Title

Does NAFLD mediate the relationship between obesity and type 2 diabetes risk? evidence from the multi-ethnic study of atherosclerosis (MESA)

Authors

Rodriguez, Luis A
Kanaya, Alka M
Shiboski, Stephen C
et al.

Publication Date

2021-11-01

DOI

10.1016/j.annepidem.2021.07.005

Peer reviewed

HHS Public Access

Author manuscript

Ann Epidemiol. Author manuscript; available in PMC 2022 November 01.

Published in final edited form as:

Ann Epidemiol. 2021 November ; 63: 15–21. doi:10.1016/j.annepidem.2021.07.005.

Does NAFLD Mediate the Relationship Between Obesity and Type 2 Diabetes Risk? Evidence from the Multi-Ethnic Study of Atherosclerosis (MESA)

Luis A. Rodriguez, PhD[a,b,*], **Alka M. Kanaya, MD**[a,c], **Stephen C. Shiboski, PhD**[a], **Alicia Fernandez, MD**[d], **David Herrington, MD**[e], **Jingzhong Ding, MD**[f], **Patrick T. Bradshaw, PhD**[g]

[a]University of California, San Francisco, Department of Epidemiology & Biostatistics, San Francisco, CA, USA

[b]Kaiser Permanente Northern California, Division of Research, Oakland, CA, USA

[c]University of California, San Francisco, Division of General Internal Medicine, San Francisco, CA, USA

[d]University of California, San Francisco, Department of Medicine, San Francisco, CA, USA

[e]Wake Forest School of Medicine, Department of Internal Medicine, Winston-Salem, NC, USA.

[f]Wake Forest School of Medicine, Sticht Center on Aging, Winston-Salem, NC, USA.

[g]University of California, Berkeley, School of Public Health, Division of Epidemiology & Biostatistics, Berkeley, CA, USA

Abstract

Purpose: To estimate the effect of obesity on type 2 diabetes (T2DM) risk and evaluate to what extent non-alcoholic fatty liver disease (NAFLD) mediates this association.

Methods: Data came from 4,522 adults ages 45–84 participating in the Multi-Ethnic Study of Atherosclerosis cohort. Baseline obesity was defined using established BMI categories. NAFLD was measured by CT scans at baseline and incident T2DM defined as fasting glucose ≥126 mg/dL or use of diabetes medications.

[*]Corresponding author: Luis A. Rodriguez, PhD, University of California, San Francisco, 550 16th Street, 2nd Floor, Box 0560, San Francisco, CA 94143, Luis.Rodriguez@ucsf.edu.

Author Contributions:

L.A.R. analyzed the data, drafted and revised the manuscript and had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis; L.A.R., P.T.B. and A.M.K. designed the research and obtained funding; P.T.B. and S.C.S. provided statistical expertise and contributed to the analysis of the data; A.F. contributed to the interpretation of the data; D.H. is a principal investigator of the MESA cohort and was responsible for the data collection procedures; J.D. is a principal investigator of a MESA ancillary study and was responsible for the data collection and procedures; all authors critically reviewed and approved the final manuscript. Parts of this study were presented in abstract form at the Society for Epidemiological Research Meeting in Minneapolis, Minnesota, 18–21 June, 2019.

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Results: Over a median 9.1 years of follow-up between 2000 and 2012, 557 new cases of T2DM occurred. After adjusting for age, sex, race/ethnicity, education, diet and exercise, those with obesity had 4.5 times the risk of T2DM compared to normal weight (hazard ratio [HR] = 4.5, 95% confidence interval [CI]: 3.0, 5.9). The mediation analysis suggested that NAFLD accounted for ~36% (95% CI: 27, 44) of the effect (direct effect HR = 3.2, 95% CI: 2.3, 4.6; indirect effect through NAFLD, HR = 1.4, 95% CI: 1.3, 1.5).

Conclusions: These data suggest that the association between obesity and T2DM risk is partially explained by the presence of NAFLD. Future studies should evaluate if NAFLD could be an effective target to reduce the effect of obesity on T2DM.

