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Weight Change and the Development of Non-Alcoholic Fatty Liver Disease in Metabolically Healthy Overweight Individuals

Short title: Weight change and NAFLD

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Author contributions:

Y.C. and S.R. planned, designed, and directed the study, including quality assurance and control. S.R. analysed the data and designed the analytic strategy of the study. E.S. supervised the field activities. All authors conducted a literature review and prepared the Materials and Methods and Discussion sections of the text. I.Y.C. and Y.C. drafted the manuscript. I.Y.C., Y.C., E.S., W.S., J.-H.K., H.S., and S.R. interpreted the results. All authors, including C.D.B. and S.H.W., contributed to the critical revision of the manuscript. All authors have read and approved the final manuscript.

Abbreviations:

BMI, body mass index; CI, confidence intervals; FIB-4, fibrosis 4 score; HR; hazard ratios; HS, hepatic steatosis; hsCRP, high sensitivity C-reactive protein; HOMA-IR, homeostatic model assessment–insulin resistance; MHO, metabolically healthy obesity; NAFLD, Non-alcoholic fatty liver disease; NFS, NAFLD fibrosis score.

Abstract

Introduction: To investigate the effect of weight change on hepatic steatosis (HS) incidence with or without liver fibrosis in metabolically healthy overweight or obese individuals.

Methods: A cohort of 14,779 metabolically healthy men and women who were overweight or obese (BMI ≥23 kg/m^2) and free from HS and an intermediate or high probability of fibrosis at baseline were followed for a median of 5.2 years. Metabolic health was defined as freedom from the components of metabolic syndrome and a homeostasis model assessment of insulin resistance <2.5. Weight changes were calculated as differences from baseline at the next subsequent visit. The outcome was HS incidence, with or without liver fibrosis, as assessed by liver ultrasound and two non-invasive fibrosis scores.

Results: During 76,794.6 person-years of follow-up, 3,539 cases of HS incidence were identified. The multivariable adjusted hazard ratios (95% confidence intervals) for HS incidence by weight change group, <-5.0%, -5.0%–1.0%, 1.0%–5.0%, and >5.0%, relative to the no weight change group (-0.9% to 0.9%) were 0.52 (0.44–0.60), 0.83 (0.75–0.92), 1.21 (1.10–1.33), and 1.51 (1.36–1.69), respectively. Clinically relevant weight loss of >5% was also associated with a lowered risk of HS with intermediate or high probability of advanced fibrosis. In mediation analyses, associations remained significant, although adjustment for metabolic risk factors was attenuating.

Discussion: Clinically relevant weight loss was associated with a reduced risk of developing non-alcoholic fatty liver disease with or without intermediate or high probability of advanced fibrosis in metabolically healthy overweight or obese individuals.

Keywords: body mass index; non-alcoholic fatty liver disease; metabolically healthy obesity; obesity; overweight.

Introduction

Non-alcoholic fatty liver disease (NAFLD) is the most common chronic liver disease worldwide.[1] Although NAFLD is known to occur in lean individuals, obesity is the most important risk factor for NAFLD.[2, 3] Obesity is frequently accompanied by metabolic abnormalities such as type 2 diabetes (T2DM), metabolic syndrome, or insulin resistance, all of which are also closely associated with NAFLD.[4-6] However, a unique subset of obese individuals who do not have metabolic abnormalities in spite of their excessive adiposity, those with so-called metabolically healthy obesity (MHO), are of considerable interest,[7, 8] because the future health implications of MHO remain uncertain.[9] Contrary to some previous observations that individuals with MHO are not at an increased risk of cardiovascular disease (CVD) compared to normal weight metabolically healthy individuals, MHO has been reported to be associated with a greater risk of all-cause mortality,[9] and NAFLD development[10] with liver fibrosis progression,[11] even in the absence of metabolic abnormalities. Moreover, development of NAFLD predicts the transition from MHO to metabolically unhealthy obesity,[12, 13] is associated with metabolic syndrome,[14] and is an independent risk factor for both T2DM[15] and CVD.[16] While lifestyle modification to achieve weight loss is known to be beneficial in NAFLD and is a first-line intervention for patients with NAFLD,[17, 18] it is not known whether subjects with MHO benefit from the effects of weight loss for reducing their risk of developing NAFLD.

