1.81)	0.19
Difference		0.74		0.85		0.70
Full follow up period	1.25 (0.92-1.70)	0.16	1.31 (0.96-1.80)	0.089	1.31 (0.95-1.80)	0.095
Cardiovascular morbidity						
First 2 years	1.30 (0.80-2.14)	0.29	1.42 (0.64-3.13)	0.39	1.47 (0.65-3.36)	0.36
After 2 years	1.19 (0.94-1.51)	0.15	1.01 (0.73-1.39)	0.95	1.04 (0.75-1.45)	0.80
Difference		0.73		0.43		0.44
Full follow up period	1.06 (0.78-1.44)	0.71	1.06 (0.78-1.43)	0.72	1.09 (0.80-1.49)	0.59

Values are hazard ratios, HR (95% confidence intervals) from multivariate Cox regression analysis of the association between weight loss in kg/year (modeled as the slope of a regression line through all the measured weights for each patient) and the outcomes. The covariates are: age, gender, education, BMI at diagnosis, smoking change, physical activity change, medication change, and the Charlson comorbidity score in the monitoring period. 1) mean HbA1c in the 6 years monitoring period. 2) Values at diagnosis. 3) HR

for mortality in the first 2 years period of follow up. 4) HR for mortality in the remaining 11 years period of follow up ('After 2 years'). 5) Difference in HR between the first 2 years and the remaining 11 years of follow-up. 6) The total follow up period was 13 years.

Table 15. *Sensitivity analyses of mortality risk attributable to one kg of weight loss per year – in patients with intention to lose weight.*

	Additionally adjusted for systolic blood pressure at diagnosis		Narrowed definition of intention to lose weight1 (n=158)		Only patients without macro-vascular disease at diagnosis (n=157)	
	HR (95%CI)	p	HR (95%CI)	p	HR (95%CI)	p
All-cause mortality						
First 2 years2	1.41 (0.83-2.38)	0.20	1.71 (0.79-3.72)	0.18	1.39 (0.69-2.78)	0.36
After 2 years3	1.23 (0.96-1.59)	0.10	1.04 (0.76-1.43)	0.80	1.33 (0.99-1.79)	0.06
Difference4		0.65		0.25		0.91
Full follow up period5	1.27 (1.00-1.61)	0.05	1.12 (0.83-1.50)	0.46	1.33 (1.02-1.79)	0.04
Cardiovascular mortality						
First 2 years	1.61 (0.83-3.23)	0.16	2.15 (0.82-5.66)	0.12	1.61 (0.75-3.45)	0.23
After 2 years	1.23 (0.84-1.82)	0.28	1.09 (0.64-1.83)	0.76	1.56 (0.93-2.63)	0.09
Difference		0.48		0.21		0.97
Full follow up period	1.30 (0.92-1.85)	0.13	1.26 (0.79-2.02)	0.33	1.59 (1.00-2.50)	0.05
Cardiovascular morbidity						
First 2 years	1.52 (0.77-3.03)	0.23	1.30 (0.50-3.43)	0.59	1.22 (0.53-2.86)	0.64
After 2 years	1.06 (0.89-1.28)	0.49	0.68 (0.42-1.11)	0.13	1.11 (0.92-1.33)	0.28
Difference		0.32		0.22		0.82
Full follow up period	1.09 (0.91-1.30)	0.35	0.75 (0.48-1.19)	0.22	1.11 (0.93-1.33)	0.25

Values are hazard ratios, HR (95% confidence intervals) from multivariate Cox regression analysis of the association between weight loss in kg/year (modeled as the slope of a regression line through all the measured weights for each patient) and the outcomes. The covariates are: age, gender, education, BMI at diagnosis, smoking change, physical activity change, medication change, and the Charlson comorbidity score in the monitoring period. 1) Narrowed definition of intention to lose weight: 6 or more recorded goals of weight loss, and at least one of these goals should still be recorded in one of the 3 last consultations. 2) HR for mortality in the first 2 years period of follow up. 3) HR for mortality in the remaining 11 years period of follow up ('After 2 years'). 4) Difference in HR between the first 2 years and the remaining 11 years of follow-up. 5) The total follow up period was 13 years.

Simulated bias in baseline BMI: With 'No bias' the mean baseline BMI is the same in the weight loss as in the constant weight category. Then one BMI unit is systematically added to the baseline BMI in the weight loss group (Bias=1, Bias=2, …)

Table 16. *The values are the estimated odds ratios for mortality in the weight loss group (true OR=1).*

	No bias Initial BMI=25	Bias=1 initial BMI=26	Bias=2 Initial BMI=27	Bias=3 Initial BMI=28	Bias=4 Initial BMI=29
OR for mortality in the weight loss group	1.00	0.85	0.70	0.58	0.51

Interpretation

In this model (with only one covariate) increasing the bias (overestimation of baseline BMI in weight losers) in the covariate incrementally decreased the weight losers OR for mortality (misleadingly, since the OR=1). The effect of the bias in baseline BMI was acting through a confounding effect on the weight loss-mortality association.

DISCUSSION

WEIGHT CHANGES IN THE GENERAL POPULATION

In the INSUAP cohort weight changes over 9 years were mainly determined by age, sex, education, baseline BMI, and change in smoking status. Age was a powerful determinant of weight change with high weight gain rates in young adults, lower weight gain rates in middle aged adults, and an average weight loss in subjects older than 60. The analyses of weight history suggest that, on average, weight is gained throughout adulthood to the age of 60-65 years. We have called this age-related weight change pattern the 'normal weight development'. Lifestyle factors like physical activity, the total energy intake, or the macronutrient composition of the diet at baseline were not associated with long-term weight changes, except the intake of protein that was associated with weight gain. However, a lifestyle factor like smoking status and in particular smoking cessation was highly predictive of weight change. The 'smoking cessation weight change model' demonstrated the effects of smoking and smoking cessation in relation to the normal weight development. One or two comorbidities, as measured with the Charlson comorbidity index, was associated with weight gain compared with healthy participants, whereas multimorbidity (a Charlson score of 3 or more) seemed to be related to weight loss.

Limitations

Article 1 and the evaluation of weight change in the 'general population' were based on the INSUAP cohort. However, the cohort is community-based, and the participants may not be entirely representative for the Danish population. On the other hand, the age and gender distribution of the baseline participants was close to the national average (Table 3) and the high participation rate and the completeness of the data most likely ensured a relatively good representation of different segments in the local population.

Altogether 85% of the baseline participants participated in the follow-up study (Table 4). Even though the 200 subjects not attending the follow-up study were somewhat younger than those attending, this younger age group was overtly well represented in the baseline study (Table 3), and there were no dissimilarities between attenders and non-attenders in the follow-up study regarding the other baseline characteristics or Charlson score during the follow-up period. Thus, it is probably reasonable to regard the INSUAP cohort as reasonably representative for the background population in these studies of weight changes.

Several assumptions and limitations are listed in article 1. A general limitation not mentioned is that the focus on average weight change hides a major individual variability. However, the use of means is a fundamental condition in epidemiology, and the

variability (SD) in weight changes among for instance the different categories of smokers in article 1 was similar (Table 5). Nonetheless, the predictive power of the model for a given individual that quits smoking is probably low.

The baseline weight was obtained by clinical examination but the follow-up weight was self-reported. In general, self-reported weight is underestimated compared with clinical examination, and this bias may differ between men and women as well as for other characteristics in the population (64). In a general practice population the average self-reported weight was underestimated by 1.2 kg in both men and women compared with measures taken by the general practitioner, and there was no age bias (63). On the other hand, the weight history depicted in Figure 8 illustrates that the birth cohorts 48-68 reported a higher weight one year prior to the baseline study than the weight that was measured at baseline, despite these birth cohorts could be expected to have gained a little weight on average. Still, it seems reasonable to anticipate that the weight change in the period is probably underestimated by approximately 1 kg on average. One study of overweight employees found that non-smokers underreported their weight by 0.2 kg more than smokers (1.4 kg vs. 1.2 kg) (65). However, this estimate is not directly transferable to the smokers in the rather unselected INSUAP cohort, but it indicates that weight-reporting bias by smoking status is not large.

The normal weight development

The correlation between age and weight gain rate was strong in all subgroups examined. A similar weight gain rate pattern can be deducted from the weight change tables in the NHANES 2 survey with 10 years follow-up (28). The analyses of weight history combined with the baseline and follow-up assessments indicate that weight was gained throughout adulthood in all birth cohorts examined. The lower panels in Figure 8 further indicate that the weight peaked around 60 years in the oldest birth cohorts. These findings are in accordance with the weight change pattern (based on numerous data points) described in male physicians by Barone et al (22). In INSUAP the growth appears to be almost linear with a steeper slope in the younger birth cohorts. This finding indicates that the very large 9 year weight gain rates seen among the young adults in Figure 5-7 may partly be explained by a birth cohort effect. It is alarming that all of the birth cohorts examined gained approximately 10 kg on average (estimated from Figure 8) since they were 20 years old as the younger birth cohorts may not have reached their peak yet. Some of the high weight gain rate among the young adults may also be explained by selection, as young adults in the higher education system may be underrepresented due to rural setting of the study. Still, age seems to be a good surrogate marker for an underlying apparently normal physiological processes resulting in a general weight gain till the age of 60-65 years and a plateau or decline thereafter. Therefore this phenomenon will be referred to as 'the normal weight development'.

It should be emphasized that the ideas regarding the normal weight development are priliminary and still in the exploration phase. The results are unpublished and needs further work-up before they will be presented in a scientific article.

It may be argued that it is misleading to talk about a normal weight development in the midst of an obesity epidemic. In Denmark the development of the obesity epidemic is overtly well described by for instance data from the mandatory draft board examinations in two regions resulting in a practically unselected sample of 95% of all young men born since 1943 (99). Analyses on this cohort revealed that the 95th percentile increased by 2 BMI points between subjects born in 1943 and subjects born 1972-74, whereas the median was reduced by 0.2 BMI point and the population mean was increased by 0.2 BMI point between these birth cohorts. Thus, it appears that the obesity epidemic affected mainly the heaviest individuals and that the population mean was largely unchanged in this population of young men. However, there is no doubt that the average height and weight in the general population increased in the INSUAP follow-up period (100) . On a national level the increase took place in all age groups (based on a number of cross sectional surveys) and among both men and women.

A study using data from both the draft board examinations and the mandatory in-school health examinations in Copenhagen from 1930-1983 identified two sharp increases in the prevalence of obesity in the birth cohorts 1940 to 1955 and from birth cohort 1970 to 1980 and two plateaus (37). These periods are depicted in Figure 13. The steady increase in baseline BMI with older birth cohorts seems almost reversely related to the phases of the obesity epidemic. Yet, the leaps in the intensity of the obesity epidemic may not be entirely parallel in Ørum and in the Copenhagen area. But, the development was parallel in Copenhagen and North Zealand (the rural region north of Copenhagen) (99).

The normal weight development pattern could in theory also be explained by a survivor effect if old people gaining weight died more often than others, leaving behind the weight losers. Yet, multiple imputation of weight change in the dead participants did not alter the pattern observed (Figure 6 panel A). However, multiple imputation in this setting may not be entirely adequate since the missing values cannot be said to be missing at random. Regarding the correlation between age and baseline BMI there was no sign of an 'inverted U'. On the contrary, the BMI appeared to increase with age also among the oldest participants. This may at a first glance seem incompatible with the general weight loss among older participants described in the normal weight development, but the increase in BMI is probably partly explained by decreasing height with increasing age (101). Overall, the increasing BMI with age does not leave much room for a survivor effect.

Altogether, the concept of a normal weight development does not seem to be an artifact of the obesity epidemic or explained by a survivor effect. Still, the analyses of weight history indicate that the 'normal' seems to be evolving.

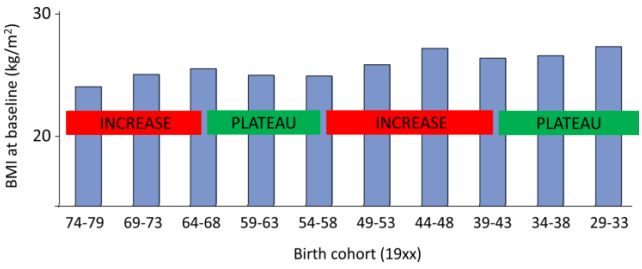

Figure 13. *Baseline BMI by birth cohort in the INSUAP population (same data as Figure 9). The red bars indicate birth cohorts with increase in the prevalence of obesity and the green bars indicate birth cohorts with unchanged prevalence (37). The development of the obesity epidemic should be 'read' from right to left.*

Comorbidity and multimorbidity

The objective of developing the Charlson comorbidity index was 'to develop a prospectively applicable method for classifying comorbid conditions which might alter the risk of mortality for

use in longitudinal studies' (72), and it is well documented that the Charlson index provides a valid method of estimating risk of death from comorbid disease in longitudinal studies. To my knowledge no one has published results or considerations regarding weight changes in relation to Charlson score. If specific diseases result in different weight changes (e.g. large negative weight change with disease B and small positive with disease A) controlling multivariate analyses of mortality for specific diagnoses would probably provide a more precise adjustment of the mortality estimate, than using the Charlson score. However, categorical adjustment for specific diagnoses requires many more degrees of freedom (depending on the number of comorbidities) as compared to just one with the continuous Charlson score. Furthermore, there may well be an additive effect of having multiple comorbidities – or multimorbidity - that cannot easily be captured by adjusting for the single entities categorically. This was indicated by our finding that a score of 1 or 2 was related to weight gain compared with healthy individuals, whereas a score of 3 or more was related to a weight loss. Thus, the Charlson index may well provide a better estimate of weight changes related to disease burden than adjustment for the single entities, but this will have to be explored further and confirmed in other cohorts. Another multimorbidity scoring system including also mental disorders could also be considered.

Lifestyle

In contrast to what most lay people may imagine (10) lifestyle factors were not convincingly associated with long term weight changes in INSUAP. Physical activity at baseline was not associated with weight change, except from the trend towards weight gain in participants with a high level of physical activity at work. This finding may to some degree be explained by residual confounding from socioeconomic status that was not captured by the education/work covariate, but it may also reflect a true increase in muscle mass.

The dietary intake examined was not associated with weight change, except the intake of protein that was associated with weight gain. This finding is in contrast to the prevailing ideas about protein intake that, by many researchers, is regarded as a key element in the dietary prevention of obesity and in weight loss maintenance diets (123;124). This could be explored further, but since it as a post hoc analysis, I regard it as a chance finding.

In summary, our measures of lifestyle did not appear to be important for the long-term weight development. Whether this is true or it is a consequence of the poor instruments for measuring lifestyle (as described in the background chapter) remains an open question.

The total energy intake and weight change

In an early stage the total energy intake was included in the INSUAP weight change model. At a first glance this made sense, but the correlation of energy intake with weight change disappeared when the model was adjusted for baseline BMI, sex and age. After rethinking the model in terms of mediators of effect, the total energy intake was left out (in analyses that did not concern the dietary intake) for several reasons:

4. In line with the energy balance theory we already know that weight changes are fundamentally caused by a larger intake than expenditure of energy. What we are really interested in, is learning about the possible explanations for, or predictors of this imbalance.
5. The total energy intake is mediating the effect of all other factors influencing the energy intake. For instance, by adjusting for total energy the effect of age will be attenuated as the energy intake to some degree explains why younger participants gained weight faster than the older.
6. Even in a change-model the adjustment for the change in total energy intake would confuse the interpretation of the analysis; for instance a weight gain attributable to smoking cessation will be partly explained by an increase in energy intake, diluting or disguising the underlying causal relation.
7. The measurement error is rather large and affected by bias dependent on sex, age, and BMI. Bias we do not want to bring into the analysis if possible.
8. The basal metabolic rate as calculated by BMI, sex, age, and physical activity is almost definitely a better estimate of the energy intake and expenditure. When the analysis is already adjusted for these variables it may be argued that adjustment for the total energy intake is unnecessary.

In conclusion, adjusting for the total energy intake will in this context make the analysis of weight change difficult to interpret, induce bias, it will not add useful information, and dilute the associations we are really interested in. Of notice, these arguments are not valid in analyses of the macronutrient composition of the diet and an outcome (102).

BIAS IN PROCESSING OF DIETARY DATA

In article 2 it was demonstrated how median imputation methods were suboptimal when processing dietary data with many missing values. The article suggested solutions on how to reduce bias caused by imputation. The Coca method is a simple and intuitively meaningful method. Along with the multinomial regression method, Coca provided the best way to handle missing values by the use of multiple imputation. The specific example was missing portion sizes, but the methods can be applied on other types of missing values in epidemiology. The results from article 2 are carefully discussed in the paper, and the application of the methods is described in APPENDIX 2.

Limitations

The DANHES aimed to establish a population based cohort, but probably ended up with the more health conscious 1/3 of the population including 59% women. The subsample used in article 2 cannot be said to be a proper population based sample as the participants were selected through multiple steps of health conscious behavior (i.e. attending the survey, showing up for physical examination, completing long questionnaires). It may be argued that the external validity of the methods can be questioned as the included subjects differed slightly from the excluded. However, the question is not whether the included and the excluded were comparable, but rather whether the relation between physiology and portion sizes was different among the included and excluded, which does not seem very plausible.

All available data may be used to construct the imputations. There is no requirement that the imputation model necessarily mimics a data generating mechanism, e.g. data collected later than the FFQ data may be used for imputation (if a month later another FFQ was handed out, now with portion sizes, then this portion size data would be very informative for the missing portion size data in the previous FFQ. Multiple imputation requires that the missingness is not informative, i.e. that the fact that a value is missing is not dependent on the value that is missing. This assumption is unfortunately untestable – knowledge about the

data collection is necessary – and generally it is justified by just including a lot of information in the imputation model. Of notice, the no-informative-missing requirement is fulfilled in the present use of Coca where ALL portion sizes are missing regardless of their value. However, the Coca method has the limitation that it breaks down when too many informing variables are used – the classes become too small – so if many informing variables must be used to justify that the missingness is random, Coca may not be the method of choice.

Analyses of the dietary intake in the INSUAP dataset with multiple imputation of portion sizes from KRAM

The results obtained by the use of multiple imputation techniques did not differ much from the results obtained with the use of standard portion sizes. Analyses of soft drinks were virtually unchanged. This is not surprising as the portion size of a soft drink was fixed, and only the total energy intake covariate vas affected. In the analyses of macronutrient intake both the exposure variable and the total energy covariate were affected. Compared with the analyses with standard portions, the estimates obtained with multiple imputation analyses seemed to be closer to zero. These results do not necessarily imply that the value of multiple imputation techniques is overrated. If there in fact is no relation between dietary composition and weight change an effect cannot be measured no matter how good the data are.

BIAS IN BASELINE BMI AND THE SIMULATION STUDY

In the background chapter it was suggested that CPS-1, and possibly other studies with retrospectively estimated weight loss, may have overestimated the baseline BMI in the weight loss group.

Two mechanisms could potentially contribute to this bias.

1. Measurement error: A participant could truthfully report a weight loss of 10 kg in the last year. However, with our knowledge about the difficulties related to maintaining a weight loss, the participant may well have regained some (or even all of the) weight at the time of the query. The baseline BMI in the weight loss group ('initial BMI') was calculated with the reported body weight and height at the time of the query and the claimed weight loss. If any weight was regained since the reported weight loss the initial BMI was over estimated. This mechanism is illustrated in Figure 1 and 2.
2. Information bias: The participants may have reported a larger weight loss than they actually achieved. I don't know if this is true, but I think it is more likely that the participants overestimated their weight loss than they underestimated it. This hypothesis finds some support in Williamson 2000 paper (84) where the average weight loss (in the intentional weight loss group) in the last year or two was 10.9 kg, which is certainly very impressive in a population without any systematic weight loss intervention program.

The data simulation study demonstrated that a bias (that is pertained solely to the exposure group) in a confounder that is associated with the outcome, may affect the relation between the exposure and the outcome. I think this is quite straight forward epidemiology: some of the mortality in the weight loss group was incorrectly accounted for by a faulty higher baseline BMI. This is of cause only a problem in studies of real populations if baseline BMI is in reality associated with mortality. This is well documented in the background population (1), but may not be the case in patients with type 2 diabetes. But, I'll restrain from opening the discussion regarding the 'obesity paradox' in patients with diabetes.

The results of the data simulation study support the hypothesis as an overestimation of baseline BMI in weight losers would falsely decrease the weight losers OR for mortality, at least in studies of subjects unselected for disease. The effect of this bias in baseline BMI was acting through a confounding effect on the weight loss-mortality association. However, the model contained only one covariate, and it is difficult to predict how much this mechanism would have affected the outcome in the exposure group in real data in a full model with more covariates and uncertain relations between these.

And for the nerds: If the exposure variable (weight loss) had been continuous, an overestimation of the initial BMI would also have resulted in an 'exposure-effect'. This additional mechanism was included in an earlier version of the simulation study, and unveiled that the 'exposure effect' did not affect the outcome estimate, but the flaw in the estimated OR was attributable solely to the bias in BMI (Appendix 1).

THERAPEUTIC WEIGHT LOSS AND MORBIDITY/MORTALITY IN PATIENTS WITH TYPE 2 DIABETES

In article 3 intentional therapeutic weight loss was not associated with reduced incidence of CVD or mortality in patients with type 2 diabetes. On the contrary, there was a trend towards increased mortality. This was an unexpected finding. Our 'a priori' hypothesis was that therapeutic weight loss would decrease mortality and that weight gain would increase mortality as illustrated in Figure 14. Additional results from the sensitivity and subgroup analyses are presented in the results chapter above and discussed below in the section 'Interpretation of article 3 in relation to the literature in the field'. First, the material, methods, limitations, Harrington's meta-analysis, and the Look AHEAD trial will be carefully evaluated.