Keywords

causal mediation analysis; diabetes mellitus; marginal structural model NAFLD; obesity

INTRODUCTION

Type 2 diabetes (T2DM) currently affects one in seven adults in the United States (US)[1]. If trends continue, it is projected that T2DM will affect as many as one in three US adults by 2050[2]. Obesity is a well-known risk factor for T2DM[3–8] and in the past three decades, the obesity epidemic has contributed to the increase in T2DM[1,9,10]. Despite the clear obesity-T2DM relationship, the precise mechanisms that connect these conditions remain unclear. It is hypothesized that at least three mechanisms link obesity and insulin resistance to T2DM[5]: 1) increased production of adipokines/cytokines (e.g. tumor necrosis factor-alpha), promoting insulin resistance; 2) mitochondrial dysfunction, resulting in insulin resistance and B-cell dysfunction; and 3) increased ectopic fat deposition, leading to dysmetabolic sequelae. The third mechanism is of particular interest given the recent rise in non-alcoholic fatty liver disease (NAFLD)[11].

The NAFLD-specific role in the obesity-T2DM link may be due to an exacerbation of hepatic insulin resistance and alteration in the secretion of hepatokines and inflammatory biomarkers, that may promote the development of T2DM[11–14]. Prior studies have found obesity to also be an established risk factor for NAFLD[11,15–23]. NAFLD in turn has been shown in multiple observational studies[24–38], and two Mendelian randomization studies[39,40], to be associated with an increased T2DM risk. All of these observational studies[24–38] have adjusted for obesity in an attempt to estimate independent associations between NAFLD and T2DM risk, however none have used principled analytical techniques to quantify how much of the obesity-related risk for T2DM is mediated through NAFLD. Understanding the possible mediating role that fatty liver has on the obesity-T2DM relationship may be of interest as NAFLD prevention or management could be a promising target to reduce the obesity-related burden of T2DM[41].

We hypothesize that the association between obesity and T2DM risk is explained at least in part by the degree of fat in the liver. To test this hypothesis, we used longitudinally collected data from the Multi-Ethnic Study of Atherosclerosis (MESA) cohort to estimate the overall effect between obesity on risk of T2DM, and decompose this into the portion

of the relationship mediated, and not mediated, by the degree of liver fat accumulation (i.e. indirect and direct effects, respectively).

MATERIAL AND METHODS

Participant population

Our observational study includes data from the well-characterized MESA cohort. MESA objectives and design have been described in detail elsewhere[42]. Briefly, 6,814 participants aged 45–85 years free of known cardiovascular disease (CVD) were recruited in the years 2000–2002 from six communities in the United States and followed until present time. Participants were seen at six US university clinics (Columbia, New York, NY; Johns Hopkins, Baltimore, MD; Northwestern, Chicago, IL; University of California, Los Angeles, CA; University of Minnesota, Twin Cities, MN; and Wake Forest, Winston-Salem, NC). The cohort includes those of White, African American, Hispanic, and Chinese American descent. For this study we used data from exam visits 1 through 5, conducted between July 2000 and February 2012. Informed consent was obtained from all study participants and institutional review board approval at the sites conducting MESA was obtained.

Exclusions

We excluded participants with prevalent diabetes at baseline (n = 859) defined as having a fasting glucose ≥126 mg/dL and/or reporting using any diabetes medications (see Web Appendix Table 1 for baseline characteristics of these excluded participants). We further excluded participants whose computed tomography (CT) imaging did not extend inferiorly sufficiently to measure liver attenuation (n = 75), participants with a history of high alcohol use (average of >1 serving/day in women and >2 servings/day in men; n = 322), history of liver cirrhosis (n = 6) and use of oral steroids and class 3 antiarrhythmic medications (n = 84)[43], those who failed to return to at least 1 follow-up visit (n = 294) or those with missing covariates of interest (n = 652). Our final sample size was 4,522.

Exposure: obesity

Obesity at baseline was defined using body mass index (BMI, weight in kg/squared height in m) as a measure of generalized obesity and, in separate models, using waist circumference (WC) as a proxy for abdominal obesity. Weight and height were measured[44], and BMI categorized according to established criteria[4,45]: normal (<25 or <23 kg/m^2 for Chinese Americans), overweight (25-<30 or 23-<27.5 kg/m^2 for Chinese Americans), or obese (≥30, or ≥27.5 kg/m^2 for Chinese Americans). Waist circumference (cm) was measured and categorized using established sex-specific cut-points >88 cm for women and >102 cm for men[46]. In sensitivity analysis, we re-classified waist circumference using sex- and race/ethnic-specific cut-points >80 cm for women and >94 cm for White and African American men and >90 cm for Hispanic and Chinese American men[47].