To date, no studies have longitudinally analyzed the effects of weight loss on the incidence and progression of NAFLD in individuals with MHO. Therefore, we aimed to investigate the association between weight change and the development of hepatic steatosis (HS) both with and without intermediate to high probability of advanced liver fibrosis, in a large sample of metabolically healthy overweight and obese individuals.

Methods

Study Population

This cohort study, a part of the Kangbuk Samsung Health Study, included men and women who participated in comprehensive health screening exams at the Kangbuk Samsung Hospital Total Healthcare Centers in Seoul and Suwon, South Korea[10, 19] (further details of the study population and measurements collected are provided in **Supporting Documents**). We included study participants who were overweight or obese, had an initial baseline and subsequent visit, which comprised the baseline period for estimating weight change, and had at least one follow-up visit between March 2011 and December 2019 (n =126,354). Exclusion criteria are shown in **Figure 1**.

A total of 14,779 subjects were retained in the final analysis, all of whom were overweight or obese, were metabolically healthy, without excessive alcohol use (threshold of ≥20 g/day for women and ≥30 g/day for men),[2] and did not have either NAFLD or intermediate or high fibrosis scores at baseline. This study was approved by the Institutional Review Board of Kangbuk Samsung Hospital (IRB 2020-04-034), which waived the requirement for informed consent because we used a pre-existing de-identified dataset that was routinely collected during the health screening process.

Assessment of Metabolic Health Status

In addition to being free from NAFLD, metabolic health was defined as absence of each of the following: (1) fasting blood glucose (FBG) ≥100 mg/dL or use of glucose-lowering medications, (2) BP ≥130/85 mmHg or use of BP-lowering medications; (3) triglyceride level ≥150 mg/dL or current use of lipid-lowering medications; (4) high-density lipoprotein

cholesterol (HDL-C) <40 mg/dL and <50 mg/dL in men and women, respectively, and (5) homeostatic model assessment-insulin resistance (HOMA-IR) ≥2.5.

Assessment of NAFLD and Fibrosis

HS diagnosis was based on abdominal ultrasound examined by experienced radiologists who were blinded to the aim of this study. Abdominal ultrasound examination was a routine part of the comprehensive health examinations and performed at all initial and subsequent visits. To assess the risk of having progressed during follow-up into more severe NAFLD, two non-invasive indices of liver fibrosis were used: the fibrosis 4 score (FIB-4) and NAFLD fibrosis score (NFS)[20, 21] (further details in Supplement 1, Supplementary Digital Content 1).

Assessment of Weight Change

Weight change during the baseline period was computed as the difference in weight from visit 1 to visit 2. Weight change, calculated as 'visit 2 weight' minus 'baseline weight,' was categorized into <-5.0%, -5.0% to -1.0%, -0.9% to 0.9 % (stable weight as reference), 1.0% to –5.0%, and >5.0%. Since more than 5% weight loss from the baseline is widely accepted as being clinically relevant[22], we assessed weight change of >5.0%, compared with <1.0% weight change as the reference. Because this was an observational cohort study, subjects did not receive specific interventions for weight loss targeted to prevent NAFLD. Instead, they all uniformly received general recommendations to reduce their weight and maintain ideal weight (body mass index (BMI) <23 kg/m^2) in their health examination results whenever their BMI was ≥23 kg/m^2.

Statistical Analyses

The primary endpoints were: a) incidence of HS, and b) incidence of HS plus intermediate or high probability of advanced fibrosis at follow-up, according to two non-invasive fibrosis markers (FIB-4 and NFS). The incidence of HS and the incidence of HS with progression of each non-invasive fibrosis marker were treated as separate endpoints and analyzed independently. For analysis of the association between weight change and the incidence of HS, if HS was identified during follow-up, subsequent observations were not included in the analysis. Visit 2 was the start of follow-up for all our study participants regardless of the weight change category. Person-years of follow-up were calculated from the date of visit 2 until the date of the primary endpoint (HS or HS with intermediate or high fibrosis scores, separately) or the last screening exam (December 31, 2019), whichever came first. Since incidences of HS occurred at unknown time points between the visit at which HS was diagnosed and the previous visit, we used a parametric proportional hazards model to account for this type of interval censoring.[23] The baseline hazard function was parameterized with the restriction of cubic splines in log time with four degrees of freedom for these models. We estimated the adjusted hazard ratios (HR) with 95% confidence intervals (CI) for HS incidence by comparing weight change categories against the no weight change category.