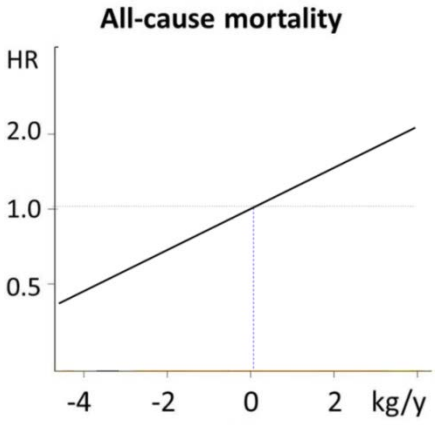

Figure 14. *A sketch of our hypothesis*

MATERIAL – DCGP

The DCGP cohort is truly a population based sample of patients newly diagnosed with type 2 diabetes in Denmark 1989-92. The patients and their treatment are in many ways comparable with diabetes patients and their treatment today, as the intervention –

structured care – has become the general treatment strategy with only few alterations; the lifestyle intervention is largely unchanged. However, risk factors for CVD are treated much more aggressively today than they were 20 years ago. Drugs like statins were uncommon, but are now standard treatment. Also most diabetes patients today are diagnosed at an earlier disease stage than the DCGP participants were. Compared with other studies of intentional weight loss the DCGP cohort was overly well-monitored. No other studies in this field have continuous measurements of weight, continuous descriptions of the goal for weight change, and physical examinations every third month. DCGP is an inception cohort, that included patients >40 years when they were diagnosed in general practices throughout Denmark. In terms of disease severity, this inclusion strategy resulted in a relatively homogeneous cohort of newly diagnosed patients. In contrast, all other studies of weight change in diabetes patients – including the Look AHEAD trial – included patients at different stages of a therapeutic runway. Therefore, these participants may well have been included in other weight loss programs for diabetes patients prior to the inclusion. In DCGP none of the patients received any diabetes medication or treatment prior to the study. However, the patients were not necessarily at exactly the same stage of disease as the disease in some cases may have been undiagnosed for several years.

THE EXPOSURE

To my knowledge only three earlier studies in this field analyzed weight change by means of linear regression within the strata of participants intending to lose weight (66;83;85). Most studies only reported the categorical comparison of weight maintainers and intentional weight losers. An advantage in this latter method may be that in studies with just 2 self-reported weights, the categorization into maintainers and losers may accommodate some of the measurement error. In DCGP there was an average of 13 clinical examinations with registrations of weight per participant, and it seemed reasonable to use the β-coefficient of a regression line through the measured weights as a continuous exposure (Figure 10).

MULTIVARIABLE MODELING OF A CHANGE

When analyzing the association between weight changes and mortality the exposure (weight change) is per definition taking place over a period of time. Adjusting the analysis for smoking status at baseline or at follow-up would not be sufficient as a change in smoking status in the exposure period would affect the outcome but also the exposure – as demonstrated in article 1. The same is true for physical activity and other covariates reflecting lifestyle. Also changes in medication may cause changes in body weight and at the same time improve the diagnosis or be predictors of mortality e.g. insulin therapy. However, adjusting for a change is only relevant for some covariates. Sex is constant, and socioeconomic status at follow-up is probably sufficient.

POTENTIAL CONFOUNDERS OF THE CORRELATION BETWEEN WEIGHT CHANGE AND MORBIDITY/MORTALITY

In the multivariable regression analyses of an association between weight change and mortality/morbidity, a number of potential confounders were considered. Those that were believed to have a causal effect on both weight change and mortality/morbidity were included, unless they were mediating effects covered by other covariates.

Age, sex, baseline BMI, change in physical activity, and socioeconomic status

Age is powerful predictor of weight change and death. Sex is also related to longevity and weight change (27). BMI is a predictor of the outcomes and is associated with the exposure, as an absolute weight loss of for instance 10 kg is relatively small for an obese person compared to a normal weight person. Also change in physical activity is related to weight change and future health. It is reasonable to assume that direction of the causality is from these covariates to the exposure and to the outcome. Socioeconomic status is a well-known determinant of longevity, and short education and low income are predictors of weight gain (32;103).

Intention to lose weight

Confounding from wasting disease or weight loss preceding death seems to be the greatest challenge in cohort studies of weight loss and mortality, and in the prevailing literature separating intentional weight loss from unintentional weight loss is an important strategy (71). Whether prospective or retrospectively described intention is better is not obvious. A prospectively described intention is probably less affected by information bias but will only rarely result in a long-term weight loss. The intention could likely be changed (consciously or unconsciously) if a disease arose, and thus the discriminating power of the prospective intention would be compromised. On the other hand, a retrospectively described intention is affected by recall bias, that may well result in a 'healthy weight loser effect' where those who lost weight with no side effects and are still healthy at the time of the query may tend to report that their weight loss was intentional, whereas those with ill health (which could in theory be caused by the weight loss) may be more likely to describe their weight loss as unintentional. This potential bias is only a problem if the group of intentional weight losers is compared with another group; for instance those maintaining weight. This is worth noticing as the results from Harrington's analyses are a comparison between the mortality rate in subjects with intentional weight loss and the mortality rate in participants with unchanged weight and unknown intention. Mette Kildevæld Simonsen et al evaluated the literature in a review article of intentional weight loss among healthy subjects (104). Rather than making a meta-analysis Simonsen et al gave points to the existing publications on 10 predefined qualities. They gave 2 points (maximum) for retrospectively assessed intention and 1 point for prospectively assessed intention, but the justification for this weighing was not well-described. In our article 3 the intention is prospectively described multiple times during the monitoring period. We chose to define 'intention to lose weight' as 3 or more well-described goals of weight loss (6 or more in the sensitivity analysis) throughout the 6 years of monitoring, whereof at least one goal was set within one of the three last check-ups. We do consider this way of categorizing intention as more valid than earlier methods, because it is free of recall bias and the multiple data points in each subject makes it more likely that the intention is truly present for the duration of the weight monitoring period.

Smoking status

The results from article 1 implicate that studies of weight loss and mortality should be adjusted for changes in smoking status, rather than smoking status at baseline. In Williamson's studies this was done indirectly as the information on smoking (like the weight change) was collected retrospectively and the 'former smoker' category was subdivided into (<1,1-4,>5yr). In contrast, Gregg's and Yaari's studies were apparently not adjusted for

changes in smoking status, which may have diluted a potential favorable effect of weight loss (83;85;88).

Medication

The different diabetes medications may cause changes in weight and at the same time they are indicators of disease severity. Insulin is known to induce weight gain and is/was only given to patients whose blood sugar could not be controlled with oral agents (105). On the other hand, metformin (without insulin) may induce a small weight loss and is/was given to patients with milder disease. In DCGP the changes in medication were carefully registered, and we adjusted for the use in categories compared with those that were not treated with antidiabetic medication in the monitoring period. None of the studies included in Harrington's meta-analysis were adjusted for changes in diabetes medication, which could have biased the results towards a favorable effect of weight loss.

Comorbidity

Most of the existing studies of weight loss and mortality have either excluded 'unhealthy' individuals, or adjusted for a range of self-reported health conditions at baseline. In article 1 and 3 data on prevalent and incident comorbidity was from the Danish National Patient Register (73;95). The evaluation of the Charlson comorbidity index in the INSUAP cohort demonstrated that mild disease (a score of 1 or 2) was associated with weight gain compared with healthy subjects, whereas a score of 3 or more was related to weight loss (Table 10). Even though not all these trends in the data reached statistical significance, the results indicated that light morbidity was associated with weight gain, whereas severe morbidity was associated with weight loss. Among participants with one or more diseases (a Charlson score greater than zero; n=291) there was a trend towards an association between the continuous Charlson score and weight loss (- 0.317 kg / point / 9 years, p=0.09) (Table 11). As everyone had diabetes in the DCGP cohort we used the incident continuous Charlson score during the monitoring period to adjust for disease related weight loss in article 3. To my knowledge this is the first time the Charlson score is used as covariate in weight studies, and I perceive this as a new and relevant way to reduce confounding from wasting and disease severity. However, as can be seen in Figure 12a compared with 12c the use of the Charlson score as covariate did not change the spline estimate much compared with adjusting for CVD in three categories.

Other potential confounders

In the review article by Simonsen et al studies adjusting for marital status, home conditions, and life quality measures were rewarded with points. However, we did not regard these factors as being obvious confounders or we perceived them as adequately covered by other covariates. Some studies also included the total energy intake at baseline. The total energy intake is obviously causal in relation to weight change, but is it (within a normal range) associated with morbidity/mortality? Long-term studies in rhesus-monkeys suggest that permanent energy restriction may increase survival compared with ad libitum diet (106). However, a parallel study did not find an increased survival in energy restricted rhesus-monkeys (107), and an experimental set up in animal studies does probably not reflect a realistic diet pattern in man. The long-term effect of energy restriction in humans is uncertain. Thus, the total energy intake is not a clear cut confounder, and like in studies of determinants of weight change adjustment for the total energy intake may distort the interpretation of the results.

The DCGP study had no data on genotype or family predisposition, or early life events.

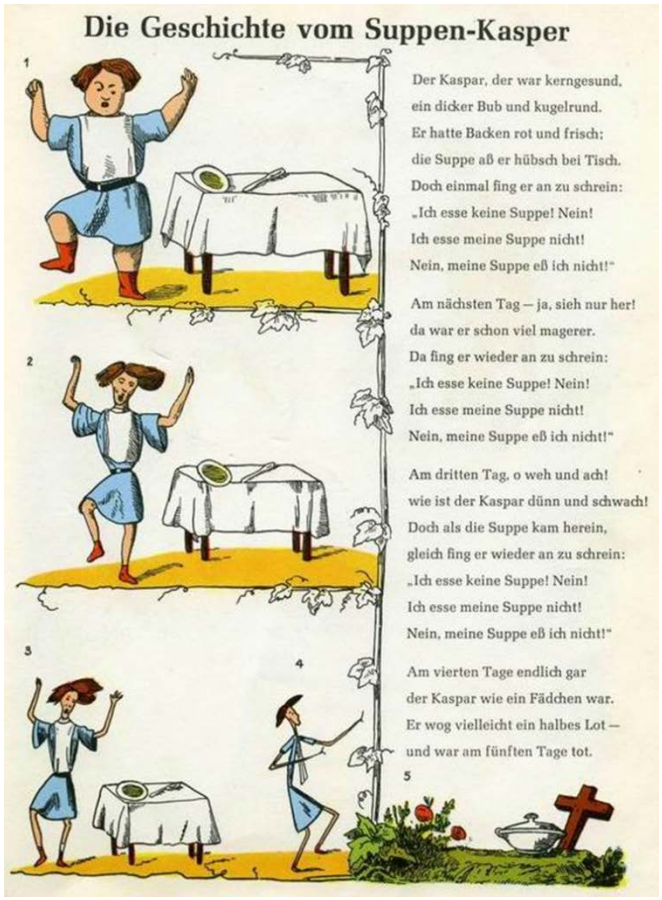

Figure 14. *The total energy intake is obviously causal in relation to weight change, but is it (within a normal range) associated with morbidity/mortality?*

LIMITATIONS IN THE DESIGN OF ARTICLE 3

Reverse causality

Despite the intensive attempt to get rid of, adjust for, or unveil weight loss caused by wasting, there is no way to exclude that residual confounding from this source has affected the results. The problem concerning reverse causality gets an extra twist when the subjects in question already have got at least one chronic disease: type 2 diabetes, which may in itself be a wasting disease - at least in the year prior to diagnosis (108). The DCGP population had an average decline in weight in the 6 years after the diagnosis of approximately 2 kg. The patients were encouraged to lose weight and the average loss may be seen as either wasting from disease severity or a success for the DCGP intervention. However, in the light of the high mean age and the magnitude of the weight loss, the loss is probably mainly explained by the normal weight development.

The exposure

There was a considerable variation in the weight development for each individual, and a slope of a regression line may seem too simple to describe the individual weight change pattern. For instance, a patient with high compliance would lose 6 kilos fast and then maintain the weight for 6 years. This would result in a relatively flat slope compared with a patient losing 1 kg a year, which would result in a steeper slope. Consequently, the slow weight loser would get a higher numeric value for the exposure. This latter pattern may carry more residual confounding from disease severity than the first. However, the spline estimate (Figure 12a) is particularly steep (towards increased mortality) at exposure values close to zero, indicating that smaller changes around zero (e.g. 0.5 -> 1.0 kg/year) induced more hazard than larger changes more distant from zero (e.g. 1.0 -> 2.0 kg/year). Under any circumstance, this method certainly better describes the general weight change than retrospectively self-reported weight changes or the difference between two measurements as earlier studies of intentional weight loss have used.

A recent study modeled weight change patterns with latent class trajectories in the Whitehall study in participants that later developed diabetes (109). This data driven method appeared at a first glance appealing but since 94% of the subjects ended up in the same category, the clinical usefulness may be limited in the present context and we did not go further into this technique. However, it could be interesting to evaluate this method on the DCGP data on a later occasion.

Weight loss method

The weight loss method was not recorded. Losing weight by energy restriction may be less healthy than losing weight by exercise. However, exercise was not as common among elderly persons in the start of the 90s as it is today, and the main emphasis of the intervention was put on changes in diet (98). Therefore, most of the weight loss was probably caused by energy restriction, rather than by increased energy expenditure. The analyses were adjusted for changes in physical activity, and the resulting estimates are probably best interpreted as weight loss caused by energy restriction independent of changes in physical activity.

The patients

The selection of patients was an almost perfect population based sample. Only few patients were excluded due to severe somatic or psychiatric disease. Only 36 rejected to participate. However, today patients with diabetes are, in most settings, diagnosed earlier than they were 25 years ago. In 1990 screening for diabetes was uncommon, and the patients were diagnosed – and included – primarily because they had some kind of symptom and were seeking medical attention. Thus, the patients had clinical diabetes as opposed to most patients diagnosed today who have no symptoms. Thus, the results can only be generalized to patients with clinical diabetes.

Only the intervention group from the original trial was included as intentions for weight change were not recorded in the control group. However, the study was randomized and thus the included patients are still representative for the population with incident diabetes (98). In comparison, the participants in Williamson's diabetes study and the Look AHEAD trial were not representative for patients with type 2 diabetes in the background population.

Missing values

Although this research group should know better, missing values in article 3 were not imputed with a suitable method, but the analyses were done with complete cases only. It is uncertain if or how this may have affected the result. However, there were relatively few participants with missing values in the relevant covariates as described in article 3 and no loss to follow-up.

HARRINGTON'S META-ANALYSIS REVISITED

Harrington meta-analysis and many of the included studies have some potential methodological flaws.

The underlying studies of 'unhealthy' participants

Potential bias from retrospectively assessed intention to lose weight

Gregg et al demonstrated that the mere 'intention to lose weight' in subjects both with and without diabetes was associated with reduced mortality regardless of whether they actually lost weight or not (83;85). In Gregg's diabetes article the trend towards a lower mortality in patients with intentional weight loss seemed to be entirely explained by confounding from intention as weight loss was not associated with reduced mortality in the strata of patients intending to lose weight (83). As a matter of fact, the mortality rate was significantly reduced in subjects with diabetes who, despite intention, failed to lose weight. It is obviously unlikely that intending to lose weight was in is self-protective, but it may be a good proxy for true explanatory factors like health literacy, compliance, and a healthy lifestyle in general.

On the other hand, intention to lose weight was not associated with mortality in DCGP (adjusted for actual weight change and the other covariates). However, the setting with newly diagnosed patients in general practice and our very conservative way of determining intention was very different from the retrospectively assessed intention in CPS-1 and NHIS. Rather than being an effect of health literacy, the association between intention to lose weight and reduced mortality found by Gregg may be a consequence of the retrospective assessment, or a 'healthy weight loser effect' as described above on page 61.

This is to some degree supported by a study of healthy individuals (participants with a disease except hypertension were excluded) by Sørensen et al where the intention was assessed prospectively and intention to lose weight was not associated with reduced mortality (66).

Bias from potentially overestimated 'initial BMI'

All of Williamson's and Gregg´s studies used 'initial BMI' and 'present BMI' for weight losers and weight maintainers, respectively. The data simulation study indicated that a potential overestimation of baseline BMI in intentional weight losers would result in an artificially low mortality risk in this group compared with those maintaining their weight. The participants may also have overestimated the size of their weight loss. In any case, it appears rather unlikely that more than 1/3 of the diabetes patients in Williamson's study (the intentional weight loss group), who were contacted at a random time point in their disease, within the last year or two lost 24 pounds on average (=10.9 kg) or 11% of their body weight. This is certainly better than most intervention studies. The authors stated that the largest limitation of the studies based on CPS-1 data was the instrument for assessing weight change (68).

I think the 'initial BMI' was generally overestimated in these studies. Partly because of recall bias, but also because some participants may well have reported a weight loss that was actually regained at the time of the query (Figure 2). Any such measurement error would probably result in overestimation of the 'initial BMI' in the group of intentional weight losers and a biased estimate of the mortality risk. This may point to a general design problem in most of the studies with retrospective weight loss information.

The underlying studies of 'Healthy' participants

Most of the studies included in Harrington's meta-analysis of 'healthy' subjects were evaluated and graded in Simonsen's review (104). The review mainly focused on the choice of covariates included in the multivariate analyses in the different studies and did not take into account more fundamental aspects of the study design. All of these studies may have bias from comparing participants with intention to lose weight with participants with unknown intention, as most of the studies had retrospectively assessed weight loss and thus also possible bias from 'initial BMI'. The intention to lose weight was prospectively described in two of the included studies (66;88).

The meta-analysis

Harrington's meta-analysis is a brave attempt to summarize the conflicting results in very diverse populations from the above-mentioned studies. However, in the meta-analysis of intentional weight loss among obese patients, it is a problem that 3/4 of the diabetes population from Williamson's diabetes study (2000) study were also included in Williamson's 1995 and 1999 publications that appear in the same analysis and in the same Forrest-plot. In the 1995 and 1999 publications subgroup analyses demonstrated the most pronounced favorable effect of weight loss among the patients with diabetes. Thus, the subgroup with the largest effect was included twice, flawing the results in direction of a favorable effect of intentional weight loss in 'unhealthy' patients.

Summing up Harrington's

The general results from Harrington's analysis are to some degree compromised by potential bias from 'initial BMI' and possible confounding from increased survival in participants with retrospectively assessed intention to lose weight in the underlying studies. The results for unhealthy participants were compromised by the fact that the patients with the largest effect (the patients with diabetes) were included twice. All of which were flawing the results towards a more favorable effect of weight loss.

THE LOOK AHEAD STUDY REVISITED

After the publication of the main results from Look AHEAD many comments and letters have been posted and published. Most commenters seek to explain why the expected healthy effect of weight loss was not reflected in a reduction in the primary outcome. The most prevalent explanations are listed here.

1. Cardio-protective drugs were more frequently used in the control group. Baseline statin use was 44% in both groups, and at 10 years it had increased to 74% in the control group compared with 71% in the intervention group (111;112). Consequently, the LDL levels were 1.6 mg/dL (0.04 mmol/L) lower in the control group according to Annuzzi et al (112). They did not regard this difference as trivial, and computed that the difference would correspond to a 0.8% reduction in major CVD events. However, there were 418 events in the control group and 403 in the intervention group and 3-4 more events in the control group would probably not have changed the p value of 0.51 even though pleiotropic effects of statins could play an additional role. Other commenters judged the difference in LDL cholesterol to be only 1.2 mg/dL (0.03 mmol/L) (113).
2. The weight loss was inadequate. The intervention group reduced their baseline weight by 8.6% during the first year, but at year 10 the weight loss had shrunk to 6.0%. A larger weight loss could maybe have had a larger effect on the outcome. However, there is not much evidence to support this theory, and in the light of the very professionally and thoroughly conducted lifestyle intervention in a relatively health conscious selection of participants, is seems unlikely that a larger average weight loss can be achieved in for instance an unselected general practice population.
3. The control group also lost weight, which diminished the difference between the groups to be clinically unimportant. The control group had lost 0.7% of their baseline weight at year 1 and had lost 3.5% at year 10; one commentator wondered: "By contrast the smaller weight loss in the control group was gradual and consistent over the course of the study" (114). The Look AHEAD authors described this pattern as the "combined effects of diabetes and age" (115). But, this should come as no surprise for the reader of this thesis, as it is consistent with the normal weight development. The roughly 0.35% point weight loss per year is largely corresponding to the average change observed in overweight and obese participants in this age category in the INSUAP cohort (Figure 7). In Look AHEAD the mean age at baseline was 59 years, and accordingly the mean weight was expected to fall regardless of any intervention. The weight loss trajectories stratified on pattern of weight change is depicted in a recent publication from the Look AHEAD group (115). The weight loss rates increased with age in all strata, and data regarding the mean age in the different trajectories would have added valuable information to this otherwise nice article. Thus, both groups in the study were under influence of the normal weight development, and in accordance with the argument above, it seem unlikely that a larger difference in weight loss can realistically be achieved in a pragmatic clinical setting.
4. The follow-up time was not long enough. This may be true; in DCGP for instance, the reduced risk of myocardial infarction caused by the intervention was not evident until after 19 years of follow-up (116). But, as article 3 indicated this reduction in myocardial infarctions was probably not attributable to the weight loss.