Mediator: liver fat

At the baseline visit, participants received two consecutive CT scans. Liver attenuation by CT scan has been shown to be inversely correlated with liver fat deposition by liver biopsy (correlation coefficient: −0.9; p-value <0.001)[48]. Likewise in another study, unenhanced CT

scans showed a R^2 value of 0.649 against histologic fat content in linear regressions[49], showing that CT scanning provides a useful non-invasive method for identifying fatty liver. Degree of liver attenuation was measured in three consistent regions in the parenchyma of the right hepatic lobe (each measuring about 1 cm^2) and calculated as the average density[50]. Liver fat was categorized into quartiles of Hounsfield units (HU), and inverted so that the highest quartile represented the lowest liver fat content as the referent group.

Outcome: type 2 diabetes

Individuals were considered as having T2DM if they had a fasting glucose ≥126 mg/dL and/or reported using any diabetes medications during in-person clinic exams at any point during follow-up. The outcome, time to T2DM, was specified as time of first observation of T2DM at any time during follow-up.

Confounders

Informed by our directed acyclic graph[51], we assumed that the same set of covariates potentially confound the relationship between obesity and T2DM, obesity and fatty liver, and fatty liver and T2DM as shown in the Figure 1. Measured confounders included baseline age, sex, race/ethnicity, education (less than high school, completed high school, some college, or bachelor's degree or higher), exercise (quartiles), dietary quality (quintiles) and total caloric intake (quintiles). Exercise was calculated from the duration and intensity of total intentional exercises using metabolic equivalent minutes (MET-min) per week and was measured using a detailed, semi-quantitative questionnaire adapted from the Cross-cultural Activity Participation Study[42,50]. Usual diet intake over the previous 12 months was quantified using a food frequency questionnaire (FFQ)[42,52] from which dietary quality was calculated using the Alternative Healthy Eating Index-2010 (AHEI) Score, based on the evidence for its strong link with CVD and T2DM[53]. Daily energy intake (kcal/day) was also estimated from the FFQ's. In a sensitivity analysis, we also adjusted for cigarette smoking (current, former and never smoker), serum triglycerides (quartiles), hypertension, antihypertension medication, statins, lipid-lowering medications, field center, and alcohol consumption, however adjusting for these did not meaningfully alter our findings, thus they were excluded from the final models.

Statistical analysis

Our study's objective was to quantify how much of the effect between obesity and T2DM was potentially mediated by fatty liver. Conventional mediation analysis using the Kenny and Baron mediation approach[54] works in the special case of linear models without interactions, but is otherwise flawed[55]. To accomplish our objective, we conducted a causal mediation analyses to decompose the overall effect into two separate effects: (1) the direct effect (i.e. the effect of obesity on T2DM that is not mediated by fatty liver), and (2) the indirect effect (i.e. the effect of obesity on T2DM that is mediated by fatty liver)[56].

We estimated these effects with inverse probability weighted marginal structural models according to the method of Lange et al.[57]. The steps were as follows: first, we used multinomial logistic regression to model the categorical mediator (quantiles of liver fat) as a function of obesity and assumed confounders (age, sex, race/ethnicity, education, exercise

and diet). This model was used to obtain predicted counterfactual mediator values for each level of the exposure in each individual, so that for any individual, three mediator values were predicted when BMI was the exposure: the potential mediator value *had* the individual been normal weight, the potential mediator value *had* the individual been overweight, and the potential mediator value *had* the individual been obese. This was operationalized by constructing an extended data set by repeating each observation three times and including an auxiliary exposure variable for each counterfactual level of exposure (normal, overweight, and obese BMI categories). The original exposure variable and the auxiliary exposure variable were then weighted by dividing the probabilities corresponding to the counterfactual value observed for the mediator by using the auxiliary exposure, by the probabilities corresponding to the value actually observed for the mediator. The stability of the calculated weights was evaluated by inspection of a histogram of the final weights and verifying no extreme values[58] (near zero or excessively large) (see Web Appendix Figures 1–3). A marginal structural model for the relationship between obesity and T2DM outcome was then estimated by fitting a parametric proportional hazards model with a Weibull distribution with robust SEs, and incorporating weights estimated in the first stage of modeling. Instead of estimating a separate model for the exposure conditional on confounders, we included the same set of covariates from the liver fat model, which results in weights that are typically much more stable as these do not involve inverse probability weighting of the exposure distribution[57]. In MESA, T2DM event times were not observed exactly; events were known to occur within some interval of time (i.e. between any two exam visits) but the exact time of the event was unknown. Correspondingly, we used estimation methods that accounted for the interval-censored event times[59–61]. Ninety five percent confidence intervals (CIs) for the total, indirect, and direct effects were estimated using 1,000 bootstrapped samples. The proportion mediated by the mediator was calculated as the ratio of the natural indirect effect to the total effect[62]. Separate models were repeated using waist circumference as a proxy for central obesity following the same steps as described above.