The models were initially adjusted for age and sex, and then adjusted for additional confounders: study center (Seoul, Suwon), year of screening examinations, level of education, smoking status (never, former and current smokers), alcohol consumption (none, >0 to <10 g/day, and ≥10 g/day), physical activity, BMI (continuous), and total energy intake (in quintiles or unknown). To determine the linear trends of risk, we included the median value of each weight change category as a continuous variable and tested its statistical significance in each model. We also used a parametric proportional hazards model with weight change category, alcohol intake, smoking status, physical activity, and total energy

intake as time-dependent categorical variables and baseline age, sex, center, year of screening exam, BMI, and education level as time-fixed variables. Weight changes were calculated for each subject as the differences in weight from visit 2 to baseline (visit 1), from visit 3 to visit 2, from visit 4 to visit 3, and in the same way afterwards. We assessed the proportional hazards assumption by examining graphs of estimated log (−log(survival)) versus log of survival time graph: no violation of the assumption was found.

Additionally, we differentiated intentional weight loss from unintentional weight loss based on the self-reporting of subjects who had >1% weight loss; specifically, subjects were asked whether they had intended to lose weight during the previous year. Predefined subgroup analyses were also performed by sex (men vs. women) since women usually have more adipose tissue than men (although fat accumulation in gluteo-femoral adipose tissue, is more common in women and is favorably associated with metabolic health.[24]) Interactions between weight change categories and subgroup characteristics were tested using likelihood ratio tests comparing models with versus without multiplicative interaction terms.

To evaluate whether the association between weight change and incidence of HS was mediated by insulin resistance, inflammation, and other metabolic factors on an *a priori* basis, we performed a mediation analysis including HOMA-IR, high sensitivity C-reactive protein (hsCRP), and metabolic components in a multivariable-adjusted model (criteria for mediation analysis in **Supplement 1**, **Supplementary Digital Content 1**). We also assessed whether the risk differed by BMI status (BMI <25 vs. ≥25 kg/m^2).

All analyses were performed using STATA version 16.0 (Stata Corp LP, College Station, Texas). Statistical tests were two-sided, and P <0.05 were considered statistically significant.

Results

During 76,794.6 person-years of follow-up, 3,539 cases of HS incidence were identified (incidence rate 46.1 per 10^3 person-years). The median follow-up duration was 5.2 years (interquartile range, 3.8–6.8) for the overall subjects, 4.2 years (interquartile range, 3.4–5.9) for those who developed HS, and 5.7 years (interquartile range, 3.9–7) for those who did not. For those who developed HS, the median number of visits until development of incident HS was 3 (interquartile range 3–4); and 3 (interquartile range 2–5) for those who did not develop HS, until the end of the follow-up period. Baseline characteristics according to weight change category among metabolically healthy individuals who were overweight or obese are shown in **Table 1.** At baseline, the mean (SD) age and BMI of study participants were 36.4 (6.2) years and 24.6 (1.4) kg/m^2, respectively. The >5% weight loss group tended to have a higher BMI at baseline than the no weight loss group, were less likely to be male, and tended to have higher levels of total cholesterol, LDL-C, triglycerides, and ALT at baseline. Overall, among those who reported the intent to lose weight during the previous year (n=8,466), 33.7% achieved a >1% weight loss from their baseline weight; 11.3% achieved a >5% weight loss from their baseline weight, and 22.4% achieved 1%–5% weight loss from their baseline weight.