In contrast to the lack of effect on the main outcome measure, Look AHEAD revealed other beneficial effects of the intensive lifestyle intervention: improvement of sleep apnea symptoms, quality of life, and mobility, and a non-significant reduction in all-cause mortality. However, increased exercise was a fundamental part of the 'intensive lifestyle intervention' besides the caloric restriction. Thus, the effects on the clinical conditions above cannot be attributed to the weight loss alone. All of the clinical conditions could also have been improved by the increase in physical activity, increased attention, or better care in general.

INTERPRETATION OF ARTICLE 3 IN RELATION TO THE EXISTING LITERATURE IN THE FIELD

In contrast to most other observational studies in the field, the main result of article 3 is based on a continuous exposure. The mortality among intentional weight losers was not compared with participants with stable weight and unknown intention or intention to maintain weight. Instead the weight change was correlated to the outcome within the group of patients intending to lose weight. In this way a possible bias from intention was avoided. For each patient included in the main analysis, the goal to lose weight was prospectively described at least 3 times during the 6-year monitoring period. This very conservative definition of intention ensured that the intention was present throughout the monitoring period.

It is hard to tell how much these potential bias sources may have flawed the results of earlier cohort studies, but it can be argued that results from article 3 are based on better data and an analysis method with fewer potential sources of bias.

In the look AHEAD trial the combined effect of energy restriction and increased exercise was explored in intention to treat analyses. Unlike this, article 3 was an analysis of the weight change itself. The weight change was correlated with the incidence of the outcome independent of weight changes attributable to age, sex, BMI at diagnosis, education, and changes in smoking, changes in medication, and changes in physical activity.

In article 3 weight loss was not associated with CVD incidence or CVD mortality, and in the rest of this section will focus on all-cause mortality.

The nature of a cohort study does not allow for making causal inferences, as long as confounding factors cannot be excluded. The major concern in this context is residual confounding from pathological weight loss. To avoid this we took several precautions.

1. All patients with prior or incident cancer were excluded.
2. Only data from patients with intentional weight loss were used in the main analysis.
3. The multivariate model was adjusted for change in antidiabetic medication.
4. The model was adjusted for the Charlson comorbidity score during the 6 years of monitoring.

The follow-up period (after the 6 years of monitoring) was separated into the first two years and the remaining 11 years. The hypothesis was that if there was no difference in mortality attributable to weight loss between the two periods, mortality caused by wasting (i.e. death in the first 2 years) was sufficiently adjusted for by exclusions, intention, medication and the comorbidity score. In the main analysis of all-cause mortality in patients with intention to lose weight, there was no difference in HR between the periods (p=0.75), indicating that confounding from wasting was absent or at least modest.

The estimates in Tables 12-15 (and Table 2 in article 3) denote linear correlations like the one hypothesized in Figure 14 (but with a negative β-coefficient). In most of these linear response estimates the association between intentional weight loss and mortality was not significantly different from a HR of 1.0, even though there was a trend towards an increased risk in all subgroups except in patients with intention to lose weight and BMI<30. But, as demonstrated in Figure 12a the correlation between weight change and mortality was V shaped rather than linear. Analyzing the estimate as two separate linear estimates (one for weight loss and one for weight gain) would probably have yielded some significant linear results.

Apparently weight stability prompted the best prognosis. Weight gain tended to increase the risk, but the confidence intervals were wide and included 1.0 throughout the spectrum of weight gain. In contrast, weight loss in the interval from approximately 0.5 kg/y to 3.0 kg/y was associated with increased mortality. However, this specific interval should be interpreted cautiously. The cubic spline estimate is most precise around zero, whereas it is less reliable towards the ends of the x-axis, as indicated by the very broad confidence intervals. Probably not too much emphasis should be put into the details regarding curves and twists. What is interesting is that there was a very robust trend towards increased risk and not, as anticipated, decreased risk.

The subgroup analyses revealed that intentional weight loss among patients with BMI>30, microalbuminuria, or microvascular disease (only - and not macrovascular disease) at diagnosis was associated with mortality in the linear model. However, the non-linear spline estimate indicated that intentional weight loss was in fact worse for patients with macrovascular disease compared with patients with microvascular disease only (Figures 12f and 12g). Even among active patients (Figures 12d) or patients without CVD at diagnosis (Figure 12e) there was no sign of a favorable effect of intentional weight loss, but the confidence intervals were wide. Figure 12b demonstrates how weight loss was unmistakably associated with increased mortality in patients not clearly intending to lose weight ('intention to maintain weight').

Thus, if there was a positive effect of therapeutic weight loss on survival it was so small, that it was completely diluted in a modest residual confounding from wasting in all subgroups analyzed. Alternatively, intentional weight loss was without any effect on survival or even harmful in these patients.

The increased risk of all-cause mortality in patients with BMI>30, microalbuminuria at diagnosis, or microvascular disease contradict the findings from Harrington's meta-analysis: that among unhealthy obese subjects weight loss was associated with a reduced mortality. Article 3 basically supports the results from look AHEAD: that weight loss is not an efficient therapy for reducing CVD or mortality in patients with type 2 diabetes. Thus, indicating that the findings from Look AHEAD can be extrapolated to the general population.

Interestingly the CVD incidence was significantly reduced in the PREDIMED study (50% with diabetes), and in the Da Qing study (all with prediabetes) the mortality was apparently reduced. Both prevention studies used lifestyle interventions other than weight loss (or at least the weight loss intervention in Da Qing was inefficient). In Look AHEAD weight loss was the main contributor to the improvements in intermediate outcomes, and increases in exercise only contributed marginally to this (117). Despite the favorable effect on intermediate outcomes, one may dare hypothesize that weight loss could be directly harmful and that weight loss + exercise counterbalanced each other explaining the Look AHEAD null result. However, the look AHEAD intervention also resulted in a non-significant reduction in all-cause mortality (HR 0.85, P=0.11). This could be attributable to the increase in exercise, but together with the results from the underpowered ADAPT trial it does leave a bit of support for a beneficial effect of weight loss on all-cause mortality brought about by other mechanisms than reductions in CVD. However, I think that the body of evidence from RCTs points to a much smaller – and possibly non-existing – effect of weight loss on morbidity and mortality than most people – laypeople and health professionals – do anticipate.

In the light of the evidence from RCTs and the methodological critique raised in this thesis against Harrington's meta-analysis and the underlying studies, it seems unlikely that weight loss has a clinically important impact on anyone's risk for cardiovascular

disease or mortality; healthy or unhealthy, overweight or obese. At best the effect seems to be clinically irrelevant or harmless.

Alternatives to weight loss

Other lifestyle factors seem to be more important than weight loss in preventing CVD or premature death in patients with type 2 diabetes or impaired glucose tolerance and in healthy overweight subjects. The PREDIMED study demonstrated the efficacy of a Mediterranean diet with vegetables, wine, and olive oil in only 5 years. On top, the long-term compliance is almost certainly better with this regimen than with energy restriction, weight loss medications and low-fat foods. The Da Qing indicated a long-term effect of diet and exercise in subjects with impaired glucose tolerance. A low level of physical activity is associated with increased mortality in patients with type 2 diabetes (118). The Aerobics Center Longitudinal Study of physically active men with type 2 diabetes, demonstrated that a cardio-respiratory fitness rather than BMI predicted mortality (119). Warburton's review of health benefits of physical activity found that exercise interventions in patients with type 2 diabetes resulted in small but consistent reductions in CVD risk factors, and that evidence from cohort studies shows a strong association between exercise and reduced rates of death from any cause in these patients (120). However, like the weight loss cohort studies, observational exercise studies are also at risk for being confounded by reverse causality, as inactivity may be a marker of ill health. However, it is outside the scope of this thesis to systematically scrutinize the observational evidence in the field of exercise and mortality.

Bottom line is that lifestyle interventions without weight loss seem to be more efficient than lifestyle interventions with weight loss in secondary prevention of CVD and premature death.

Quality of life

Often improvements in quality of life and self-esteem are used as arguments for weight loss therapies (now that there is no convincing evidence that it reduces the risk of CVD or mortality), and truly weight loss has been documented to result in improvements in a range of psychosocial factors right after a successful weight loss (125). However, in many studies physical activity and interventions not resulting in weight loss also improved the same psychosocial factors (125), and many weight loss studies has a high drop-out rate, inferring bias in favor of psychosocial improvement. Still, there seems to be a clear improvement in these factors right after a successful weight loss – at least in the short term. In this thesis it has been discussed how only very few are able to maintain a weight loss, and the main concern regarding quality of life in this context is that when the large majority of weight losing patients in general practice most likely regain their weight, the initial improvements in psychosocial factors might be reversed. Altogether, it does not seem very likely that short term improvement in quality of life weighs up the massive burden of stigmata, stress and guilt the weight loss paradigm has put on many overweight people (125;126). On the contrary, the narrow focus on weight loss in many health conditions dealt with in general practice, may decrease self-esteem and life quality in the long run among the majority of patients who are not able to maintain a weight loss. Still, if we assume that weight loss actually results in permanent improvements in quality of life and a sustainable reduction in the incidence of diabetes it can be argued that it is still not ethically unproblematic to recommend it to healthy overweight patients as Harringtons' metaanalysis suggests an increased risk of death associated with intentional weight loss in these subjects.

IMPLICATIONS FOR CLINICAL WORK AND RESEARCH

The statisticians from MetLife Insurance were right: obesity is deadly. There is just no convincing proof that weight loss reduces the risk. Weight loss cannot be regarded as evidence based treatment to reduce CVD or mortality in unhealthy obese people, including patients with type 2 diabetes. Consequently, therapeutic weight loss is not documented to reduce the incidence of CVD or improve survival in neither healthy nor unhealthy overweight subjects. Even though lifestyle intervention with weight loss delayed the diagnosis of diabetes in two large prestigious diabetes prevention studies, the hard outcomes were not affected and several attempts to replicate the studies in community-based settings did not achieve the same weight reductions or success with the prevention as in the original trials (93). A recent meta-analysis concluded that there is no evidence that interventions delivered within primary care settings by generalist primary care teams trained in weight management achieve meaningful weight loss (127).

This implies that the present DSAM treatment guidelines for diabetes and overweight have to be reconsidered. However, weight loss had an independent favorable effect on knee osteoarthritis and may also be beneficial for other outcomes like sleep apnea etc. If the goal of the weight loss is to reduce symptoms from these diseases or other evidence based indication, weight loss may be considered for this, even if the weight loss is transient. From a health perspective, it seems like there is no point in monitoring weight with bathroom scales at home. Benefits in sleep apnea or other conditions may as well be monitored by the clinical progress. However, some patient groups, for instance patients with congestive heart failure, may have good reasons for monitoring weight. But, for CVD prevention in the general population, monitoring health is most likely more efficient with a pedometer. Thus, the take home message to the population could be, to get rid of your bathroom scales and get a pedometer; "Don't count kilo's – count kilometers".

The normal weight development can be considered when following patients over time, which is the tradition in Danish general practice. The normal weight development can also be taken into account when evaluating studies of weight loss. Individuals that quit smoking will likely gain weight, but on average they will end up weighing the same as if they never started smoking because everybody (less than 60 years) gains weight over time. In other words, they will return to their 'normal' weight trajectory – the weight gain seems to be natural.

Population studies of food intake and epidemiological studies in general should use the best available methods to handle missing values. In studies with many missing values imputation of median values is not a satisfactory method. Among other stochastic methods, Coca is a doable method for use with multiple imputation in future studies

PERSPECTIVES

The lean esthetically ideal body, the obesity epidemic, and the resulting weight loss paradigm all together constitute a powerful drift in our society. The health care sector has been pulling on this cart, while others, including a sizable weight loss industry, have been pushing. Based on the present evidence it cannot convincingly be excluded that weight loss therapies may increase the risk of mortality. Primum non nocere – it is essential to rule out whether weight loss can be unsafe. We owe our patients and the population proper evidence. Is weight loss good, fruitless, or even harmful?

What we need are large scale trials that are designed like the ADAPT study, so the long-term effects of weight loss by energy restriction independent of exercise, and the effect of exercise independent of weight loss, can be untangled and documented in the general population. Ideally, groups using different weight loss methods, specific diets and exercise regimens should be included in a mega trial set in for instance general practice in Denmark.

For a start, the Look AHEAD data could be reanalyzed for the independent effect of the weight loss. Regression analyses of the association between the weight change as a continuous exposure and mortality, adjusted for physical activity and randomization group would add valuable information. Analyses like this, and other future cohort studies of weight change and mortality/morbidity, may well reduce confounding from disease severity by adjusting for the Charlson's comorbidity index or another score for multimorbidity including also mental disorders.

What we would really like to know is whether a persistent weight loss is healthy, but since the primary effect of a therapeutic weight loss for most people is weight cycling, this phenomenon might also be worth taking a closer look at. The consequences of weight cycling needs to be further elucidated in better cohort studies with numerous clinical measurements of weight and information on weight loss methods and intention.
Nutritional epidemiology has some fundamental methodological problems. A systematic elaboration on the methods for processing dietary data may improve the field along with better instruments for assessing the diet. To my knowledge no FFQ with participant related outcome measures (food intake) have been validated with item response theory, which could be a way forward.

For my own part, I am planning two projects that I, among other tasks, will work on in the next year:

MUVIR – Motion Uden Vægttab I Rødovre (Exercise without weight loss in Rødovre – a Copenhagen suburb)
MUVIR is a pilot randomized trial of the isolated effect of weight loss independent of physical activity. Thirty obese persons are recruited in a general practice in Rødovre and randomized to either an exercise program alone or the same exercise program + dietician inducing weight loss with energy restriction. The 'dietician group' will be monitored monthly with body weight at check-ups at the general practitioner, whereas the 'exercise only' group will be monitored monthly by physical activity (accelerometer score) also at check-ups at the general practitioner. The municipality, a fitness center chain, and general practice will cooperate in this pragmatic setting. Intermediate outcomes will be evaluated at the follow-up after 12 month. The perspective is that if this intervention is feasible, it can be used to raise funds for a full-scale study with long-term intervention and long-term follow-up on hard endpoints.

Weight cycling study
This study will evaluate the association between weight cycling and general mortality among patients with type 2 diabetes from DCGP intervention arm cohort. Like in article 3 the slope of a regression line denotes the general change in body weight in the monitoring period (Figure 15). Weight cycling is defined as the standard deviation around the regression line: SD = root (sum residuals2/ number of registrations). The SD is correlated to all-cause mortality in a cox model adjusted for the β-coefficient denoting the line's slope and other confounders. The preliminary analyses suggest that weight cycling, as defined here, is an independent risk factor for mortality in overweight patients with diabetes trying to lose weight.

CONCLUSIONS

- The dietary intake of fructose or soft drinks sweetened with sugar was not associated with long term weight changes.
- Age is a powerful determinant of long term weight changes. Historical weight data suggest that the body weight

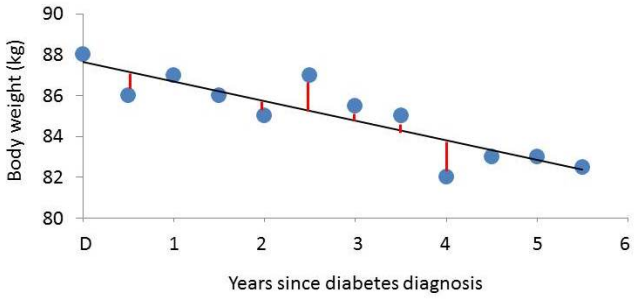

Figure 15. *Example of weight registration in one patient. We defined weight cycling as the standard deviation (SD - the mean distance from the measurement points to the regression line).*

  increases throughout life to the age of 60-65years. This 'normal weight development' can be taken into consideration when evaluating weight studies, and when following patients over long time.
- Also smoking status and in particular smoking cessation are strong determinants of weight change. Smokers weigh less than comparable never-smokers, but by quitting they regress towards their 'normal weight' had they never smoked. This is illustrated in the graphic "smoking cessation weight change model". This model may find use as a clinical tool to communicate with weight-worried patients who are thinking of giving up smoking.
- Handling missing portion size values in food frequency questionnaires with imputation of median values induce bias in the resulting estimates of energy intake. Missing values in epidemiological studies are better handled with stochastic methods like the Coca method and multiple imputation.
- Intentional therapeutic weight loss in patients with type 2 diabetes, supervised by a medical doctor, was not associated with decreased long-term risk for CVD, CVD-mortality or all-cause mortality.
- The contradictions between our results and the prevailing observational evidence may be explained by methodological weaknesses favoring weight loss in the earlier studies, and there is no convincing evidence from RCTs backing that weight loss reduces CVD or mortality.
- Thus, there is no good evidence to support that intentional weight loss reduces the risk of CVD or mortality in any group of patients in general practice or in the general population. Still, weight loss may benefit other clinical conditions like for instance knee arthritis, prevent (or delay) the onset of diabetes, and may result in improvements in a range of psychosocial factors. However, since most people are not able to maintain a weight loss, initial improvements in for instance quality of life are most likely temporary and the stress that the weight loss paradigm has put on overweight people may well result in a long term reduction of life quality. Ultimately

we don't know whether weight loss is harmful or beneficial in terms of mortality, and it does not seem likely that short term positive effects weighs up this uncertainty in general. Consequently, weight loss strategies in general practice should be reconsidered. Rather than aiming at weight loss, general practice could focus on lifestyle changes like exercise and Mediterranean diet in overweight patients.

ARTICLE 1

BACK ON TRACK – SMOKING CESSATION AND WEIGHT CHANGES OVER NINE YEARS IN A COMMUNITY-BASED COHORT STUDY

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ABSTRACT

Objective
To examine the impact of smoking cessation on body weight compared with long term weight changes in the general population, and to propose a 'smoking cessation weight change model' for use in clinical work when health providers discuss post cessation weight gain (PCWG) with patients who are smokers.

Design
Community-based cohort study.

Setting
General practice.

Participants
All adults (20-70 years) in a rural town in Denmark were invited to take part in a study of lifestyle and health in 1998-2000; 66% participated (n=1374). After nine years 1121 participants took part in the follow-up study.

Main outcome measures
Weight and weight changes were compared using multivariable regression models.

Results
The mean baseline weight of never-smokers was 76.4 kg (SD 16.0), and the adjusted weight of smokers and ex-smokers differed by -4.2 kg (95%CI [-5.9]-[-2.6]), and -0.7 kg (CI [-2.5]-1.1), respectively. The adjusted mean yearly weight gain rate (g/year) of never-smokers, continuous smokers, and ex-smokers was 213, 127, and 105, respectively. The absolute PCWG was 5.0 kg (SD 7.0), and the mean adjusted PCWG was 2.8 kg (95%CI 1.7-3.9) compared with never-smokers, and 3.5 kg (2.3-4.8) compared with continuous smokers. At follow-up there was no difference between the weight of new quitters and never-smokers (0.1 kg [-2.4-2.6]). A graphical model illustrates the results.

Conclusion
Smokers weighed less than comparable never-smokers and by quitting they approached the weight of never-smokers. The weight gain rates differed by smoking status. Consequently, the PCWG depended on the length of follow-up, and on whether the PCWG was defined as the absolute gain or a gain relative to continuous smokers or never-smokers. Therefore, PCWG seems to be best described in a graphical model rather than as a single number. The graphical 'smoking cessation weight change model' may help smokers and health providers to understand the weight dynamics of smoking cessation in relation to normal weight development.

INTRODUCTION

Cigarette smoking is a major risk factor for morbidity and remains the leading cause of preventable death in developed countries (1). Although post cessation weight gain (PCWG) may limit some of the health benefits of smoking cessation, it is widely acknowledged that the net benefit of quitting on overall health greatly outweighs the potential risks (2-4). In disease prevention PCWG is a concern mainly because patients' fear of weight gain is a major barrier to smoking cessation, and this fear discourages 50% of female and 26% of male smokers from attempting to quit (5).

Experimental evidence suggests that smoking cessation causes a decrease in the basal metabolic rate and an increase in appetite which, taken together, explain most of PCWG (2, 6-9). These effects are likely to be caused by nicotine stimulation of the sympathetic nervous system (6) and overall smoking appears to lower the body weight set point (10).

PCWG appears to take place primarily during the first year after cessation (2). The magnitude of PCWG is disputed (11) and population studies have reported average PCWG ranging from 1.3 kg to 6.6 kg (12, 13). The large variation in PCWG may be due to: different lengths of follow-up periods after quitting, varying from a few weeks to 11 years (14-16); different methods of participant selection across studies (11, 12, 17); and the choice of comparison groups. Some studies report absolute weight gain in the group of quitters (2), most studies compare quitters with continuous smokers (3, 12, 15, 18), and a few studies also compare quitters to never-smokers (11, 14).

Perhaps a better understanding of long term weight changes in comparable never-smokers and smokers could relieve some of the fear that discourages many smokers from attempting to quit, and support quitters with a rational accepting of the physiological changes that is often the consequence of nicotine abstinence.

The objective of this study is to describe the impact of smoking cessation on body weight compared with the common age-related weight changes in never-smokers and smokers over nine

years in the general population, and to propose a 'smoking cessation weight change model' for use in daily clinical work when health care providers discuss PCWG with patients who smoke.