We also assessed whether indirect and direct effects differed between racial/ethnic groups using covariate-by-exposure interactions as described by Lange et al.[57], and tested their significance using a Wald test. We likewise assessed for possible exposure-mediator interactions. None of the interactions were statistically significant ($p>0.05$) (see Web Appendix Tables 2a and 2b) thus we removed interactions from final models. All statistical analyses were done in Stata v.15 (StataCorp, College Station, TX).

RESULTS

Descriptive characteristics by BMI category among 4,522 adults in MESA are presented in Table 1. The mean age (SD) of the population was 62 (10) years, and roughly half were female. Over a median 9.1 years of follow-up, 557 new cases of T2DM occurred (12%). Incidence rates were 5.5 (95% CI: 4.2, 7.2), 14.3 (12.5, 16.4), and 29.8 (26.6, 33.3) per 1,000 person-years among those in normal, overweight, and obese BMI categories, respectively. There was an association between liver fat quartile and BMI category (correlation coefficient: 0.24; p-value <0.001); among those in the obese category, more participants were in the highest quartile of liver fat.

Results of models for the mediators are presented in Web Appendix Tables 3–5. Estimates of the total, natural direct and natural indirect effects of obesity and T2DM risk are shown in Tables 2 and 3. After covariate adjustment, those with BMI-defined obesity were at 4.5 times the risk of T2DM compared to those with normal weight (total effect hazard ratio [HR] = 4.5, 95% CI: 3.0, 5.9). The mediation analysis suggested that NAFLD was responsible for ~36% (95% CI: 27, 44) of the relationship between obesity and T2DM risk (indirect effect of BMI through NAFLD, HR_{NAFLD} = 1.4, 95% CI: 1.3–1.5; direct effect, $HR_{BMI\text{-}obese}$ = 3.2, 95% CI: 2.3, 4.6). Those with overweight had more than twice the risk of T2DM compared to those with normal weight (HR = 2.2, 95% CI: 1.5, 3.0), with NAFLD responsible for ~27% (95% CI: 18, 41) of this relationship (indirect effect, HR_{NAFLD} = 1.2, 95% CI: 1.1, 1.3); direct effect, $HR_{BMI\text{-}overweight}$ = 1.9, 95% CI: 1.3, 2.7). Similarly, when using waist circumference as a proxy for central obesity, those with elevated waist circumference were at 2.7 times the risk of T2DM compared to those with normal waist circumference (total effect HR = 2.7, 95% CI: 2.2, 3.2), with NAFLD responsible for ~32% (95% CI: 24, 40) of this relationship (indirect effect (HR_{NAFLD} = 1.2, 95% CI: 1.1, 1.3); direct effect, $HR_{WC\text{-}high}$ = 2.2, 95% CI: 1.8, 2.6).

Sensitivity analysis

In a sensitivity analysis, when using waist circumference according to the International Diabetes Federation[47], the estimated effects of central obesity on T2DM risk were marginally higher compared to when using the Third Report of the National Cholesterol Education Program classification[46], but the proportion explained by fatty liver was similar (~27%, 95% CI: 21, 33 of the total effect) (Table 4).

DISCUSSION

In this multi-ethnic population-based cohort study of 4,522 adults in the US, our mediation analysis suggests that NAFLD mediates around 30% of the effect between obesity and incident T2DM. To our knowledge, this is the first analysis to decompose the complex links between obesity, NAFLD and incident T2DM.

There is prior evidence for the prospective associations between each of these factors (obesity-T2DM, obesity-NAFLD, and NAFLD-T2DM). First, consistent with prior studies[3–8], we found strong evidence of a total effect between obesity and incident T2DM. Second, longitudinal studies of different populations have shown the importance of obesity, or weight gain in NAFLD onset[17–23]. Furthermore, two of these studies demonstrated an increased rate of NAFLD remission among those who had weight loss during the observation periods[17,22]. And third, the presence of NAFLD has been shown in multiple observational studies to be associated with an increased risk of incident T2DM across different racial/ethnic groups[24–38]. Likewise, a Mendelian randomization study found that liver fat was causally associated with insulin resistance, a precursor of T2DM, as well as with a small but significant increase in T2DM risk[39]. However, in this last study, these associations were observed only among individuals with fibrosis[39]. In our analysis, due to lack of histology, we were unable to distinguish between simple steatosis from advanced

liver disease. Future studies should distinguish between these conditions and assess their mediating role separately.