After adjustment for age, sex, BMI, and other confounders (**Table 2**), the multivariable adjusted HR (95% CI) for HS incidence comparing weight loss 1.0%–5.0% and >5.0% to no weight change (-0.9% to 0.9 % as reference) were 0.83 (0.75–0.92) and 0.52 (0.44–0.60), respectively. The multivariable adjusted HR (95% CI) for incident HS comparing weight gain 1.0%–5.0% and >5.0% to no weight change were 1.21 (1.10–1.33) and 1.51 (1.36–1.69), respectively. In time-dependent models introducing weight change, BMI, and confounders as time-varying covariates, the associations between weight change and HS incidence became stronger compared to the original analysis. These associations were observed in both men and

women, and the associations did not differ by sex (P for interaction = 0.413, **Supplementary Table 1**).

Table 3 shows the risk of HS incidence plus intermediate or high fibrosis score. During 82,708.3 person-years of follow-up, 171 cases of HS incidence plus intermediate or high FIB-4 were identified (incidence rate 2.1 per 10^3 person-years). After adjustment for age, sex, BMI, and other confounders, the multivariable adjusted HR (95% CI) for incident HS plus intermediate or high FIB-4 comparing weight loss 1.0%–5.0% and >5.0% to no weight change (-0.9% to 0.9 % as reference) were 0.96 (0.63–1.46) and 0.29 (0.10–0.80), respectively. Similarly, the risk for HS incidence plus intermediate or high fibrosis markers based on NFS significantly decreased with increasing levels of weight loss. Conversely, the positive association between weight gain and HS incidence plus intermediate or high FIB-4 was not significant, whereas HS incidence plus intermediate or high NFS increased with increasing levels of weight gain.

When we differentiated intentional weight loss from unintentional weight loss based on self-reporting (**Supplementary Table 2**), the association between weight loss and decreased risk of HS with or without intermediate or high fibrosis scores did not differ by intentionality; however, the decreased risk of HS and HS plus intermediate or high FIB-4 tended to be slightly stronger in the intentional weight control group.

Finally, in mediation analyses (**Supplementary Table 3**), further adjustment for either HOMA-IR, hsCRP, or each metabolic component did not significantly alter the association between weight change and HS with or without intermediate or high fibrosis scores. When analyzing NAFLD risk in subjects with BMI <25 vs. ≥25 kg/m^2 at baseline, the results did not differ according to BMI category (p for interaction = 0.931 for HS, 0.184 for HS with intermediate/high FIB-4, and 0.815 for HS with intermediate/high NFS).

Discussion

Our study is the first to demonstrate that in metabolically healthy individuals who were overweight or obese and NAFLD-free at baseline, clinically relevant weight loss of >5% may help reduce the risk of developing HS both with and without intermediate or advanced fibrosis. In contrast, weight gain was associated with an increased risk of HS. These associations persisted after adjustment for potential confounders.

In our study, a higher percentage of weight loss was associated with a lower risk of HS incidence, with stronger associations in time-dependent analyses. Previous studies have mainly assessed the effect of weight loss in patients with baseline NAFLD,[17, 18, 25] showing NAFLD remission with varying degrees of weight loss. Based on these studies, NAFLD guidelines recommend at least 3%–5% weight loss to improve HS, and 7%–10% to improve features of steatohepatitis including fibrosis in patients who already present with NAFLD.[2] However, before our study, it was uncertain whether weight loss in individuals with MHO helps prevent HS. To the best of our knowledge, our study is the first to demonstrate that clinically relevant weight loss of >5% and even weight loss of 1%–5% may help reduce the risk of developing HS in overweight and obese individuals without baseline NAFLD who are metabolically healthy.

Previous studies of weight loss and fibrosis regression have also been conducted only in NAFLD patients. A meta-analysis of NAFLD patients showed that >7% weight loss led to improvement in histological disease activity,[17] and a prospective study showed that ≥10% weight loss led to biopsy-proven fibrosis regression in 45% of patients,[18] demonstrating that weight loss is beneficial for improvement of HS and fibrosis in patients already presenting with NAFLD. Contrary to the small-sized studies of only NAFLD patients, our study

included a large cohort without baseline NAFLD, and our results suggest that >5% weight loss, which is clinically relevant for improving other chronic diseases,[22] may lead to reduced risk of fibrosis progression in individuals with MHO who are commonly considered healthy besides being overweight.