METHODS

Participants and design

The baseline data for this population-based cohort study were collected between 1998 and 2000 in a Danish general practice, which included all adults in the local community (19). Of the 2082 citizens aged 20-69 years in the rural town of Ørum, 1374 (66%) agreed to participate in a study of lifestyle and metabolic health (Fig. 1). The same investigator (PEH) carried out a physical examination of all 1374 baseline participants. They were weighed in their underwear and the weight was registered to the nearest 100 g (Seca®Electronic 0 –200 kg). All participants answered questions from a comprehensive questionnaire which included general health, lifestyle, weight history, physical activity, as well as education and occupation. After nine years, in 2007-2008, the participants received a letter with questionnaires and instructions on how to measure their body weight in underwear. Thus, the follow-up weight was self-reported. A detailed history of smoking behaviour was obtained both at baseline and at follow-up. The exact date of quitting was registered for 117 (89%) of the 131 subjects who stopped smoking during the follow-up period, and for 226 (86%) of 262 who had stopped before the baseline examination. The study was approved by the regional Research Ethics Committee and informed consent was given by all participants.

Definition of smoking categories

For the purposes of our study, we defined six categories of participant. These are:

1. Never-smokers: no history of smoking.
2. Continuous smokers: smoking at baseline and at follow-up.
3. Ex-smokers: stopped smoking before the baseline study, and were not smoking at follow-up.
4. New quitters: smoking at baseline, but stopped smoking between baseline and follow-up.
5. New smokers: no history of smoking at baseline, but were smoking at follow-up.
6. Relapsed smokers: stopped smoking before the baseline study, but were smoking at follow-up.

Statistics

For the longitudinal analysis of weight change over nine years of follow-up, we used a multivariable linear regression model for body weight at the two time points (baseline and follow-up after nine years), with a person random effect to adjust for the inherent correlation between weight measurements on the same subject. The exposure of interest was the smoking category, as defined above. The longitudinal analysis was controlled for sex, age, baseline BMI, education, physical activity, and historical weight one year before the baseline study (to adjust for regression towards the mean). The design of the analysis was based on our knowledge of smoking and weight change physiology and was not controlled for intermediate variables. The analysis was not adjusted for covariates such as 'pack year' as we wanted a model for weight change that was also valid for never-smokers.

To compare mean body weight in the different smoking categories at baseline and at follow-up, we did cross-sectional analyses with an ordinary multivariable linear model adjusted for age, height, sex, and education.

In an analysis of 'super gainers' (which we defined as a person gaining 10 kg or more during the nine year follow-up period) we used a multivariable logistic regression model to identify independent risk factors. In the model we included smoking category, age, sex, education, baseline BMI, and physical activity.

Education level was categorized as: high school degree, skilled worker, or unskilled worker. Physical activity was self-reported with Baecke's questionnaire and used as a continuous variable (20). Twenty four baseline ex-smokers relapsed and were smoking again at follow-up; they were included in the multivariate analyses but their results are not reported. Only two participants among the baseline never-smokers reported they had started smoking during the follow-up period, and they were subsequently omitted from the analyses. T-tests were used for univariate analyses of continuous covariates, and Chi2-tests were used for univariate analysis of categorical covariates. Missing data in the covariates, but not in the exposure and outcome variables, were handled with multiple imputation. All statistical analyses were conducted with the use of SAS statistical software, version 9.3.

RESULTS

Subjects

Of the 1374 individuals who participated in the baseline study, 1122 also participated in the follow-up survey after a mean of 8.6 years (SD 0.6; Range 7.5-10.1), including 374 (78%) of 478 baseline smokers (Figure 1). Of these, 243 (65%) were still smoking and 131 (35%) had stopped; in this group of new quitters the mean time since quitting was 5 years (59 months). The baseline characteristics of the participants, grouped according to their smoking status at baseline, are described in Table 1.

Cross-sectional weight analyses

The mean baseline weight of never-smokers was 76.4 kg (SD 16.0). The adjusted mean baseline weight of smokers differed by -4.2 kg (95%CI [-5.9]-[-2.6]), whereas the weight of ex-smokers was not significantly different (-0.7 kg [-2.5 - 1.1]) compared to the never-smokers. Table 2 presents the mean body weight at baseline and at follow-up, stratified according to smoking status at follow-up and age. At follow-up never-smokers still weighed significantly more than continuous smokers, whereas there was no significant difference between the weight of never-smokers and new quitters, or between never-smokers and continuous ex-smokers.

Longitudinal weight analyses

The mean longitudinal change in body weight over nine years is reported in Table 3. For new quitters the absolute PCWG (i.e. the mean weight gain over 9 years) was 5.0 kg (SD 7.0). The mean adjusted PCWG was 2.8 kg (95%CI 1.7-3.9) compared with never-smokers, and 3.5 kg (2.3-4.8) compared with continuous smokers. For quitters, an analysis by age appears to be significant. For those who were younger than 50 at baseline and stopped smoking, the absolute PCWG was 6.0 kg compared to an absolute PCWG of 3.1 kg for quitters aged more than 50. For these two groups of quitters, the adjusted PCWG was 3.0 kg and 2.5 kg respectively, compared to never-smokers and 3.2 kg and 4.1 kg respectively compared to continuous smokers.

The formation of a graphical model

We calculated the adjusted mean yearly weight gain rates of continuous smokers and continuous ex-smokers compared with the crude weight gain rate of never-smokers (tab. 4). We used these

weight gain rates, the adjusted baseline and follow-up weights (tab. 2), and our adjusted estimate of the mean PCWG (tab. 3) to form the 'smoking cessation weight change model' (fig. 2 and 3). The never-smokers' weight trajectory was defined by their crude weight gain rate starting at the mean baseline weight in year zero. The quitters' trajectory was modeled with the adjusted weight gain rate of continuous smokers for the first four years. At year 4 we modeled smoking cessation with a PCWG taking place during that year (2). For the subsequent four years the quitters' trajectory was modelled by using the adjusted weight gain rate of continuous ex-smokers. We used the adjusted follow-up body weights and the adjusted PCWGs to calibrate the relation between the weight trajectories of the quitters, never-smokers, and continuous smokers at year 9.

Table 1. *Baseline characteristics of the participants*

	Smoking status at baseline				
	Never-smoker (n=562)	Smoker (n=478)	P diff	Ex-smoker (n=331)	P diff
Age, years	41.9 (12.3)	45.3 (12.7)	<0.0001	47.9 (12.6)	<0.0001
Sex, female	296 (52.7)	231 (48.3)	0.17	152 (45.9)	0.05
BMI, kg/m^2	26.2 (5.2)	25.0 (4.4)	<0.0001	26.3 (4.4)	0.75
Education					
High school	163 (29.1)	92 (19.3)	<0.001	85 (25.7)	0.28
Skilled worker	288 (51.3)	235 (49.4)	0.53	162 (48.9)	0.49
Unskilled worker	110 (19.6)	149 (31.3)	<0.0001	84 (25.4)	0.04
Physical activity, METs	8.4 (1.3)	8.2 (1.3)	<0.01	8.3 (1.3)	0.31
Pack years	0	25.5 (23.6)	-	16.1 (20.1)	-

Values are means (SD) or numbers (%). METs: Metabolic Equivalent of Task from Baecke's questionnaire.

Table 2. *Body weight at baseline and at follow-up*

Body weight, kg	Smoking status at follow-up			
	Never smoker (n=458)	Continuous smoker (n=243)	New quitter (n=131)	Continuous ex-smoker (n=262)
Baseline body weight				
Level – unadjusted				
All	76.4 (16.0)	72.8 (14.6)	73.5 (15.7)	77.3 (14.3)
20-50 years	75.8 (16.1)	72.5 (13.4)	72.8 (15.3)	76.7 (14.4)
50-70 years	77.8 (15.7)	73.4 (16.4)	74.9 (16.4)	78.1 (14.3)
Difference – adjusted*				
All	ref	-4.1 (-6,2 - (-2.1))†	-3.3 (-5.8 - (-0.8))†	-0.3 (-2.3 - 0.7)
20-50 years	ref	-3.3 (-5.8 - (-0.8))†	-2.8 (-5.9 - 0.2)	-0.4 (-2.2 - 2.9)
50-70 years	ref	-5.8 (-9.3 - (-2.2))†	-4.1 (-8.6 - 0.3)	1.0 (-4.3 - 2.4)
Follow-up body weight				
Level - unadjusted				
All	78.2 (16.5)	74.1 (14.7)	78.5 (15.7)	77.6 (14.5)
20-50 years	78.6 (17.0)	75.1 (14.1)	78.8 (15.6)	77.5 (14.1)
50-70 years	77.4 (15.1)	72.7 (15.4)	78.0 (16.2)	77.7 (15.0)
Difference, adjusted*				
All	ref	-4.2 (-6.2 - (-2.2)†	0.1 (-2.4 - 2.6)	-1.1 (-3-1 - 0.9)
20-50 years	ref	-3.1 (-5.7 - (-0.6)†	0.5 (-2.5 - 3.6)	-1.3 (-3.9 - 1.3)
50-70 years	ref	-6.2 (-9.5 - (-3.0)†	-0.6 (-4.7 - 3.6)	-0.6 (-3.7 - 2.5)

Age is from the baseline examination. Unadjusted values are means (SD). Adjusted values are adjusted means (95% confidence intervals). *) A cross-sectional general model was used to estimate the adjusted mean differences in body weight compared with never-smokers (95% confidence intervals). The differences were adjusted for age, height, sex, and education. †) P<0.05

Table 3. *Change in body weight over 9 years*

Change in body weight, kg	Smoking status at follow-up: Never smoker (n=458)	Continuous smoker (n=243)	New quitter (n=131)	Continuous ex-smoker (n=262)
All participants				
Unadjusted	+ 1.8 (6.0)	+ 1.3 (6.4)	+ 5.0 (7.0)	+ 0.4 (6.1)
Adjusted*	-	- 0.7 ((-1.7) – 0.2)	+ 2.8 (1.7 – 3.9)†	- 0.9 ((-1.8) – 0.0)†
20-50 years				
Unadjusted	+ 2.7 (6.0)	+ 2.6 (6.2)	+ 6.0 (6.5)	+ 1.0 (6.9)
Adjusted*	-	- 0.2 ((-1.4) – 1.0)	+ 3.0 (1.5 – 4.5)†	- 1.6 ((-2.8) – (-0.4))†
50-70 years				
Unadjusted	- 0.3 (5.6)	- 0.8 (6.2)	+ 3.1 (7.5)	- 0.4 (4.9)
Adjusted*	-	- 1.6 ((-3.0) – (-0.2))†	+ 2.5 (0.8 – 4.3)†	- 0.2 ((-1.5) – 1.1)

Age is from the baseline examination. Unadjusted values are means (SD). Adjusted values are adjusted means (95% confidence intervals). *) A longitudinal random effects model was used to estimate the weight changes compared with weight changes of never-smokers. The analyses were adjusted for age, sex, education, physical activity and BMI at baseline as well as self-reported weight one year before the baseline examination. †) P<0.05

Table 4. *Weight change rates*

	Reference	Relative change at follow-up	Follow-up time	Adjusted yearly weight change rate*
	g	g	Years	g/year
All participants				
Never-smokers	(1829)	ref	8.60	213
Continuous smokers	213	- 741	8.66	127
Continuous ex-smokers	213	- 939	8.68	105
Age 20-50				
Never-smokers	(2740)	ref	8.53	321
Continuous smokers	321	-202	8.65	298
Continuous ex-smokers	321	-1575	8.58	138
Age 50-70				
Never-smokers	(- 283)	ref	8.75	- 32
Continuous smokers	-32	-1581	8.68	- 214
Continuous ex-smokers	-32	-162	8.80	- 51

*) The adjusted yearly weight change rates derive from the crude change rate in never-smokers, plus the relative weight changes of continuous smokers and continuous ex-smokers in the follow-up period compared to never-smokers (from Table 2) divided by the actual follow-up time in years.

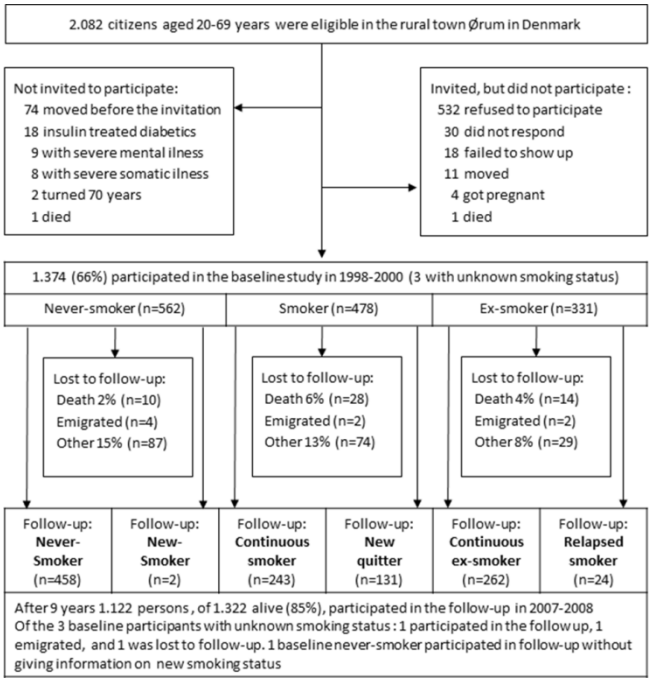


Figure 1. *Patient flow*

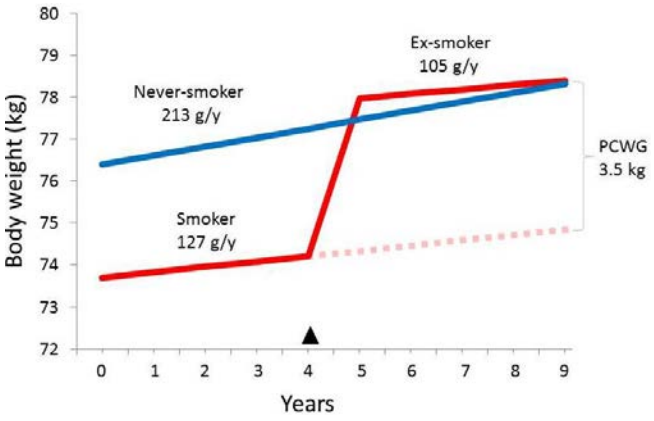


Figure 2. *'Smoking cessation weight change model'. The figure illustrates the mean weight trajectories for adults (20-70 years at baseline) according to smoking status. The triangle denotes smoking cessation.*

Supplementary analyses of super gainers

Altogether, 7% of the participants (n=90) gained 10 kg or more during the 9 years of follow-up, including 21% of the quitters, 7% of the never-smokers, and 4% of the continuous smokers. In a logistic regression analysis including all participants we identified four independent risk factors for becoming a super gainer: smoking cessation (odds ratio 3.29; 95%CI 1.85-5.85 compared to never-smokers, or 3.91; 1.94-7.87 compared to continuous smokers); younger age (1.08 per year; 1.05-1.10); reduced physical activity at baseline (1.30 per METs; 1.09-1.56); and unskilled worker compared to high school graduate (3.49; 1.57-6.35).

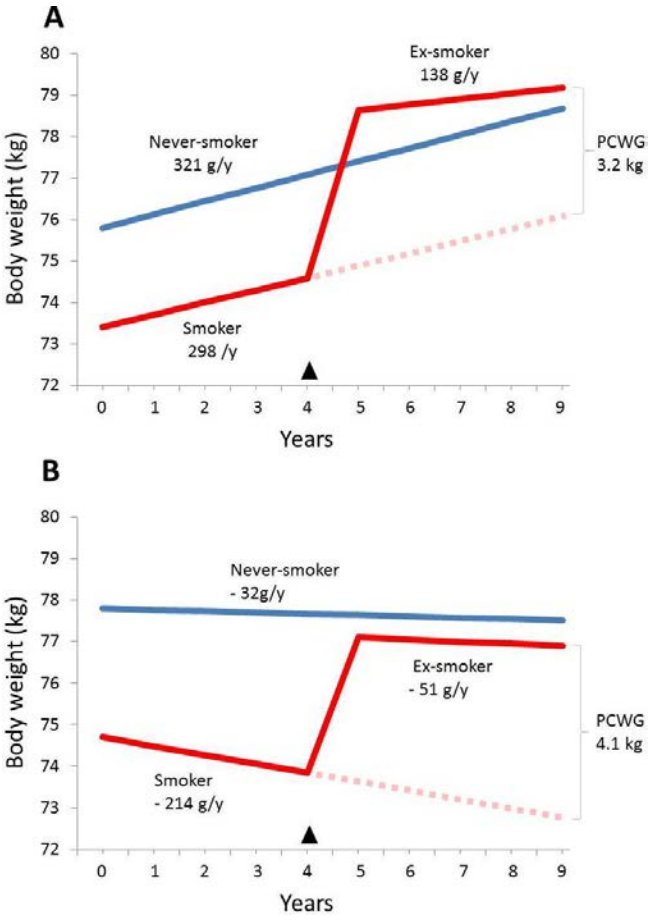


Figure 3. *'Smoking cessation weight change model'. Panel A illustrates the mean weight trajectories according to smoking status for subjects aged 20-50 years at baseline, and panel B illustrates this in subjects aged 50-70 years at baseline. The triangles denote smoking cessation.*

DISCUSSION

Principal findings

At Baseline smokers weighed less than comparable never-smokers. By quitting, they gained weight and ended up weighing the same as comparable never-smokers. While the smokers weighed significantly less than never-smokers at baseline, there was no difference between the weight of never-smokers and ex-smokers. At follow-up there was no difference between the weight of never-smokers and new quitters, or between never-smokers and continuous ex-smokers, but smokers still weighed significantly less than never-smokers. The weight gain rates differed by smoking status. Consequently, the size of PCWG depended on the length of the follow-up period, and on whether the PCWG was reported as an absolute gain or a gain relative to continuous smokers or never-smokers. In new quitters of all ages, the mean adjusted weight change was 3.5 kg compared with continuous smokers, which we consider the most reasonable way of enumerating the PCWG. However, PCWG seems best described in a graphical model rather than as a single number. For instance, the adjusted PCWG in quitters aged less than 50 years was 3.2 kg, and 4.1 kg in quitters aged over 50 years (compared to continuous

smokers), although the absolute weight gain was 6.0 and 3.1, respectively. The discrepancy between the absolute and the relative change in the two age groups was mainly explained by the decreasing (150 g/year) weight among smokers aged more than 50 years, highlighting the importance of choice of comparison group. On average, smokers, ex-smokers, and never-smokers aged 20-50 years all gained weight during the nine year follow-up period. In contrast, smokers, ex-smokers, and never-smokers aged 50-70 years all lost weight on average. Therefore, a person's age when he or she stops smoking is an important determinant of the relative PCWG. The graphical 'smoking cessation weight change models' (fig. 2 and 3) clearly illustrates this complexity.

Our results compared with other studies

Other studies have found that cigarette smokers have a lower average body weight than nonsmokers, and that cessation of smoking is associated with weight gain (10, 14, 21, 22). John et al found that the proportion of obesity among individuals who had quit smoking within the last 12 months was not higher than that of never-smokers, and that former smokers did not differ according to prevalence of overweight or obesity (10). Reas et al and Munafo et al also found that the average body weight of quitters tends to stabilize over time to levels of never-smokers (14, 21). Our study confirms these findings and adds a graphical tool for understanding the weight dynamics of smoking and smoking cessation in general as well as in different age groups. The temporal dimension in the graphical model may also explain some of the discrepant findings in the literature regarding the magnitude and clinical significance of PCWG (12, 13).

The lower baseline body weight in smokers (compared with never-smokers) can only to a limited degree be explained by the lower yearly weight gain rates that were observed in our data. Whether a related rapid weight loss occurs when people start smoking is not well described in the scientific literature. In experimental studies nicotine appears to acutely increase the metabolic rate (23), but observational studies have generally failed to demonstrate weight loss in subjects starting to smoke (24).

How to use the 'smoking cessation weight change model'

It is our hope that the proposed graphical model can be used by health care professionals as a clinical tool to communicate with weight-worried patients who are thinking of giving up smoking. Our model may help smokers understand that smoking causes an abnormally low weight, and any weight gain that may be caused by cessation is therefore natural. After a few years the quitters on average will approach their natural weight defined as their weight had they never smoked. Yet, the usefulness as a clinical tool will have to be evaluated in practice, for instance by making an interview study of general practitioners and patients presented for the tool.

Additionally, the graphical model may be useful for epidemiologists in better understanding weight trajectories related to smoking. An implication for future research of body weight changes using multivariable analysis could be to adjust for changes in smoking status, rather than adjusting only for baseline smoking status.

Considerations about the design of the 'smoking cessation weight change model' – strengths and limitations

In our study the mean time since participants stopped smoking was five years, and we assumed that quitters followed the weight trajectory of smokers in the first four years, and then the weight trajectory of ex-smokers in the last four years. We also assumed that the entire cessation related weight gain occurred during year five. The latter assumption is based on a meta-analysis of 62 trials where the average PCWG primarily occurred in the first three month after smoking cessation. The weight gain rate was highest right after smoking cessation and after one year it approached the mean weight gain rate of ex-smokers observed in our study (2).