Accumulating evidence implicates free fatty acids (FFAs) as the primary culprit of liver injury[63]. Accumulation of fat in the liver can be caused by obesity-related factors including an influx of FFAs into the liver, an imbalance of adipokines (increased proinflammatory cytokines or decreased adiponectin) as well as increased *de novo* lipogenesis from excessive carbohydrates and certain amino acids[63,64]. It is hypothesized that the NAFLD-specific role (especially non-alcoholic steatohepatitis [NASH] with varying levels of fibrosis) on the obesity-T2DM link could be due to an exacerbation of hepatic insulin resistance and alteration in the secretion of hepatokines, such as retinol-binding protein (RBP)-4, fetuin-A, fibroblast growth factor (FGF)-21, or of inflammatory biomarkers such as C-reactive protein, tumor necrosis factor (TNF)-alpha and interleukin-6 (IL)-6[11–14]. These hepatokines and inflammatory cytokines negatively affect hepatic gluconeogenesis, glycogen synthesis and insulin signaling, which in turn directly affect the risk of T2DM[11].

Our results should be interpreted in light of several assumptions required in the analytic approach. First, mediation analyses assume all confounders of the obesity-T2DM, obesity-NAFLD, and NAFLD-T2DM relationships were identified and accounted for. Second, valid estimation of natural effects also assumes that there are no mediator-outcome confounders that are caused by exposure. These are restrictive assumptions. We carefully considered relevant causal factors of obesity, NAFLD and T2DM and adjusted for major confounders using a directed acyclic graph. Additionally, sensitivity analyses included additional possible confounders, nevertheless the possibility of residual and unmeasured confounding cannot be completely excluded. Third, interpretation of these effects as etiological relationships requires the assumption of consistency, or well-defined exposures. Although BMI has been criticized in this regard[65], in populations with elevated BMIs, studies that target weight reduction have consistently found similar health benefits, including reduced T2DM risk, or improved T2DM management, regardless of the intervention[5–7]. Fourth, positivity, or the positive probability of the mediator observed at all levels of exposure and confounders, is required. Positivity was evaluated empirically and we found overall good overlap of all included confounders by BMI category. Fifth, temporality is necessary to establish causality[66] and as both exposure and mediator were measured at baseline, it is possible that the mediator could have preceded the exposure. However we believe this is highly unlikely as prior studies have shown that in most adults, liver fat arises due to central obesity and insulin resistance[11,67], and many epidemiological studies have consistently found obesity to precede NAFLD[17–23]. Furthermore, because our mediator was only measured at baseline, we were unable to use BMI or waist circumference as time-varying exposures. Future studies can improve on these limitations by modeling this association longitudinally. Lastly, marginal structural models via inverse probability weighting requires correct specification of models for the estimation of these weights (see Web Appendix Figs. 1–3 showing that no observation was given unreasonably large weights).

Our results should be interpreted in light of a few additional limitations. First, MESA did not include oral glucose tolerance tests, considered gold standard for diagnosing diabetes, or repeat measures of glycated hemoglobin (HbA1c), so we may have had some outcome

misclassification. Second, we only evaluated the mediating role of NAFLD, future studies can expand on this initial work and evaluate other mechanisms that mediate the obesity-T2DM relationship (e.g. visceral and intramuscular fat). Third, because presently there are no known effective NAFLD treatments that do not also include obesity reduction, we did not estimate controlled effects, which are useful in prescriptive settings[56], for instance in estimating the effect of an exposure on an outcome, holding a mediator value at a particular level (e.g. no liver fat). Fourth, missing covariates and attrition over time reduced our analytic sample by about 13%. And lastly, understanding the mechanisms linking obesity and T2DM, and the role that targeting NAFLD may play in T2DM prevention has been an area of active but inconclusive research[39,40,68,69], and this is in part due to the fact that mechanisms of NAFLD onset remain unclear[70] and additional studies, including interventional, may be necessary to more conclusively understand these links.