In contrast, weight gain was associated with an increased risk of HS, and a trend for increased risk of HS plus intermediate or high NFS scores. FIB-4 did not show such a trend, possibly due to the fewer incidences of HS plus intermediate or high FIB-4 scores in >5% weight gain category. Our study findings suggest that weight gain may lead to increased risk of HS, and possibly the probability of advanced fibrosis in MHO, although they are all free of NAFLD and metabolic abnormalities at baseline. Considering that MHO is not a static condition, and fibrosis is the most important factor associated with increased mortality and liver-related complications in NAFLD,[26] individuals with MHO may benefit from weight loss interventions, even before the onset of NAFLD. Notably, even though the study population fell into the overweight or obesity category at baseline, almost half of the individuals gained additional weight during follow-up instead of losing weight. False assurance should be avoided since MHO is not a harmless condition in terms of relation to worse health outcomes, including cardiometabolic risk and NAFLD risk.

The mechanisms for the association between weight loss and risk of HS without or with probability of advanced fibrosis are unclear. Diet- and lifestyle-induced weight loss can improve insulin sensitivity, increase expression of hepatic adiponectin and suppress pro-inflammatory cytokine expression, which are all closely involved in the pathogenesis of NAFLD.[27-29] In contrast, weight gain may be linked to increased adipose tissue accumulation, which as an endocrine organ releases cytokines and free fatty acids (FFA),[5] producing toxic by-products and contributing to the development of hepatic fibrosis.[30] The cytokines released

include adiponectin and leptin, which may contribute to NAFLD development and its progression through accumulation of liver fat, insulin resistance, and inflammatory processes.[31] In mediation analyses, further adjustment for HOMA-IR, hsCRP and other metabolic components did not significantly alter the associations between weight change and HS with or without intermediate or high fibrosis scores. Nonetheless, when examining participant characteristics at follow-up, the group with >5% weight loss showed decreased HOMA-IR and TG levels, while the group with >5% weight gain showed increased HOMA-IR and triglyceride levels (**Supplementary Table 4**), suggesting that the association between weight gain and NAFLD may be mediated by insulin resistance and increased triglycerides, which are thought to contribute to excessive fat accumulation in the liver,[32] whereas weight loss is known to reduce hepatic triglyceride content.[28] However, further studies are warranted to elucidate the mechanisms underlying the association between weight change and NAFLD risk.

Despite the strengths of our study including a large cohort of metabolically healthy overweight and obese individuals, our study also has several limitations. First, diagnoses of HS and intermediate to high probability of advanced fibrosis were assessed through ultrasonography and non-invasive fibrosis scores, instead of liver MRI, elastography, or histological diagnosis, which is the current gold standard. However, liver biopsy is not feasible in routine health screening exams, and unfortunately, in our study, data on transient elastrography or liver MRI was not available because our study was based on de-identified, retrospective cohort data from individuals who underwent routine health check-ups as part of a program in which transient elastography and liver MRI were not included. Instead, ultrasonography is commonly used in epidemiological studies, and allows for reliable and accurate diagnosis of fatty liver.[33] We also used non-invasive fibrosis scores that have been

validated for identifying advanced liver fibrosis,[20, 21] and intermediate to high fibrosis scores are known to be associated with a higher risk of liver-related mortality.[34] Second, information on how, and the reason for why weight loss was achieved was unavailable. However, in our analyses the results did not differ by reported intention to lose weight. Previous studies have recommended incorporating the intention to lose weight in analyses of weight loss and health outcomes, as different population characteristics have been identified according to intention of weight loss.[35] Nonetheless, further studies including information on how weight loss was achieved would help identify overweight people who will benefit most from weight loss and inform a realistic approach to achieving weight loss. Finally, our study subjects included relatively healthy, well-educated, young, and middle-aged Koreans; therefore, our findings may not be generalizable to other ethnic or older age groups. Future studies including older adults may help confirm whether our findings are applicable to older populations.

Conclusion

In conclusion, our results suggest that weight loss per se may help reduce the risk of developing HS and possibly HS with intermediate to high probability of advanced fibrosis, regardless of metabolic health in overweight and obese individuals. Our findings suggest that clinicians should encourage even metabolically healthy overweight and obese individuals to reduce their weight to prevent the development of NAFLD and fibrosis progression.

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Figure Legend

Figure 1 Flowchart for selection of study participants