Our model may appear experimental but it is in fact made from several estimates of weight gain rates patched together into an understandable diagram. This approach is highly illustrative, but limited by the assumptions outlined above and by the fact that general weight developments over time are not linear. These weaknesses have given rise to some inconsistencies. First, the modeled weight for smokers at year zero is not entirely consistent with our findings, as the adjusted mean baseline weight was somewhat lower than indicated (0.5 kg for all smokers; 0.3 kg for the 20-50 year olds; and 1.0 kg for the 50-70 year olds). Second, the absolute weight gain in quitters was also slightly higher than indicated in the model (0.3 kg for all quitters; 0.2 kg for 20-50 year olds; 0.9 kg for 50-70 year olds).

These inconsistencies were necessary to make the graphical proportions of the PCWG, compared with both continuous smokers and never-smokers at year 9, fit our data explicitly. Thus, our 'smoking cessation weight change model' should be interpreted with caution because of the fragmented nature of the data. However, the different elements in our model gain confidence from being in accordance with the findings of numerous other studies (14, 21, 22, 25).

Comorbidity may affect both body weight and smoking status. In our original model we adjusted for a range of incident comorbidities in the weight change period, but since it did not substantially affect the estimated PCWG or weight change rates (in any age group) we omitted it from the multivariable model.

Another limitation of the model and the study in general is the self-reported weight at follow-up. In general, self-reported weight is underestimated compared to clinical examination, and the bias varies for men and women as well as for other characteristics of the population (26). For instance in a general practice population, in both men and women the average self-reported weight was underestimated by 1.2 kg compared with measures taken by the general practitioner (27). Consequently, the weight gain rates and absolute PCWG presented in this study are probably somewhat underestimated. No earlier studies on PCWG seem to have considered differential reporting bias of weight by smoking status. If we assume that smoking status did not substantially bias the self-reported weights, our estimated relative PCWG is not affected by the underreporting. The self-reported smoking status must also be considered a limitation of the study, although it may be argued that the status of the self-reported quitters gains some confidence by the fact that 89% reported a well-defined date for stopping smoking. Also, we did not have any data on multiple quitting attempts. The data on quitting date was obtained at follow-up. Thus, the self-reported smoking status at follow-up (in relation to the smoking status at baseline) determined the smoking category.

Conclusions and policy implications

At baseline smokers weighed less than comparable never-smokers. By quitting, they gained weight and ended up weighing the same as the never-smokers. Weight gain rates differ by smoking status. Consequently, the PCWG depends on the length of the follow-up period, and on whether the PCWG is defined as the abso-

lute gain or a gain relative to continuous smokers or never-smokers. The 'smoking cessation weight change model' illustrates the complexity of weight changes related to smoking status in different age groups, and we found that PCWG is better described in a graphical model rather than as a single number.

We believe that the 'smoking cessation weight change model' may help smokers and health providers to understand the weight dynamics of smoking cessation in relation to normal weight development. An implementation study must determine whether the 'smoking cessation weight change model' is suitable in a clinical setting and useful in encouraging smokers to quit.

Contributors: RKR and CP contributed to the conception and the design of the study. PEH acquired the data. RKR conducted the analyses assisted by CP and VS. RKR, CP, VS, JEH, PEH, BLH, and NO analysed and interpreted the data. RKR and CP drafted the article, and PEH, VS, JEH, BLH, and NO revised it critically for important intellectual content, and approved the final version to be published. RKR is the guarantor.

ARTICLE 2

MISSING PORTION SIZES IN FOOD FREQUENCY QUESTIONNAIRES - ALTERNATIVES TO USE OF STANDARD PORTIONS

Abstract

Objective Standard portions or substitution of missing portion sizes with medians may generate bias when quantifying the dietary intake from food frequency questionnaires (FFQs). This study compared four different methods to include portion sizes in FFQs.

Design

We evaluated three stochastic methods for imputation of portion sizes based on information about anthropometry, sex, physical activity, and age. Energy intakes computed with standard portion sizes, defined as sex-specific medians (median), or with portion sizes estimated with multinomial logistic regression (MLR), 'comparable categories' (Coca), or K-nearest neighbors (KNN) were compared to a reference based on self-reported portion sizes (quantified by a photographic food atlas embedded in the FFQ).

Setting

The Danish Health Examination Survey 2007-2008.

Subjects

This study included 3728 adults with complete portion size data.

Results

Compared to the reference, the rooted mean squared errors of the mean daily total energy intake (in KJ), computed with portion sizes estimated by the four methods, were (men; women): median (1118; 1061), MLR (1060; 1051), Coca (1230; 1146), KNN (1281; 1181). The equivalent biases (mean error) were (in KJ): median (579; 469), MLR (248; 178), Coca (234; 188), KNN (-340; 218).

Conclusions

The methods MLR and Coca provided the best agreement with the reference. The stochastic methods allowed for estimation of meaningful portion sizes by conditioning on information about physiology, and they were suitable for multiple imputation. We propose to use MLR or Coca to substitute missing portion size values, or when portion sizes needs to be included in FFQs without portion size data.

INTRODUCTION

Food Frequency Questionnaires (FFQs) are commonly used in large-scale nutritional epidemiology studies, but some FFQs do not have questions about portion sizes (1-3). Details concerning portion sizes or missing portion size values are rarely accounted for in scientific publications, but when calculating the dietary intake from a FFQ, standard portion sizes are often applied.

The absence of portion size questions in a FFQ can be regarded as a missing data problem. Using standard portion sizes is methodologically equivalent to applying median portion sizes for all subjects. These may be sex-specific, but the size of portions depends on several other factors than sex, for instance age, BMI and physical activity (4). Hence, the standard portion size used may well be the same for a young physically active man as it is for an elderly sedentary man.

Substituting unknown portion sizes with standard sizes may thus under- or over-estimate the "true" intake in certain segments of the population (5-7). It is now well recognized that missing data is most rationally accounted for through multiple imputation techniques, rather than with deterministic imputations like medians, to avoid flawed (too narrow) confidence intervals (8,9). Multiple imputation require an adequate method for imputation, i.e. a method with error and bias as low as possible.

In this paper we describe how physiologically meaningful portion sizes can be estimated from information on age, sex, physical activity, weight, and height by imputation from participants with complete data or from another FFQ dataset with portion sizes (from a comparable population). We invented the 'comparable categories' method (Coca), and improved the 'K nearest neighbors' (KNN) and the multinomial regression method (MLR) by making them suitable for multiple imputation. The basic idea of these advanced imputation methods are that instead of using a median value for substituting missing data, one may condition on other information available in the dataset, to better estimate a reasonable portion size.

In this study the dietary intake computed with standard portion sizes (the sex-specific median values), or with portion sizes determined by the MLR, Coca, or KNN methods were compared to a reference dietary intake, which was computed with the originally self-reported portion sizes that were quantified by a photographic food atlas embedded in the FFQ.

EXPERIMENTAL METHODS

The Danish Health Examination Survey collected dietary data from 18 065 adult Danes in 2007-2008 using an internet-based 267 items FFQ (10). This diet-inventory has been used in many Danish population studies (2,11). In the Danish Health Examination Survey the FFQ was extended with a photographic food atlas consisting of eleven picture series placed at the end of the questionnaire in order to quantify the portion sizes (11). The portion size food atlas was developed by the Danish Veterinary and Food Administration. The picture series covered 39 items (foods or meals) classified into 4 or 6 portions of varying sizes. For instance 6 photos showed increasing serving sizes of corn flakes in a bowl, and the accompanying portion size item was used to quantify all cereal frequency items (müsli etc.). Another series with 6 photos of increasing serving sizes of a meat main meal was accompanied by 5 portion size items covering hamburger steak, steak, beef, fish, or poultry. The remaining photo series covered bread, toppings for rye bread (8 items), toppings for white bread (8 items),

warm stew with meat (3 items), potatoes (4 items), pasta, rice, vegetable dishes (4 items), mixed salad, chocolate, and candy. The actual weight in gram of the food on the picture was multiplied with the frequency to obtain the total intake of the food. Leisure time physical activity was self-reported with the International Physical Activity Questionnaire in 4 classes, where 1 was hard training multiple times a week, and 4 was inactive behavior (12). We defined class 1+2 as active and class 3+4 as sedentary. Anthropometric measures were obtained by clinical examination in 9384 subjects. The present study population consisted of the 3728 subjects with complete information on anthropometry and portion sizes (no missing values). The characteristics of the study participants are described in Table 1. The involved institutions review boards have approved the study proposal.

Table 1. *Characteristics of the study participants with complete portion size data compared with the excluded subjects with incomplete portion size data*

	Men			Women		
	Included	Excluded	P for diff	Included	Excluded	P for diff
N	1546	2078		2182	3578	
Sex (%)	41	37		59	63	
Age, years (mean)	50.0	52.8	<0.001	48.4	51.3	<0.001
BMI, kg/m2 (mean)	26·1	25.9	0.12	24.9	24.5	<0.01
Physical activity, active (%)	41	36	<0.01	25	25	0.61

Statistical methods

We analyzed four methods of imputing portion sizes. The subjects were randomly divided (SAS procedure: proc surveyselect) into two dataset: a learning dataset A (n=1864) for generating data for imputation, and a test dataset B (n=1864) for analyzing the validity of the imputed data. For dataset B the 'mean daily total energy intake' (TE) was computed with the complete set of authentic self-reported portion sizes, and this TE served as the reference. The population sex-specific medians were used as standard portion sizes. With each of the three stochastic imputation methods we imputed portion sizes from dataset A to dataset B and used these estimated portion sizes to compute a new TE. This was done 10 times (on different splits of the data) and subsequently 10 TEs were computed with each imputation method.

The mean TE from each imputation method was then compared to the reference TE by the bias (defined as the mean error) and by the rooted mean squared error (RMSE). In this paper the 'error' is defined as the reference value minus the estimated value. Spearman's rho was used to compare the ranking of the subjects; comparing the reference TE with the TE calculated with imputed portion sizes. T-statistics was used to determine the bias in TE related to TE (Figure 1). Energy intakes and nutrients were computed with FoodCalc® (13), and the Danish national food composition tables (14).

The four imputation methods were:

1. 'The median method' or 'Standard portion sizes'. Imputation of median values is equivalent to applying a standard portion size as it implies uniform portion sizes for all subjects (here 39 medians - one for each of the 39 portion size items). In this model we used the sex-specific median values from the entire sample (from dataset A+B) to define 39 sex-specific standard portion sizes in dataset B (using the sex-specific median from dataset A only would induce bias as explained in the supplemental material chapter 4).

Based on earlier reports and physiological reasoning we hypothesized that portion sizes depend on age, sex, physical activity, weight, and height (4,6). Individual data from these five variables are readily available in most epidemiological studies and they informed the following three more advanced imputation methods that are all based on stochastic principles:

2. The 'Comparable categories' (Coca) method. The subjects were divided into 32 categories. Online supplemental Table S1 demonstrates how the categories were created by first dividing the subjects by level of physical activity (in active or sedentary), then dichotomized on approximate median values of height (166 cm), then divided by sex, split on rough median values of weight (74 kg), and age (48 years). Each of these categories contains individuals sharing approximately the same physiological characteristics e.g. in category 13 everyone were sedentary, >166 cm, female, <74 kg, and <48 years. For each subject in dataset B the portion sizes were substituted by a complete set of portion sizes from one random subject in the 'comparable category' in dataset A.
3. The 'K nearest neighbors' (KNN) method (15). A missing portion size in dataset B was substituted by a random value from the K – a predefined number – most similar observations ('neighbors') in dataset A. The similarity is defined as the proximity measured by Euclidean distance between the informing variables (here age, sex, physical activity, weight, and height). While traditional KNN would impute the portion size most prevalent among the K neighbors, our version of KNN imputed a random value among the K neighbors with probability proportional to the proximity making it suitable for multiple imputation. K > 20 yielded no extra accuracy.
4. The 'Multinomial logistic regression' (MLR) method. MRL models were constructed based on dataset A: age, weight, and height were continuous covariates, sex and physical activity were categorical covariates, and the portion sizes were the categorical outcomes. Portion sizes in dataset B were determined by probability sampling from the prevalence of the categorical portion size values obtained by inserting the dataset B values for age, weight, height, sex and level of physical activity in the regression model.

The set-up was run by SAS 9·2 statistical software, but the methods can be applied on any type of software. SAS codes for KNN, MLR, Coca and a wrapper for (linear) regression analysis combining the results from multiple imputed (by any method) datasets are given in the online supplemental material.

RESULTS

More women than men participated in the Danish Health Examination Survey. The subjects included in this study were a little younger than the excluded subjects. Furthermore, the included men were more active and the included women were slightly heavier. However, differences were numerically small (Table 1). Overall, compared to the reference energy intakes, the RMSEs were equally low with the median method and MLR, and equally high with Coca and KNN. The bias of the median method was numerically larger than in any of the other methods. Table 2. KNN had a negative bias in men (overestimating the portion sizes), but a positive bias in women (underestimating the portion sizes). The bias of MLR and Coca were equally low in both men and women.

More results are presented in the supplemental material (Online supplemental Table S2), including 'non sex-specific' standard portion sizes and different versions of Coca (with different informing variables and less categories). Results with selected micronutrients and macronutrient subtypes were essentially similar to the analyses of macronutrients (results not shown).
All the methods had high spearman's rank correlation, but median and MLR imputation performed slightly better than KNN and Coca. All correlations were >0·90 and all CI's between 0·89 and 0·97 (Online supplemental Table S3).

Figure 1 illustrates how all the methods resulted in a bias of TE dependent on TE, i.e. an underestimation of TE in subjects with a high energy intake, and an overestimation of TE in subjects with a low energy intake. The magnitude of this bias (the T-value) was markedly higher with median imputation than with the other methods. Figure 2 shows that when stratifying by BMI group, age group, and physical activity class, a larger variation was seen among men than women regarding the accuracy of the imputation methods. The mean total energy intake was 12·5 MJ calculated with maximum portion sizes for all and 7·5 MJ with minimum portion sizes for all. Thus, up to 40 % of the calculated energy intake was potentially determined by the portion sizes. However, Figure 2 indicates that the mean energy intakes calculated differed with up to 2 MJ (18%) in men between the methods and up to 0.75 MJ (9%) in women.

DISCUSSION

Overall, the MLR method provided the best agreement with the reference dietary intake. However, the differences between the stochastic methods were small and the confidence intervals of the bias in MLR and Coca were overlapping in most segments of the data. In MLR and Coca the bias did not differ substantially between men and women, whereas in KNN the bias was negative in men and positive women. The median method (equivalent to sex-specific standard portion sizes) had relatively low RMSEs but was inferior to the other methods in terms of bias. All the methods underestimated the reference dietary intake, except KNN that overestimated the portion sizes in men. The use of standard portion sizes systematically underestimated the energy intake of subjects with large portion sizes; a bias that diminished for instance differences in dietary intake between age groups. E.g. a young man was assigned the same standard portion size as an elderly man even though we know that age is a determinant of energy intake as demonstrated in Figure 2, and by the fact that age is an input variable in calculating the basal metabolic rate (16). This bias may well affect parameter estimates in multivariate analyses (17). On the other hand, the median method performed better than the other methods in spearman's rank test. However, the confidence intervals were overlapping with MLR, and Coca and KNN also had high correlations with the reference energy intake.

Figure 2 demonstrates how all imputation methods were better in predicting portion sizes in women than in men. The greater variation in men is in part explained by the higher energy intake, but probably also by a greater variation in portion sizes in men.

Evaluation of the methods

We used 'sex-specific median imputation' as 'standard portions'. Standard portions can of course be defined differently, but any deterministic portion size will contain the same sort of bias, and the median sizes were probably a reasonable choice.

The simple Coca method worked surprisingly well and compared to the other stochastic methods the computer run time was much faster. Depending on the size of the learning dataset and the number of categories, empty or tiny categories may occur. This can be solved by fitting cut-off values in the dichotomization or by merging related categories. The relatively basic categorization can probably be altered to improve performance. More considerations about the different versions of the methods are presented in the online supplemental material.

External validity

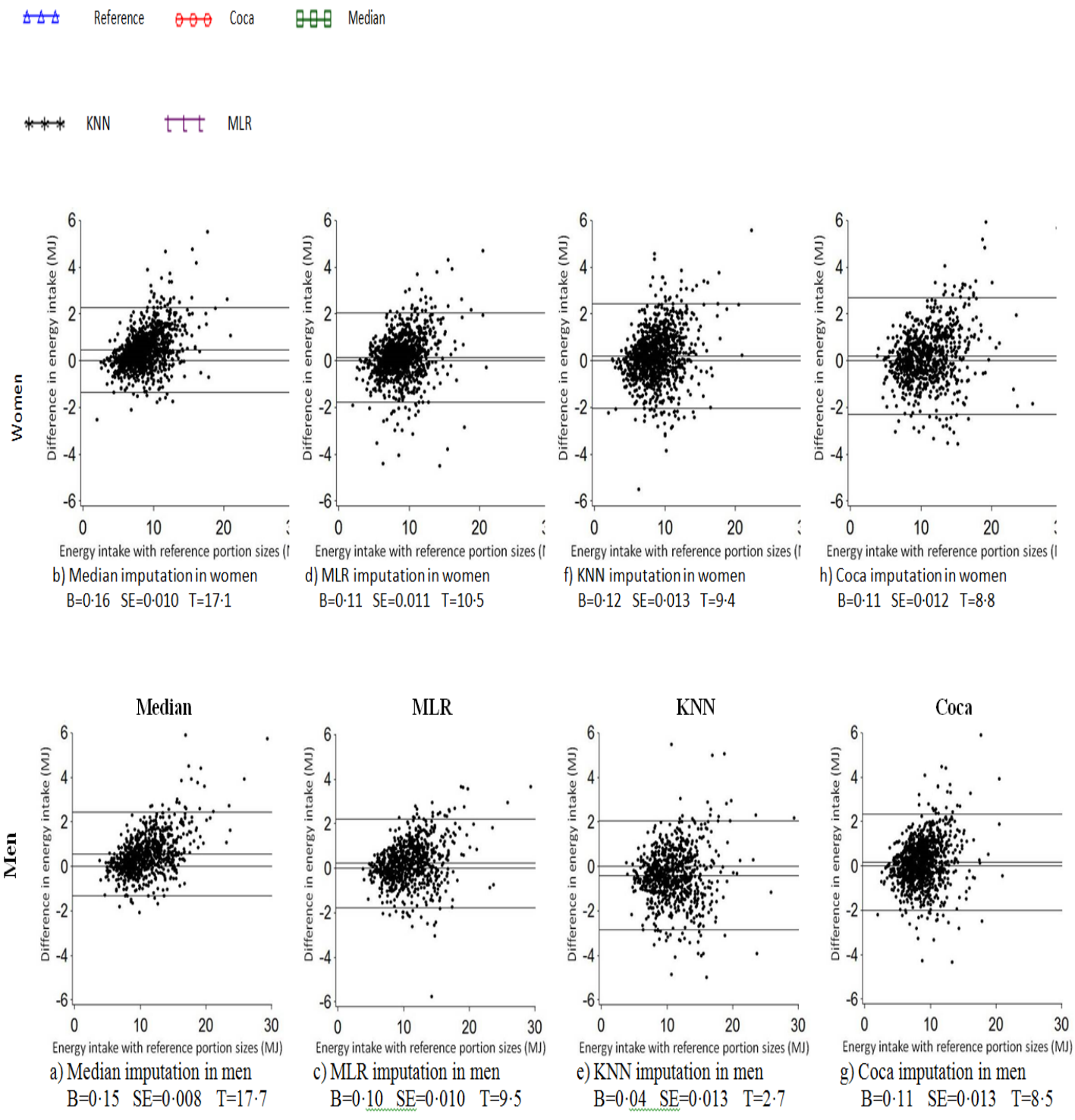
The variables physical activity, sex, age, height and weight informed the three multiple imputation methods. Consequently, the three models had access to the same information. We also tested the methods including resting heart rate and "number of potatoes with warm meals". By including the latter, all of the methods performed slightly better, and by including heart beat rate all of the methods performed slightly worse, but the methods performed approximately equal. The present five informing variables were chosen as they are readily available in most datasets.

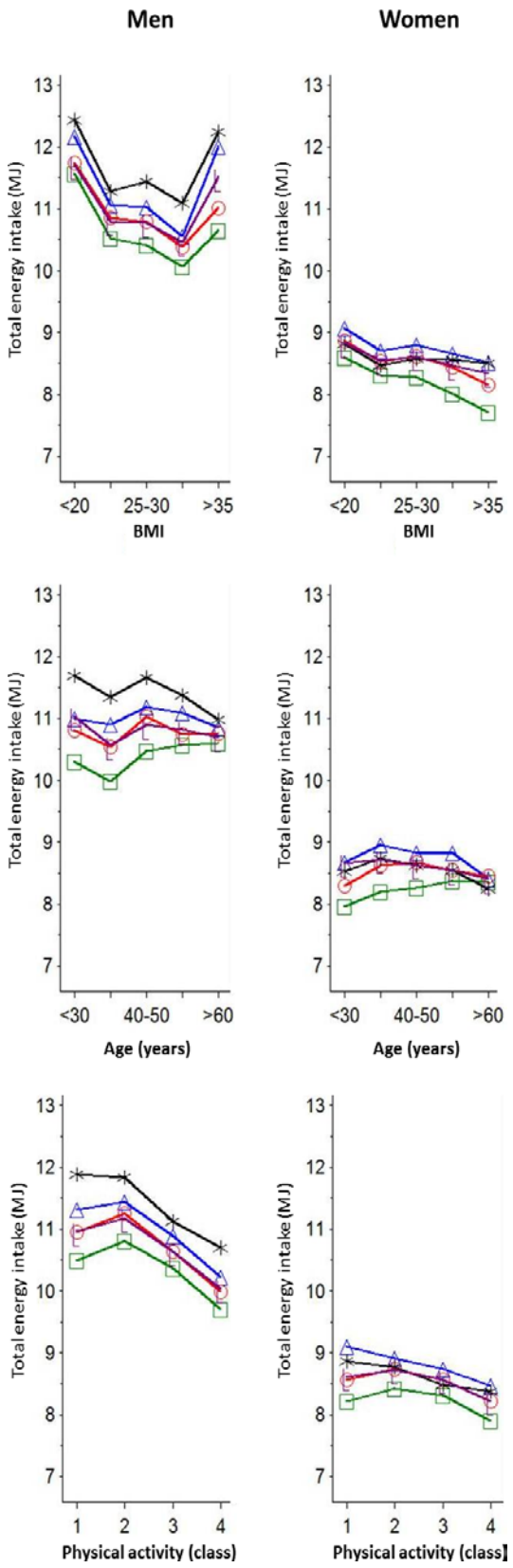
The external validity of the methods may be questioned as the included subjects differed slightly from the excluded. However, the question is not whether the included and the excluded were comparable, but rather whether the relation between physiology and portion sizes was different among the included and excluded, which does not seem very plausible.

Our reference or 'gold standard' was calculated from self-reported FFQ data with varying portion sizes, and did not take into account information bias. It is well documented how self-reported values only to some degree reflect true intakes and that reporting of specific macronutrients may be differentially biased according to sex, weight and BMI (18,19). All the methods were affected by this reporting bias. Median and MLR are model-based and thereby the reporting error affected the model and had an overall effect on all imputations i.e. possible over- and underreporting will be spread out over the whole data. In contrast, Coca and KNN imputations are based on pairing similar individual observations and hence, a systematic error will persist within the corresponding segments of the data.

Figure 1 *(next page). The total energy intake (TE) computed with reference portion sizes (x-axis), is plotted against the difference between the reference and the TE computed with portion sizes from each imputation method (y-axis). In this variation of a Bland-Altman plot the x-axis denotes the reference value (and not the mean) as the error pertains solely to the imputed measure. The horizontal lines denote zero, the mean difference, +2SD, and –2SD. B=the slope of a regression line: y=Bx+c. SE=standard error. T=B/SE. Thus, T denotes the tendency to underestimate portion sizes in subjects with high TE (and the reverse). High values of T denote stronger tendencies; the significance is implicit as T>1•95 implies P<0•05. NB: a positive value on the y-axis indicates an underestimation of the reference energy intake.*

Figure 2 *(page 39). The mean daily total energy intake is plotted against BMI, age and level of physical activity, separately for men and women. The reference – blue triangle – is computed with the originally reported portion sizes. The total energy intake has been computed with portion sizes determined by four different imputation methods: green squares - median (equivalent to sex-specific standard portions); purple L's – multinomial logistic regression; red circles – comparable categories; black stars – K nearest neighbors. The results presented are mean values of 10 imputations with each method (on random splits of the data).*


Reference
Coca
Median
KNN
MLR
Women
Men
Difference in energy intake (MJ)
Energy intake with reference portion sizes (MJ)
Median
MLR
KNN
Coca
b) Median imputation in women
B=0·16 SE=0·010 T=17·1
d) MLR imputation in women
B=0·11 SE=0.011 T=10·5
f) KNN imputation in women
B=0·12 SE=0·013 T=9·4
h) Coca imputation in women
B=0·11 SE=0·012 T=8·8
a) Median imputation in men
B=0·15 SE=0·008 T=17·7
c) MLR imputation in men
B=0·10 SE=0·010 T=9·5
e) KNN imputation in men
B=0·04 SE=0·013 T=2·7
g) Coca imputation in men
B=0·11 SE=0·013 T=8·5



Missing single values

Concerning FFQs with individual portion size questions, the MLR, Coca, or KNN methods can be used to substitute missing single values. In the Danish Health Examination Survey, from where the present data derive, 17.7% of the questions on portion sizes were missing which is not uncommon in a FFQ (20). Currently, most studies probably 'fill in the blanks' with median values or standard portions (21). As demonstrated in the present study median imputation generates bias. If only few values are missing the resulting bias may be negligible, but the impact of median imputation bias increases with the number of missing values. A comparable dataset is always available (e.g. the sub-set of the data with no missing). We have supplied Coca SAS codes for this use in the online supplemental material.

FFQs without portion sizes

MLR or Coca may be used to include portion sizes in FFQ without individual portion size questions. In this case the portion sizes will have to be imputed from a comparable dataset with portion sizes. Often traditional FFQs have later been improved with portion size questions, and if the populations are similar, data from newer semi-quantitative FFQs can be used as learning dataset. We have supplied SAS codes for this use, also in the online supplemental material.

Multiple imputation

When applying multiple imputation, the multivariate analyses are run on multiple, e.g. 10, datasets each with different imputed values. The resulting parameter estimates are then the mean values of the 10 analyses (7). In this paper we did not test our imputation methods' ability to predict parameter estimates, but solely the ability to predict the reference TE, using 10 imputations for each method. In the online supplemental material SAS codes are provided on how to do multiple regression modeling with multiple datasets.

In summary

MLR and Coca are both valuable methods for including portion sizes in FFQs or substituting missing portion size values. The KNN method seemed less attractive due to the differential bias in men and women, and the relatively high RMSE. In general, these three stochastic methods allowed for estimation of meaningful portion sizes by conditioning on information about physiology, and they were suitable for multiple imputation. Application of sex-specific standard portion sizes inferred more bias than the other methods tested, and diminished for instance age differences. We propose to use the MLR or Coca methods to substitute missing portion size values, or when portion sizes needs to be included in FFQs without portion size data.

Table 2 *(next page). The four methods were compared by their ability to predict the Reference. The Reference energy intakes were computed with a set of complete reported portion sizes. Median: sex-specific median imputation which is equivalent to using sex-specific standard portion sizes. Coca: comparable categories. KNN: K nearest neighbors. MLR: multinomial logistic regression. RMSE: rooted mean squared error. Bias: mean error. The results presented are mean values of 10 imputations with each method (on random splits of the data). Please note that a positive bias indicates an underestimation of the reference, and a negative bias indicates an overestimation.*

	Men							Women						
	Energy		95%CI	RMSE	95%CI	Bias	95%CI	Energy		95%CI	RMSE	95%CI	Bias	95%CI
	%	MJ	MJ	KJ	KJ	KJ	KJ	%	MJ	MJ	KJ	KJ	KJ	KJ
Total energy														
Reference	-	10·97	10·81-11·13	ref	-	ref	-	-	8·81	8·69-8·92	ref	-	ref	-
Median	-	10·45	10·37-10·50	1118	1098-1139	579	563-596	-	8·28	8·26-8·30	1061	1011-1111	469	455-482
KNN	-	11·37	11·32-11·41	1281	1262-1299	-340	-365-(-315)	-	8·53	8·51-8·56	1181	1129-1234	218	191-244
MLR	-	10·78	10·73-10·83	1060	1028-1092	248	223-274	-	8·57	8·55-8·60	1051	997-1105	178	161-195
Coca	-	10·80	10·75-10·83	1230	1196-1264	234	207-261	-	8·56	8·54-8·59	1146	1087-1205	188	166-210
Fat														
Reference	31·2	3·43	3·36-3·49	ref	-	ref	-	29·9	2·64	2·60-2·68	ref	-	ref	-
Median	31·8	3·32	3·31-3·34	375	364-386	124	119-130	30·8	2·55	2·54-2·56	305	292-317	67	65-70
KNN	31·7	3·61	3·59-3·63	502	491-513	-161	-175-(-146)	30·0	2·56	2·56-2·57	395	387-404	56	47-64
MLR	31·2	3·37	3·36-3·39	392	381-403	75	68-82	30·0	2·57	2·56-2·58	345	330-361	45	39-51
Coca	31·3	3·38	3·36-3·39	473	458-489	70	59-81	30·0	2·57	2·56-2·58	392	377-407	49	43-54
Protein														
Reference	16·1	1·77	1·74-1·80	ref	-	ref	-	16·3	1·44	1·42-1·45	ref	-	ref	-
Median	16·5	1·72	1·71-1·73	210	205-215	57	54-60	16·7	1·38	1·38-1·38	191	188-193	49	47-50
KNN	16·3	1·86	1·84-1·87	273	267-279	-78	-87-(-69)	16·1	1·38	1·37-1·38	251	246-257	53	48-58
MLR	16·2	1·74	1·73-1·75	220	214-225	37	32-42	16·4	1·41	1·40-1·41	211	205-216	21	18-25
Coca	16.2	1·75	1·73-1·76	271	263-278	34	27-40	16·4	1·41	1·40-1·41	249	243-256	23	20-26
Carbohydrates														
Reference	42·2	4·63	4·57-4·68	ref	-	ref	-	44·0	3·88	3·83-3·92	ref	-	ref	-
Median	41·0	4·28	4·26-4·30	613	598-627	362	354-371	42·7	3·54	3·52-3·55	675	616-733	319	307-330
KNN	41·6	4·73	4·71-4·76	672	656-688	-92	-111-(-73)	44·1	3·77	3·75-3·78	693	636-750	88	70-105
MLR	41·9	4·52	4·49-4·55	580	560-599	122	106-138	43·8	3·75	3·77-3·77	640	576-704	100	86-114
Coca	41·9	4·53	4·50-4·55	602	585-621	116	104-128	43·8	3·75	3·73-3·77	652	595-708	105	89-121

ARTICLE 3

INTENTIONAL WEIGHT LOSS AND LONGEVITY IN OVERWEIGHT PATIENTS WITH TYPE 2 DIABETES: A POPULATION BASED INCEPTION COHORT

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ABSTRACT

Background

Weight loss is recommended to all overweight (BMI≥25 kg/m2) patients with diabetes type 2 but has not been proven to reduce mortality or cardiovascular morbidity. The objective of this study was to estimate the influence of prospectively planned intentional weight loss on long-term morbidity and mortality.

Methods

Weight and prospective intentions for weight change were monitored every 3rd month for 6 years in a cohort of 761 consecutive patients (≥40 years) newly diagnosed with diabetes in general practices throughout Denmark in 1989-92. We analyzed the 444 patients who were overweight at diagnosis and alive at the end of the monitoring period with multivariate regression modelling. The outcomes were from national registers during the 13 years that followed the 6 years monitoring period.

Results

Overall weight loss was associated with increased all-cause mortality (P<0.01). The adjusted hazard ratio for all-cause mortality, cardiovascular mortality, and cardiovascular morbidity attributable to 1 kg of intentional weight loss was 1.21 (95% CI 0.97-1.50, P=0.09), 1.26 (0.93-1.72, P=0.14), and 1.05 (0.77-1.41, P=0.77), respectively. The non-linear spline estimate suggested a V-shaped association between weight change and all-cause mortality, prompting the best prognosis for those who maintained their weight regardless of intention.

Conclusions

In overweight patients with diabetes type 2 neither mortality nor cardiovascular morbidity was reduced by prospectively planned intentional weight loss supervised by a medical doctor.

INTRODUCTION

Weight loss is recognized as an important first-line treatment of overweight individuals (BMI ≥25 kg/m2) with type 2 diabetes. Losing weight has a well-documented short-term positive effect on intermediate outcomes such as glycemic control, blood pressure,

and dyslipidemia (1), and weight loss is by many clinicians regarded as an effective secondary prevention for cardiovascular disease in overweight patients with diabetes. This approach is scientifically based on improvements in intermediate outcomes, and on data from observational studies suggesting that intentional weight loss reduces the risk of death in these patients (2-6). Until recently no clinical trials had reported the effect of weight loss on longevity (7). Look-AHEAD included a selection of relatively healthy overweight adults with type 2 diabetes. Surprisingly, this large randomized trial was stopped prematurely after 9.6 years of intervention with diet and exercise in order to obtain weight loss (8). Despite a greater weight loss in the intervention group, neither the mortality, nor the cardiovascular morbidity was reduced. The authors state that the results cannot be generalized as the participants were not representative for the background population of diabetes patients.

The randomized clinical trial Diabetes Care in General Practice (DCGP) included a population-based sample of consecutive patients with newly diagnosed diabetes in 1989-92. The present observational study explored the implications of changes in body weight among the overweight patients in the well-monitored intervention arm of DCGP. Our main interest was the strata of patients with a prospectively well-described intention to lose weight, as unintentional weight loss is associated with mortality seemingly due to confounding from wasting (5;6;9). Thus, our objective was to estimate the long-term all-cause mortality, cardiovascular mortality and cardiovascular morbidity risk attributable to weight change in a population-based sample of overweight patients with newly diagnosed diabetes, stratified on intention to lose or to maintain weight.

MATERIALS AND METHODS

The subjects in this observational study were the 761 participants in the intervention arm of the DCGP study where 1381 consecutive patients newly diagnosed with diabetes in general practices throughout Denmark were randomized to routine care or the intervention: structured personal care (Figure 1)(10). The patients were 40 years or older. Every third month, the patients in the intervention group were invited to control visits at their general practitioner during a period of 6 years. Among other measures, body weight and a prospective goal for intended weight change in the next 3 months were recorded at each visit. The doctors in the intervention group were supported by clinical guidelines and continuing medical education. In overweight patients, they were prompted to get an agreement on a small realistic weight reduction, record it and follow up accordingly. It was suggested to the intervention doctors that they recommend increased physical activity and simple dietary rules: to increase the intake of complex carbohydrate to at least 50% of the diet, and in particular to increase the intake of water soluble fiber, reduce fat intake to a maximum of 30%, reduce alcohol intake, and eat 5-6 meals a day (11). The recommended management of patients did not differ according to the patients' level of overweight. The median number of weight registrations was 13 per patient. The median time between consultations was 106 days. After 6 years of intervention (as well as before the intervention) there was no statistically significant difference in body weight between the two randomization arms. The mean weight loss was 2.6 kg in the intervention arm vs. 2.0 kg in the control arm.

In the present cohort study, overlaid the original trial, the 6 year intervention period is referred to as the monitoring period. We included the 444 overweight patients in the intervention arm (BMI at diagnosis ≥25 kg/m2) who were alive at the end of the monitoring period and had at least 3 valid measurements of weight. The patients in the control arm were not included as they were not monitored every three month with body weight and intention. However, since the study was randomized the intervention arm was still a representative sample of patients with incident diabetes and they were treated with what later became the standard care for diabetes patients in Denmark. Patients diagnosed with cancer (not including benign skin cancers) at any time before or during the monitoring period were excluded (Figure 1). The study was approved by the research ethics committee of Copenhagen and Frederiksberg and informed consent was given by all patients.

Definition of exposure

For each patient the weight change was modeled with a regression line through all measured weights in the 6 year monitoring period. The exposure of interest in our study is the slope of this regression line (Figure 2).

Definition of outcome

Information on all-cause mortality, cardiovascular mortality, and cardiovascular morbidity was from the Danish Civil Registration System, the Danish National Patient Register, and the Danish Register of Causes of Death (12-14). The patients were followed up in these registers until January 1st 2009, for a total of 13 years after the end of the monitoring period. Cardiovascular mortality was defined as fatal myocardial infarction (ICD-10: I20-I25 or I50), fatal stroke (I60-I69 or G45), fatal peripheral vascular disease (I70.2 or E10.5 or E11.5 or E12.5 or E13.5 or E14.5), or sudden death (R96-R99) after the monitoring period. Cardiovascular morbidity was defined as a fatal or non-fatal incidence of myocardial infarction, stroke, or peripheral vascular disease, as defined above, after the monitoring period.

Definition of intention for weight change

The patients were categorized in four groups according to their intentions for weight change. The categorization is hierarchal, starting with no. 1:

1. Aberrant weight pattern (n=5): The patient had a goal of weight gain at any time (n=1), or a weight loss rate of > 20 kg/year between the two last measurements (equivalent to 5 kg in 3 months), not combined with a goal of weight loss for the specific period (n=4).
2. Intention to lose weight (n=209): The patient had at least 3 recorded goals of weight loss, and at least one of these goals was recorded in one of the 3 last consultations.
3. Intention to maintain weight (n=210): The patient had at least one recorded goal of maintaining weight (and up to two recorded goals of weight loss).
4. Intention of weight change not well-described (n=20): The patient had no recorded goals of maintaining weight (but up to two recorded goals of weight loss).

Definition of potential confounders

Height was measured at diagnosis and weight was measured approximately every third month (without shoes) by the general practitioner with the scales available in the clinic. BMI: baseline value (continuous). Smoking: self-reported at diagnosis and at the end of the monitoring period. We used change in smoking status (categorical: never smoker, continuous smoker, ex-smoker at diagnosis, quitter in the monitoring period, or starter in the monitoring period). Physical activity: Leisure time physical activity was

self-reported at diagnosis and at the end of the monitoring period. We used change in physical activity during the monitoring period (categorical: remained sedentary, became sedentary, remained active, or became active). Education: <10 years of school or ≥10 years. Medication: Start of antidiabetic medication in the monitoring period (categorical: oral, insulin or none). The Charlson comorbidity index: score during the 6-year monitoring period (continuous) (15;16).

Statistical methods

The multivariate analyses were designed a priori to control as effectively as possible for confounding from wasting. All analyses were stratified on 'intention to lose weight' or 'intention to maintain weight'. Thus, weight change for a given individual was compared only to patients within his or her own strata of intention. The association between weight change and mortality/morbidity was analyzed with a Cox regression model adjusted for age, gender, education, BMI at diagnosis, and changes in smoking, physical activity, medication, and the Charlson comorbidity score during the monitoring period. Only patients who were not registered as having had a cardiovascular event by the end of the monitoring period were included in the multivariate analyses of cardiovascular morbidity.

In the main model we included only covariates with an anticipated causal effect on both the exposure (weight change over 6 years) and the outcomes. Intermediate variables like the mean HbA1c in the monitoring period and the blood pressure, or triglyceride level at diagnosis, were included in additional sensitivity analyses to further adjust for disease severity. Also a narrowed definition of intention to lose weight was tested (6 or more recorded goals of weight loss, and at least one of these goals should still be recorded in one of the 3 last consultations), and we performed subgroup analyses of 'patients with intention to lose weight' stratified on physical activity (remained or became active vs. remained or became sedentary), BMI (<30 vs. BMI≥30), microalbuminuria (≥15 vs. <15 mg/L), or established macrovascular disease (+/-) at diagnosis.

The total follow-up period was 13 years. As we expected most patients with a wasting disease to die within the first two years, this period was analyzed separately. We regarded the association between weight change in the monitoring period and mortality the remaining 11 years of follow up as less confounded by wasting. As we a priori did not expect the associations of interest to necessarily be linear we planned to depict the relations by restricted cubic splines (17). For 62 patients (14%), information on one or more of the potential confounders was missing, and they were omitted from the analyses. There was no loss to follow up. All statistical analyses were conducted by the use of SAS statistical software, version 9.2.

RESULTS

In the 6 years of monitoring following the diabetes diagnosis (inter quartile range 5.7-6.3 years) there was, despite intentions, a mean weight gain in the group with 'intention to lose weight' of 0.13 kg/year, and an average weight loss in the group of patients with 'intention to maintain weight' of 0.55 kg/year (difference = 0.68 kg/year, 95% CI 0.44-0.91). Table 1 shows the patient characteristics at diagnosis and changes in risk factors during the monitoring period.

The multivariate analyses of the hazard ratio (HR) for the outcomes in the following 13 year period are presented in Table 2. In the unstratified analysis, including all patients regardless of their weight change intentions, the HR for all-cause mortality attributable to 1 kg of weight loss/year was 1.18 (95% CI 1.05-1.33), whereas weight change was not associated with cardiovascular mortality or morbidity. For all–cause mortality and cardiovascular mortality the risk was higher in the first two years compared to the remaining 11 years of the follow-up period. A similar pattern was found in the strata of patients with 'intention to maintain weight'.

Among patients with 'intention to lose weight' there was no difference in mortality risk attributable to weight loss between the first two years and the rest of the follow up period (P for difference=0.75), and there was a trend towards a linear association between weight change and all-cause mortality 1.21 (0.97-1.50). In Figure 2 the adjusted HR for all-cause mortality is depicted as a spline function of the yearly weight change rate. Zero on the x-axis means that the weight on average was maintained throughout the monitoring period, whereas a negative value denotes a general weight loss. Figure 2 suggests a V-shaped association between weight change and all-cause mortality, with a significantly increased risk in those intentionally losing weight, but not in those who gained weight despite their intention.

Sensitivity analyses

For patients with 'intention to lose weight' we performed a range of sensitivity analyses to test the robustness of the associations. The inclusion of the mean HbA1c in the monitoring period, the triglyceride level, the diastolic or systolic blood pressure at diagnosis as covariates did not change the estimates substantially. A more narrow definition of 'intention to lose weight' (as defined in Methods) did not alter the associations much either. Analyses stratified on BMI showed that the association between weight loss and all-cause mortality seemed to be driven by patients with a BMI≥30 (Table 3). In these obese patients, intentional weight loss was associated with an increased 13 years risk of mortality (HR 1.36, 95%CI 1.01-1.84, P<0.05), whereas in patients with BMI<30 there was a trend towards a protective effect (0.66, 0.38-1.14, P=0.14). In patients with microalbuminuria at diagnosis intentional weight loss was significantly associated with the 13 years all-cause mortality (1.85, 1.06-3.24, P=0.03). In patients without CVD or microalbuminuria at baseline intentional weight loss was not associated with mortality (1.04, 0.63-1.70). However, the confidence interval was wide.

In all sensitivity analyses, including 2, 11, or 13 years follow up, the HR for mortality attributable to weight loss was >1.00, except in patients with BMI<30 with 'intention to lose weight'.

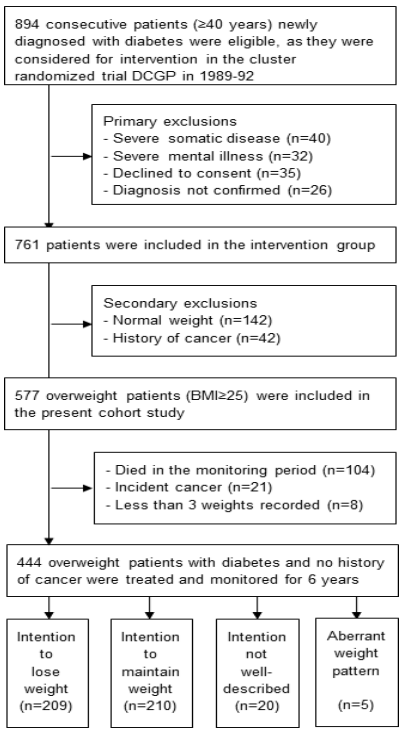
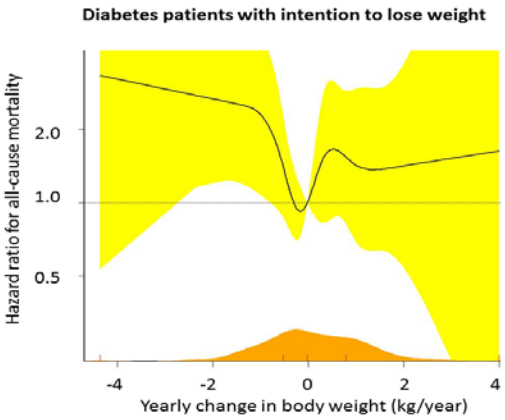


Figure 1. *Patient flow*

Figure 2. *Weight change in patients with intention to lose weight and subsequent HR for all-cause mortality (the main analysis from article 3). The spline function illustrates the association between the average yearly weight change in the 6 years monitoring period after the diabetes diagnosis, and the subsequent 13 years' hazard ratio (HR) for all-cause or cardiovascular mortality. The cox model is adjusted for age, gender, education, BMI at diagnosis, smoking change, physical activity change, medication change, and the Charlson comorbidity score in the 6 year monitoring period. The y-axis is logarithmic. Black line: cubic spline estimate, 6 data driven nodes. Yellow: 95% confidence intervals. Orange: the distribution of the patient material. Red lines: median, inter-quartile range and min/max.*



DISCUSSION

Prospectively planned intentional weight loss supervised by a medical doctor was not associated with reduced mortality or cardiovascular morbidity in this population based sample of overweight patients with type 2 diabetes. On the contrary, our results indicated an excess mortality attributable to intentional weight loss. This seemingly increased risk was most pronounced in patients with BMI≥30 or in patients with microalbuminuria at diagnosis. In patients with BMI<30 we found a slight tendency towards a decreased risk, but in all other main- and sensitivity analyses, intentional weight loss appeared to increase the risk of death (HR>1.00; with broad confidence intervals), rather than reducing it as would have been expected. A similar pattern, but less pronounced, was seen for cardiovascular death, whereas the risk for cardiovascular morbidity seemed unrelated to intentional weight loss.

The patients with 'intention to maintain weight' lost 0.55 kg/year on average. In these patients weight loss was an independent risk factor for all-cause mortality. There was a statistically significant difference between the HR in the first two years compared to the remaining 11 years of follow up, indicating that some of the weight loss in these patients might be a result of wasting disease leading to death within two years. In contrast, residual confounding from wasting in the group of patients with 'intention to lose weight' appears to be small, given the general weight gain, despite intention, of 0.13 kg/year, and given that there was no difference in mortality attributable to weight loss between the first two years and the rest of the follow up period (P=0.75 and P=0.70). The association between intentional weight loss and all-cause mortality was not statistically significant, but the spline estimate in Fig. 2 indicate that the relation investigated was not linear but V-shaped.

Table 1. *Patient characteristics at diagnosis and changes in test results after 6 years.*

	Patients with intention to lose weight (n=209)	Patients with intention to maintain weight (n=210)
Patient characteristics at diagnosis		
Male sex, no. (%)	103 (49.3)	109 (51.9)
BMI, kg/m²	32.6±4.7	30.3±4.0
Weight, kg	91.6±16.1	84.0±13.3
Age, years	58.8±9.6	65.4±10.7
10 years of school or more	55 (27.2)	32 (15.9)
Smoking	73 (35.3)	72 (35.1)
Micro vascular disease	68 (33.8)	83 (41.1)
Macro vascular disease	52 (25.9)	61 (30.2)
Weight change over 6 years*		
Weight change, kg/year	0.13±1.18	-0.55±1.27
Change in blood pressure after 6 years†		
Systolic, mmHg	-3.3±21.2	-2.2±24.8
Diastolic, mmHg	-3.6±10.9	-3.5±11.4
Change in laboratory test values after 6 years†	-0.6±2.7	-0.5±3.1
Triglycerides, mM		
Total cholesterol, mM	-0.3±1.1	-0.1±1.1
Hemoglobin A1c, fract.	-1.5±2.2	-1.3±2.3
Fasting glucose, mM	-5.0±4.7	-5.8±7.4

Values are numbers (%) or means ±SD

** The slope of a regression line through all measured weights for each patient.*

† The last measurement (after 6 years) minus the first measurements (at diagnosis).

Table 2. *Multivariate analyses of mortality and morbidity risk attributable to one kg of weight loss per year.*

	All patients		Patients with intention to lose weight		Patients with intention to maintain weight	
	HR (95%CI)	P	HR (95%CI)	P	HR (95%CI)	P
All-cause mortality	[217/379]		[92/191]		[125/188]	
First 2 years *	1.42 (1.12-1.80)	<0.01	1.30 (0.79-2.12)	0.30	1.53 (1.15-2.05)	<0.01
After 2 years †	1.13 (0.99-1.29)	0.07	1.19 (0.94-1.51)	0.15	1.13 (0.95-1.34)	0.18
Difference #		0.09		0.75		0.05
Full follow up period	1.18 (1.05-1.33)	<0.01	1.21 (0.97-1.50)	0.09	1.20 (1.03-1.41)	0.02
Cardiovascular mortality	[136/378]		[49/191]		[87/187]	
First 2 years	1.43 (1.10-1.86)	<0.01	1.39 (0.79-2.43)	0.25	1.53 (1.13-2.07)	<0.01
After 2 years	1.04 (0.88-1.23)	0.68	1.23 (0.87-1.73)	0.25	0.97 (0.79-1.20)	0.80
Difference		0.04		0.70		0.01
Full follow up period	1.12 (0.97-1.30)	0.13	1.26 (0.93-1.72)	0.14	1.09 (0.91-1.30)	0.34
Cardiovascular morbidity	[132/310]		[55/161]		[77/149]	
First 2 years	1.25 (0.91-1.73)	0.17	1.41 (0.63-3.14)	0.41	1.24 (0.85-1.82)	0.27
After 2 years	0.94 (0.79-1.13)	0.52	1.00 (0.73-1.38)	0.99	0.89 (0.70-1.12)	0.32
Difference		0.12		0.44		0.13
Full follow up period	1.00 (0.86-1.17)	0.98	1.05 (0.77-1.41)	0.77	0.96 (0.78-1.18)	0.70

*Values are [number of events/numbers of observations used] and hazard ratios, HR (95% confidence intervals) from multivariate Cox regression analysis of the association between weight loss in kg/year (modeled as the slope of a regression line through all the measured weights for each patient) and the outcomes. The multivariate analyses are stratified on intention to lose, or to maintain weight, and the covariates are: age, gender, education, BMI at diagnosis, smoking change, physical activity change, medication change, and the Charlson comorbidity score in the monitoring period. * HR for mortality in the first 2 years period of follow up.*

† HR for mortality in the remaining 11 years period of follow up ('After 2 years'). The total follow up period was 13 years. # The difference in HR between the 2 first years and the subsequent 11 years.

Table 3. *Sensitivity analyses of mortality and morbidity risk attributable to one kg of weight loss per year.*

	BMI<30		BMI>30		BMI<30 and 'intention to lose weight'		BMI>30 and 'intention to lose weight'	
	HR (95%CI)	P	HR (95%CI)	P	HR (95%CI)	P	HR (95%CI)	P
All-cause mortality	[102/167]		[115/212]		[36/63]		[56/128]	
First 2 years *	1.53 (0.90-2.60)	0.12	1.47 (1.08-2.00)	0.01	0.60 (0.13-2.90)	0.53	1.49 (0.84-2.61)	0.17
After 2 years †	0.93 (0.69-1.23)	0.59	1.17 (1.01-1.36)	0.04	0.67 (0.38-1.18)	0.17	1.33 (0.96-1.84)	0.09
Difference #		0.08		0.18		0.90		0.72
Full follow up period	1.01 (0.77-1.32)	0.94	1.22 (1.06-1.40)	<0.01	0.66 (0.38-1.14)	0.14	1.36 (1.01-1.84)	<0.05
Cardiovascular mortality	[60/166]		[76/212]		[19/63]		[30/128]	
First 2 years	1.68 (0.91-3.10)	0.10	1.46 (1.03-2.03)	0.02	0.80 (0.13-5.01)	0.81	1.38 (0.66-2.65)	0.44
After 2 years	1.04 (0.71-1.51)	0.33	1.05 (0.85-1.29)	0.68	0.91 (0.38-2.18)	0.83	1.00 (0.62-1.61)	0.99
Difference		0.16		0.08		0.90		0.49
Full follow up period	0.96 (0.67-1.38)	0.83	1.10 (0.92-1.32)	0.28	0.89 (0.40-1.98)	0.77	1.07 (0.69-1.66)	0.75
Cardiovascular morbidity	[58/135]		[74/175]		[25/55]		[30/106]	
First 2 years	0.91 (0.45-1.87)	0.80	1.40 (0.94-2.10)	0.10	1.81 (0.41-8.05)	0.43	1.71 (0.39-7.61)	0.48
After 2 years	0.76 (0.53-1.10)	0.15	1.01 (0.81-1.26)	0.92	1.11 (0.54-2.27)	0.77	1.02 (0.65-1.60)	0.92
Difference		0.64		0.15		0.54		0.51
Full follow up period	0.78 (0.55-1.11)	0.17	1.08 (0.89-1.32)	0.44	1.19 (0.60-2.35)	0.62	1.06 (0.68-1.64)	0.81

*Values are [numbers of observations used/number of events] and hazard ratios, HR (95% confidence intervals) from multivariate Cox regression analysis of the association between weight loss in kg/year (modeled as the slope of a regression line through all the measured weights for each patient) and the outcomes. The multivariate analyses are stratified on BMI, or on BMI and intention to loose or to maintain weight, and the covariates are: age, gender, education, BMI at diagnosis, smoking change, physical activity change, medication change, and the Charlson comorbidity score in the monitoring period. * HR for mortality in the first 2 years period of follow up.*
† HR for mortality in the remaining 11 years period of follow up ('After 2 years'). The total follow up period was 13 years. # The difference in HR between the 2 first years and the subsequent 11 years.

Strengths and limitations of the study

The results are based on a population-based inception cohort and the participants were prospectively monitored with numerous physical examinations. The study was carefully designed to estimate the presumed risk reduction attributable to therapeutic intentional weight loss, by minimizing confounding from wasting. Regarding residual confounding from wasting we do consider it strengths that; 1) the intentions for weight change were prospectively described together with a medical doctor, and that only data from patients with intentional weight loss were used in the main analysis; 2) the multivariate model was adjusted for the Charlson comorbidity index score i.e. incident comorbidity in the monitoring period; 3) All patients with prior or incident cancer were excluded. 4) the multivariate model was adjusted for change in antidiabetic medication.; 5) we included a range of indicators for disease severity in the sensitivity analyses, but the results were robust; 6) the 13 years follow up was divided into two periods to shed light on differences in mortality risk attributable to weight loss between the first two years and the remaining 11 years of follow up. Still, it is a limitation that there is no way to exclude that the results are partly explained by residual confounding from pathological weight loss.

Of limitations should also be mentioned that the weight loss method was not recorded, that patients less than 40 years were not included, and that patients with diabetes, in most settings, are diagnosed earlier today than they were 25 years ago. In 1990 screening for diabetes was uncommon, and the patients were diagnosed – and included – primarily because they had some kind of symptom and were seeking medical attention. Thus, the patients had clinical diabetes as opposed to most patients diagnosed today who have no symptoms. Thus, the results can only be generalized to patients with clinical diabetes.

Some may consider it a limitation that the control group was not included in the study, but since the study was randomized the included participants are still representative for patients with incident diabetes in the general population 25 years ago. On the contrary, it can be considered strength that the patients included all received the intervention that after the study became the standard care for patients with type 2 diabetes in Denmark. However, risk factors for CVD are treated much more aggressively today than they were 20 years ago, but the lifestyle intervention is largely unchanged.

For each patient included in the main analysis, the goal to lose weight was prospectively described at least 3 times during the 6-year monitoring period. This very conservative definition of intention ensured that the intention was present throughout the monitoring period.

There was a considerable variation in the weight development for each individual, and a slope of a regression line may seem too simple to describe the individual weight change pattern. For instance, a patient with high compliance would lose 6 kilos fast and then maintain the weight for 6 years. This would result in a relatively flat slope compared with a patient losing 1 kg a year, which would result in a steeper slope. Consequently, the slow weight loser would get a higher numeric value for the exposure. Still, this method certainly better describes the general weight change than retrospective weight changes or the difference between two measurements as earlier studies of intentional weight loss have used.

Comparison with other studies

The main result is in opposition to the prevailing observational literature. Harrington's meta-analysis of intentional weight loss in cohort studies found that among unhealthy obese (in this context BMI≥25-27) subjects, weight loss was associated with a reduced mortality (9). On the other hand, our results are in line with the LookAHEAD trial that found no cardiovascular protective effect of an intensive lifestyle intervention that resulted in a substantial weight loss. In the look AHEAD trial the combined effect of energy restriction, healthy diet, increased exercise was explored as intention to treat analyses. Unlike this, the present study is an analysis of the weight change itself. The weight change was correlated with the incidence of the outcome independent of weight changes attributable to age, sex, BMI at diagnosis, education, and changes in smoking, changes in medication, changes in comorbidity, and changes in physical activity. However, residual confounding from pathological weight loss cannot be excluded as mentioned above.

Williamson et al have often been referred to as evidence for weight loss as a means to reduce mortality in patients with type 2 diabetes (4;5). They found that intentional weight loss was associated with reduced mortality, while Gregg et al some years later, found that having an 'intention to lose weight' was associated with reduced mortality regardless of whether the patients actually lost weight or not (4;6). The two cohort studies were both included in Harrington's meta-analysis on weight loss and mortality (9). In contrast to most other cohort studies in the field, our main result is based on a continuous exposure. The mortality among intentional weight losers was not compared with participants with stable weight and unknown intention or intention to maintain weight. Instead the weight change was correlated to the outcome within the group of patients intending to lose weight. In this way a possible bias from intention was avoided.

A low level of physical activity is associated with increased mortality in patients with diabetes (18), and weight loss obtained by exercise may be healthier than weight loss obtained by energy restriction. A well-conducted observational study in a selected population of physically active well-educated patients with diabetes, demonstrated no independent association between intentional weight loss and longevity, when the analysis was controlled for cardio-respiratory fitness (19). Unfortunately, we have no good measure of how the individual patient lost weight in our study. However, the interventions in DCGP were relatively modest with an emphasis on healthy diet and energy restriction, and less so on exercise (11). The Look AHEAD trial showed a non-significant reduction in all-cause mortality in the intervention group, whereas we found a tendency towards increased mortality risk associated with weight loss independent of physical activity. An explanation could be that weight loss induced by calorie restriction increases the risk of death, while physical activity reduces the risk. However, this hypothesis is difficult to test in an observational setting as the bias work in opposite direction for physical activity vs. weight loss, i.e. undiscovered disease will often make weight loss look worse (wasting), whereas it will make physical activity look better (participants with undiscovered disease may engage less in physical activity).

Conclusions and policy implications

In a population-based sample of overweight patients newly diagnosed with type 2 diabetes, neither mortality nor cardiovascular

morbidity was reduced by therapeutic intentional weight loss supervised by a medical doctor. On the contrary, there was a trend towards increased mortality. It cannot be excluded that the result are partly explained by residual confounding from pathological weight loss. Still, the results indicate that the parallel findings from the LookAHEAD trial can be generalized to the general population of patients with type 2 diabetes. Recourses spend on lifestyle interventions in patients with diabetes type 2 may be used more rational by changing focus from weight loss to other modalities like increased physical activity or healthy diet.

ACKNOWLEDGEMENTS

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APPENDIX 1 – R CODE FOR THE DATA SIMULATION STUDY

```
    simdata <- func-
tion(n,OR1=1,OR2=1,ORC=1,briskc=0.5,brisk=0.1){
      bmi <- 25+(4*rnorm(n))
      beta0 <- -1*((25*log(ORC)) + (log(((1/briskc)-1))))
      xbeta <- beta0+(log(ORC)*bmi)
      probs <- 1/(1+exp(-1*xbeta))
      wd <- (runif(n)<probs)
      beta0 <- -1*((25*log(OR1)) + (log(((1/brisk)-1))))
      xbeta <- beta0+(log(OR1)*bmi)+(log(OR2)*wd)
      probs <- 1/(1+exp(-1*xbeta))
      dead <- (runif(n)<probs)
      mydata <- cbind(bmi,wd,dead)

    }

    RESULT <- array(0,c(10,10))

    for (i in 1:10){
      for (j in 1:10){
        print(c(i,j))
        mydata <- as.data.frame(sim-
data(100000,OR1=1.2,OR2=1,ORC=(1+((i-
1)/20)),briskc=0.5,brisk=0.10))
        mydata[,1] <- mydata[,1]+((j-1)*mydata[,2])
        mymodel <- glm(mydata[,3] ~ mydata[,1] + factor(my-
data[,2]),family=binomial(link = "logit"))
        RESULT[i,j] <- exp(coefficients(mymodel)[3])
      }
}
```

APPENDIX 2 – ONLINE SUPPLEMENTAL MATERIAL FOR ARTICLE 2

This appendix contains SAS codes for the imputation methods presented in the article 'Missing portion sizes in food frequency questionnaires - alternatives to use of standard portions' and short explanations on how to implement the methods. Also supplemental analyses and considerations about the use of the different imputation methods have been added.

Content

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6. Considerations about how to handle missing frequencies
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1.a. About Coca imputation

Coca is a method for substituting missing variables or missing single values with reasonable values creating less bias than using median values. This has been documented in the article 'Nutrient and energy intakes from food frequency questionnaires - alternatives to standard portion sizes'. This article will be referred to as 'the portion size article'.

The basic idea in Coca imputation is that instead of using a median value for substituting missing data, you can use information from a subject sharing approximately the same physiological characteristics as the subject with the missing data – a random subject in a 'comparable category'.

The 'comparable categories' can be used to impute a variable from dataset A to dataset B when the desired information does not exist in dataset B. The Coca method can also be used to substitute missing single values within dataset B when the desired variable does exist in B.

Coca is applicable with multiple imputation.

The Coca macro is a simple program that doesn't take much processing power or time to run on a modern computer.

We have developed the method for imputation of portion sizes in food frequency questionnaires, but the Coca-principle can be generalized to handle missing variables or missing values in other types of data as well.

1.b. Generating the comparable categories

You have to choose some meaningful variables to create suitable comparable categories. The choice of variables defining the categories depends on the data available and the variable you want to impute. In the portion size article we imputed portion sizes in food frequency questionnaires. Based on earlier reports and physiological reasoning we hypothesized that portion sizes depend on age, gender, physical activity, weight, and height. We call these 'informing variables'. In another project we used a question from the frequency chapter that was actually a portion size: 'number of potatoes with warm meals' instead of height. However, the validity of an informing variable like that depends on the cultural context. In Denmark it is obviously a good indicator for portion sizes because Danes tend to eat many potatoes whereas people from other places may eat primarily rice or other foods.

To create the categories we dichotomize the informing variables approximately at their median values. In Table S1 the princi-

ple is demonstrated. It doesn't matter in which order the informing variable enters the categorization tree. We chose to let missing values within the informing variables be categorized in the 'lower category'. This rather simple categorization can certainly be elaborated.

In the portion size article the median values of the informing variables, in the two datasets A and B were almost identical. In two unrelated but comparable datasets the median values will always differ. In this case we used the mean of the medians from the two dataset as threshold for dichotomization. For instance, in one dataset the median age was 48 years and in the other dataset the median age was 52; so we used age = 50 as cut off value in the dichotomization to create the comparable categories in both dataset. But really, any value can be used.

The subjects were divided into 32 categories. The categories were created by first dividing the subjects by level of physical activity, then dichotomized on median values of height, then divided by gender, split on median values of weight, and age. Each of these categories contains individuals sharing approximately the same physiological characteristics. For each subject in dataset B the portion sizes were substituted by a set of portion sizes from a random subject in the 'comparable category' in dataset A.

1.c. SAS code for generating the comparable categories

```
data A; set imputation.A;


    if physical_activity=<2 then PA=1;
    else if physical_activity>=3 then PA=2;

    if height=<166 then H=1;
    else if height>166 then H=2;

    if weight>=74 then W=1;
    else if weight <74 then W=2;

    if age=<50 then A=1;
    else if age>50 then A=2;


     if PA=1 and H=1 and sex=0 and W=1 and A=1 then category=1;
     if PA=1 and H=1 and sex=0 and W=1 and A=2 then category=2;
     if PA=1 and H=1 and sex=0 and W=2 and A=1 then category=3;
     if PA=1 and H=1 and sex=0 and W=2 and A=2 then category=4;
     if PA=1 and H=1 and sex=1 and W=1 and A=1 then category=5;
     if PA=1 and H=1 and sex=1 and W=1 and A=2 then category=6;
     if PA=1 and H=1 and sex=1 and W=2 and A=1 then category=7;
     if PA=1 and H=1 and sex=1 and W=2 and A=2 then category=8;
     if PA=1 and H=2 and sex=0 and W=1 and A=1 then category=9;
     if PA=1 and H=2 and sex=0 and W=1 and A=2 then category=10;
     if PA=1 and H=2 and sex=0 and W=2 and A=1 then category=11;
     if PA=1 and H=2 and sex=0 and W=2 and A=2 then category=12;
     if PA=1 and H=2 and sex=1 and W=1 and A=1 then category=13;
     if PA=1 and H=2 and sex=1 and W=1 and A=2 then category=14;
     if PA=1 and H=2 and sex=1 and W=2 and A=1 then category=15;
     if PA=1 and H=2 and sex=1 and W=2 and A=2 then category=16;
     if PA=2 and H=1 and sex=0 and W=1 and A=1 then category=17;
     if PA=2 and H=1 and sex=0 and W=1 and A=2 then category=18;
     if PA=2 and H=1 and sex=0 and W=2 and A=1 then category=19;
     if PA=2 and H=1 and sex=0 and W=2 and A=2 then category=20;
     if PA=2 and H=1 and sex=1 and W=1 and A=1 then category=21;
     if PA=2 and H=1 and sex=1 and W=1 and A=2 then category=22;
     if PA=2 and H=1 and sex=1 and W=2 and A=1 then category=23;
     if PA=2 and H=1 and sex=1 and W=2 and A=2 then category=24;
     if PA=2 and H=2 and sex=0 and W=1 and A=1 then category=25;
     if PA=2 and H=2 and sex=0 and W=1 and A=2 then category=26;
     if PA=2 and H=2 and sex=0 and W=2 and A=1 then category=27;
     if PA=2 and H=2 and sex=0 and W=2 and A=2 then category=28;
     if PA=2 and H=2 and sex=1 and W=1 and A=1 then category=29;
     if PA=2 and H=2 and sex=1 and W=1 and A=2 then category=30;
     if PA=2 and H=2 and sex=1 and W=2 and A=1 then category=31;
     if PA=2 and H=2 and sex=1 and W=2 and A=2 then category=32;
     run;
```

1.d. SAS code for Coca imputation of variables from data set A to data set B

When portion sizes (or another variable) do not exist in your dataset B and you want to impute it from another dataset A, you can do this with the Coca method. Dataset B should at least contain the variables 'id' and 'category'. Dataset A should only contain the variable 'category' and portion sizes (ps1, ps2, ps3 …). The SAS-macro ('Coca cold deck') below is sampling a set of all values (here portion sizes) from one random subject in dataset A, and assigns them to a subject in the comparable category in dataset B.

Depending on the size of the learning dataset and the number of categories, empty or tiny categories may occur. This can be solved by changing the cut-off values in the dichotomization or by merging neighbor categories. Empty categories in dataset B are ok, but an empty category in the learning dataset A will impute an empty portion size with 'Coca cold deck'. The problem can be

solved by changing the cut-off values in the dichotomization or by merging neighbor categories.

```
/*** Coca cold deck ***/


data B; set imputation.B;
run;

data A; set imputation.A;
keep category ps1 ps2 ps3 ps4 ps5 ps6 ps7 ps8 … ;
run;


%macro cocacd(setg,sett,cat,seed);
  proc sort data=&sett; by &cat;
  proc sort data=&setg; by &cat;
  ods listing close; run;
  ods output OneWayFreqs=ncat; run;
  proc freq data=&setg; table &cat; run;
  ods listing; run;
  data ncat; set ncat; keep &cat Frequency;
  proc sort data=ncat; by &cat;
  data &sett; merge &sett ncat; by &cat;
    cd=ceil(Frequency*ranuni(&seed));
   idt=_N_;
  data &setg; set &setg; by &cat;
    retain cd;
    if first.&cat then cd=0;
   cd=cd+1;
  proc sort data=&sett; by &cat cd;
  proc sort data=&setg; by &cat cd;
  data &sett; merge &sett &setg; by &cat cd;
    if idt>=0;
   drop Frequency cd idt;
%mend; run;

%cocacd(A,B,category,188789); run;

data imputation.Bps; set work.B;
proc sort; by id;
run;
```

The green number 188789 is the seed. It has to be changed every time the macro is used in order to get another random draw. The seed -1 makes a random value.

1.e. SAS code for Coca imputation of missing single values within the same dataset

You can handle the 'missing single values problem' using the Coca method. E.g. you have a dataset B with 10% missing values on physical activity and you want to impute the missing values. First you make a reasonable categorization as explained above. For physical activity we used age, gender, BMI, fasting blood sugar and 'number of potatoes with warm meals'. Dataset B should contain at least the variables 'category' and 'physical activity' (including the 10% with a missing value). The SAS-macro ('Coca hot deck') below is sampling a single value for physical activity for each missing value (in this specific variable) from a random subject in the same comparable category within dataset B.

```
/*** Coca hot deck ***/

data B; set imputation.B;
run;

%macro cocahd(var,set,cat,misslim,myimp,seed);
  data dc; set &set; if &var>&misslim;
  data dm; set &set; if &var<&misslim;          drop &var;
  ods listing close; run;
  ods output OneWayFreqs=ncat; run;
  proc freq data=dc; table &cat; run;
  ods listing; run;
  data ncat; set ncat; keep &cat Frequency;
  proc sort data=ncat; by &cat;
  proc sort data=dm; by &cat;
  data dm; merge dm ncat; by &cat;
    cd=ceil(Frequency*ranuni(&seed));
            idt=_N_;
  proc sort data=dc; by &cat;
  data dimp; set dc; by &cat;
    retain cd;
    if first.&cat then cd=0;
            cd=cd+1;
            keep cd &cat &var;
  proc sort data=dimp; by &cat cd;
  proc sort data=dm; by &cat cd;
  data dm; merge dm dimp; by &cat cd;
    if idt>=0;
            drop Frequency cd idt;
  data &set; set dm dc;
  data &set; set &set; if &var<&misslim then &var=&myimp;
drop cd;
%mend;

run;

data B; set work.B;
%cocahd(physicalactivity,B,category,-10000000,0,5207); run;

data imputation.Bpa; set work.B;
 if id<0 then delete;
run;
```

The green number 5207 is the seed. It has to be changed every time the macro is used in order to get another random draw. The seed -1 makes a random value. The 0 in (physicalactivity,B,category,-10000000,0,5207) denotes that if dataset A has got a missing value and it is imputed to dataset B it is substituted by 0. This number should be set to a reasonable value depending on the context, for instance the median, or zero if missing frequencies are imputed (se chapter 6).

1.f. Improving and developing Coca

Since height, weight, age, and gender are input variables in computing the basal metabolic rate (BMR). We tested Coca in categories of BMR combined with categories of physical activity (PA). We developed 'Coca BMR 32' with 32 categories (4 PA categories and 8 BMR categories on opproximate octiles - we had to fit the BMR cut-offs a bit in order to avoid empty categories), and 'Coca BMR 16' with 16 categories (4 PA categories and 8 BMR categories on approximate quartiles).

The results are presented in Table S2. The BMR versions did not perform better that the original Coca.

We also tested the Coca BMR 16 with a half size learning dataset: 'Coca ½ BMR 16'.The results are presented in Table S2. The performance was not reduced by the smaller learning dataset. The size of the learning dataset probably doesn't matter much as long as there's enough (the more the better, but at least a handful of subjects) in each category.

2.a. About KNN imputation

In the original 'K nearest neighbors' (KNN) method a missing value is determined by a majority vote of its nearest neighbors, being the most similar observations in the sample in terms of a set of informing variables. The proximity is measured by the Euclidean distance between the informing variables. K refers to the number of neighbors included in the vote.

The 'r' in the macro name 'knnr' indicates that we recoded the original KNN and introduced random sampling among the 'neighbors', which made KNN suitable for multiple imputation.

Missing single values or entire variables can be imputed with the KNN method. In this example we imputed all the portion sizes from dataset A to dataset B. Dataset B should contain at least the informing variables age, sex, weight, height, physical activity (or another set of relevant informing depending on the context) and an 'id'. Dataset A should contain at least the informing variables chosen and portion sizes (ps1, ps2, ps3 …).

'Cats' is the number of categories in the specific portion size item. The variable has to be categorical with no more than 6 categories.

Unlike Coca the present KNN macro does not sample all missing values in one draw, but single values in multiple draws. We also developed and tested a 'KNN random sampling- all-missing-in-one-draw' but it was not as accurate as the present macro, but the bias was more homogeneous between men and women.

The KNN method seemed to us quite appealing at a first glance and apparently less arbitrary than the Coca method. However, it proved to be very time consuming for the computers to run and less accurate than the other stochastic methods tested in the portion size article.

2.b. SAS code for KNN

```
    /*** KNN weighted probability single value sampling ***/

%macro knnr(setg,sett,imp,var,k,cats,seed);
      data &sett; set &sett; idt=_N_;
      proc sort data=&sett; by idt;
      data &setg; set &setg; myv=&imp;
      ods listing close; run;
      ods output PostTestClass=_PostTestClass;
      proc discrim data=&setg test=&sett testout=_score1
method=npar k=&k testlist;
        class myv;
        var &var;  run;
ods listing; run;
data _posttestclass; set _posttestclass;
      if _0=. then _0=0;
                  if _1=. then _1=0;
                   if _2=. then _2=0;
                   if _3=. then _3=0;
                   if _4=. then _4=0;
                  if _5=. then _5=0;
      if &cats=2 then do;
         &imp=rantbl(&seed,_0);
         &imp=&imp-1; end;
      else if &cats=4 then do;
         &imp=rantbl(&seed,_1,_2,_3); end;
      else if &cats=6 then do;
                  &imp=rantbl(&seed,_1,_2,_3,_4,_5); end;
      else
                  &imp=0;
                  idt=Obs;
     keep idt &imp;
     proc sort data=_posttestclass; by idt;
     data &sett; merge &sett _posttestclass; by idt; drop idt;
   %mend; run;

%knnr(A,B,ps1,age SEX weight height physicalactivity,20,6,-1);
run;
%knnr(A,B,ps2,age SEX weight height physicalactivity,20,4,-1);
run;
%knnr(A,B,ps3,age SEX weight height physicalactivity,20,4,-1);
run;
%knnr(A,B,ps4,age SEX weight height physicalactivity,20,4,-1);
run;
%knnr(A,B,ps5,age SEX weight height physicalactivity,20,4,-1);
run;
%knnr(A,B,ps6,age SEX weight height physicalactivity,20,4,-1);
run;

data Bps; set work.B;
proc sort; by id;
run;
```

The green number -1 is the seed, and 'cats' (here 6 or 4) denote the number of categories in each portion size item.

3.a. About MLR imputation

Missing single values or entire variables can be imputed with the Multinomial Logistic Regression (MLR) method. In the portion size article it was documented that the MLR method provided the best agreement between imputed and observed values and had less bias than the other methods tested.

In this example we imputed all the portion sizes from dataset A to dataset B. Dataset B should contain at least the informing variables age, sex, weight, height, physical activity (or another set of relevant informing depending on the context) and an 'id'. Dataset A should contain at least the informing variables chosen and portion sizes (ps1, ps2, ps3 …).

In the MLR macro we assumed 'proportional odds' between the outcome categories. The assumption of proportional odds is not necessarily correct, but we assumed that other more general models would produce more noise.

Weighted probability sampling among the portion size categories made it suitable for multiple imputation.

'Cats' is the number of categories in the specific portion size item. The variable has to be categorical with no more than 6 categories.

Unlike Coca the present MLR macro does not sample a set of values in one draw, but single values in multiple draws.

The MLR proved to be very time consuming for the computers to run.

3.b. SAS code for MLR

```
/*** MLR weighted probability single value sampling ***/
    %macro mlr(setg,sett,imp,var,cats,seed);
      data &sett; set &sett; idt=_N_;
```

```
proc sort data=&sett; by idt;
data &setg; set &setg;
data seta; set &sett &setg;
ods listing close; run;
proc logistic data=seta; model &imp=&var; output
out=_probs p=p predprobs=cumulative; run;
ods listing; run;
data _probs; set _probs; if idt>0; proc sort data=_probs; by
idt;
data _rand; set &sett; r=ranuni(&seed); keep idt r; proc sort
data=_rand; by idt;
data _probs; merge _probs _rand; by idt;  count=(r>p);
proc means data=_probs noprint sum; var count; by idt; out-
put out=_probs sum=&imp;
data _probs; set _probs; if &cats>2 then do; &imp=&imp+1;
end; keep idt &imp;
proc sort data=_probs; by idt;
data &sett; merge &sett _probs; by idt; drop idt;
%mend; run;

%mlr(A,B,ps1,age SEX physicalactivity weight height,6,-1); run;
%mlr(A,B,ps2,age SEX physicalactivity weight height,4,-1); run;
%mlr(A,B,ps3,age SEX physicalactivity weight height,4,-1); run;
%mlr(A,B,ps4,age SEX physicalactivity weight height,4,-1); run;
%mlr(A,B,ps5,age SEX physicalactivity weight height,4,-1); run;

data imputation.Bps; set work.B;
proc sort; by nr;
run;
```

4. About median imputation

We tested both non-sex-specific median imputation and sex-specific median imputation. The results are displayed in Table S2. The median methods underestimated the reference energy intake more than the other methods tested. As expected non-sex-specific median imputation grossly underestimated the energy intake in men, but surprisingly the non-sex-specific median imputation was more accurate for women than the sex-specific median imputation.

In the article 'Missing portion sizes in food frequency questionnaires - alternatives to use of standard portions' the sex-specific median portion sizes were determined from the entire sample [men n=1546] [women n=2182] and these values were imputed in all of the 10 splits of dataset B.

We checked the sex-specific median in all splits of dataset A , and in some of the splits one or two of the 39 median portion sizes actually varied one category up or down compared to the population sex-specific medians. However, the use of the specific dataset A sex-specific median would induce bias: Unlike with the stochastic methods, the imputed sex-specific median values were not random. Thus, for a median portion size in dataset A that, as a consequence of the specific split, was smaller than the population median, the corresponding 'true' reference portion size in dataset B would be larger. This bias would be a result of our study design rather than of the median method, and therefore we used the sex-specific population medians for all.

5. How to do multiple regression modeling with multiple dataset

When you have created multiple, say 10, dataset with e.g. nutrient and energy intakes, you have to create a new variable 'dataset' and give all observations in the first dataset the value 1, in the second dataset all observations should have the value 2, etc.

Then you combine the 10 dataset into one dataset using this code:

```
data mega; set i1m i2m i3m i4m i5m i6m i7m i8m i9m i10m; run;
```

To do multiple linear regression analyses (in a generalized model) of fructose as the exposure of interest and BMI as the outcome you can use this set up. The code makes 10 regression analyses, one for each 'dataset', and the resulting estimate is the mean of the 10 values (Rubin DB, Schenker N. Multiple imputation in health-care databases: an overview and some applications. Stat Med 1991;10:585-98).

```
/*** Multiple linear regression with multiple dataset ***/

data mega; set mega;

proc sort data=mega; by dataset; run;

*ods listing close; run;
ods output ParameterEstimates=mypar; run;
proc genmod;
class sex tobacco education;
model
bmi=
fructose
age
sex
tobacco
education
energi;

by dataset;
run;

*ods listing; run;

data mypar; set mypar; if Parameter="fructose";
myVar=StdErr*StdErr; run;
proc print data=mypar; run;

proc means data=mypar noprint mean; var Estimate myVar; out-
put out=myMIEst mean=MIEst MIVarW; run;
proc means data=mypar var; var Estimate; output out=myMIVar
var=MIVarB; run;

data myMIEst; merge myMIEst myMIVar; by _TYPE_;
    MIVar=MIVarW + (((_FREQ_+1)/_FREQ_)*MIVarB);
    MISE=sqrt(MIVar);
    MIEst_l=MIEst-(1.96*MISE);
    MIEst_u=MIEst+(1.96*MISE);
    MIt=-1*abs(MIEst/MISE);
    MIp=2*cdf('NORMAL',MIt);
    keep MIEst MISE MIEst_l MIEst_u MIp MIt _FREQ_;
run;

proc print data=myMIEst; run;
```

6. Considerations about missing frequencies

How to handle missing frequency data in FFQs? This is a separate question that we did not cover in the portion size article. Evidence exists that a missing frequency value is likely to be truly missing (the subject does not consume the food at all) if the food

in question is a rarely consumed food. Whereas, if the food is commonly consumed the missing value is likely missing by mistake (Frazer et al. Epidemiology. 2009). So, unlike portion size items frequency items can be truly missing (or if a subject does not eat rice, there's no harm done by imputing a rice portion size for her, since there is no frequency question to be multiplied with the proposed portion size).

However, we think it is reasonable to impute missing frequencies from the subjects within the same dataset with a complete set of portion sizes, since missing frequencies in this group were more likely truly missing (as we know the subjects filled in the portion sizes with great conscience). This is straight forward with the 'Coca hot deck' SAS code given in chapter 1.e. , but we have not validated this method. The alternative is 'complete case analysis' or to interpret all missing as the lowest frequency/zero or the median frequency.

7. Spearman's rank correlation with the four imputation methods

Spearman's rho was used to compare the ranking of the subjects; comparing the reference TE with the TE calculated with imputed portion sizes.

Table S3. Spearman's rank correlation of the subjects, comparing the reference TE with the TE calculated with imputed portion sizes
These results derive from one random split of the data (the same dataset as used in fig. 1).

All the methods had high spearman's rank correlation, but median and MLR imputation performed slightly better than KNN and Coca.

SUMMARY

Introduction

This PhD thesis is about weight changes. What determines long-term weight changes in the adult general population? Is it possible that weight loss may not always be healthy? The present clinical guidelines for general practice advice most overweight persons and patients with type 2 diabetes to lose weight. Are the guidelines based on firm evidence?

Methods

The back-bone of the thesis is constituted by three scientific articles based on three different population based cohort studies. Multivariable modeling and other epidemiological methods were used.

Results

Article 1 examined weight changes in the general population in relation to smoking status, and proposed a graphical 'smoking cessation weight change model', demonstrating the importance of time, age and smoking status in relation to long-term weight changes. Article 2 suggested new methods to improve the processing of dietary data. It was demonstrated how median imputation for missing values and assumptions about standard portion sizes were inferior to stochastic methods conditioning on information about physiology of the individual. Article 3 evaluated the influence of prospectively planned intentional weight loss on long-term morbidity and mortality in patients with type 2 diabetes. Therapeutic intentional weight loss supervised by a medical doctor was not associated with reduced morbidity or mortality.

In the general population the dietary intake of fructose and soft drinks sweetened with sugar was not associated with weight change over 9 years. Weight gain rates were large in young adults and incrementally smaller in middle aged adults. Subjects more than 60 years lost weight on average. Historical weight data suggest that the body weight increases throughout life to the age of 60-65years. A study with simulated data indicates that bias in baseline BMI may misleadingly have favored weight loss in earlier cohort studies of intentional weight loss and mortality.

Discussion

The findings regarding weight loss and mortality in patients with type 2 diabetes are in opposition to the prevailing observational literature. Harrington's meta-analysis of intentional weight loss and the underlying studies are evaluated along with the Look AHEAD trial and a number of diabetes prevention studies. Difficulties in conducting and interpreting weight change studies are discussed.

Conclusions

Surprisingly, intentional therapeutic weight loss in patients with type 2 diabetes, supervised by a medical doctor, did not seem to reduce the long-term risk for CVD, CVD-mortality or all-cause mortality. The contradictions between our results and the prevailing observational evidence may be explained by methodological weaknesses favoring weight loss in earlier studies. Consequently, there is no good evidence to support that intentional weight loss will reduce the risk of CVD or mortality in any group of patients in general practice or in the general population.

Age was a powerful determinant of weight changes and the 'normal weight development' can be taken into consideration when evaluating weight studies, and when general practitioners are following their patients over time. Compared with age, sex, education, and comorbidity, lifestyle factors like the dietary intake and physical activity seemed to be of less importance for long-term weight development. An exception to this was smoking or smoking cessation.

Based on the scientific literature in the field and on the results of article 3, it seems uncertain whether weight loss is beneficial or harmful in terms of mortality and cardiovascular morbidity in patients with diabetes and in overweight people in general. Improvements in for instance psychosocial factors and diabetes prevention may well be short term as only few are able to a maintain weight loss. Rather than going for weight loss in overweight high risk patients, it seems more rational for general practitioners to focus on other lifestyle changes like for instance Mediterranean diet and increased exercise.

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