CONCLUSIONS

These data suggest that the association between obesity and T2DM risk is partially explained by the presence of NAFLD. Understanding the relative impact of the NAFLD pathway provides valuable knowledge that can be incorporated into strategies to reduce the negative effect of obesity on T2DM at the population level. Consistent with prior studies[41], these results support that more evidence is needed to evaluate if NAFLD could be an effective target to reduce the effect of obesity on T2DM.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments:

The authors thank the other investigators, staff, and the MESA participants for their valuable contributions. A full list of participating MESA investigators and institutions may be found at www.mesa-nhlbi.org.

Funding Support:

MESA was supported by contracts 75N92020D00001, HHSN268201500003I, N01-HC-95159, 75N92020D00005, N01-HC-95160, 75N92020D00002, N01-HC-95161, 75N92020D00003, N01-HC-95162, 75N92020D00006, N01-HC-95163, 75N92020D00004, N01-HC-95164, 75N92020D00007, N01-HC-95165, N01-HC-95166, N01-HC-95167, N01-HC-95168 and N01-HC-95169 from the National Heart, Lung, and Blood Institute, and by grants UL1-TR-000040, UL1-TR-001079, and UL1-TR-001420 from the National Center for Advancing Translational Sciences (NCATS). L.A.R. was supported by the National Institute Of Diabetes And Digestive And Kidney Diseases (NIDDK) of the National Institutes of Health (NIH) under Award Number F31DK115029, by a University of California Dissertation-Year Fellowship Award, and also received funding from The Permanente Medical Group (TPMG) Delivery Science Fellowship Program and The NIDDK Grant T32DK11668401. A.F. was supported by NIDDK Grant K24DK102057. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health, the University of California, or the Permanente Medical Group.

References

1. Cheng YJ, Kanaya AM, Araneta MRG, et al. Prevalence of Diabetes by Race and Ethnicity in the United States, 2011–2016. JAMA. 2019;322(24):2389–2398. doi:10.1001/jama.2019.19365 [PubMed: 31860047]

2. Boyle JP, Thompson TJ, Gregg EW, Barker LE, Williamson DF. Projection of the year 2050 burden of diabetes in the US adult population: dynamic modeling of incidence, mortality, and prediabetes prevalence. Popul Health Metr. 2010;8(1):29–37. [PubMed: 20969750]
3. Holmes MV, Lange LA, Palmer T, et al. Causal effects of body mass index on cardiometabolic traits and events: a Mendelian randomization analysis. Am J Hum Genet. 2014;94(2):198–208. doi:10.1016/j.ajhg.2013.12.014 [PubMed: 24462370]
4. WHO | Obesity: preventing and managing the global epidemic. Published online 2000. Accessed October 8, 2016. http://www.who.int/entity/nutrition/publications/obesity/WHO_TRS_894/en/index.html
5. Eckel RH, Kahn SE, Ferrannini E, et al. Obesity and Type 2 Diabetes: What Can Be Unified and What Needs to Be Individualized? Diabetes Care. 2011;34(6):1424–1430. doi:10.2337/dc11-0447 [PubMed: 21602431]
6. Expert Panel Members, Jensen MD, Ryan DH, et al. Executive summary: Guidelines (2013) for the management of overweight and obesity in adults. Obesity. 2014;22(S2):S5–S39. doi:10.1002/oby.20821 [PubMed: 24961825]
7. American Diabetes Association. 8. Obesity Management for the Treatment of Type 2 Diabetes: *Standards of Medical Care in Diabetes — 2019*. Diabetes Care. 2019;42(Supplement 1):S81–S89. doi:10.2337/dc19-S008 [PubMed: 30559234]
8. Bell JA, Kivimaki M, Hamer M. Metabolically healthy obesity and risk of incident type 2 diabetes: a meta-analysis of prospective cohort studies. Obes Rev Off J Int Assoc Study Obes. 2014;15(6):504–515. doi:10.1111/obr.12157
9. Menke A, Casagrande S, Geiss L, Cowie CC. Prevalence of and Trends in Diabetes Among Adults in the United States, 1988–2012. JAMA. 2015;314(10):1021–1029. doi:10.1001/jama.2015.10029 [PubMed: 26348752]
10. Cowie CC, Rust KF, Byrd-Holt DD, et al. Prevalence of Diabetes and Impaired Fasting Glucose in Adults in the U.S. Population: National Health and Nutrition Examination Survey 1999–2002. Diabetes Care. 2006;29(6):1263–1268. doi:10.2337/dc06-0062 [PubMed: 16732006]
11. Byrne CD, Targher G. NAFLD: A multisystem disease. J Hepatol. 2015;62(1, Supplement):S47–S64. doi:10.1016/j.jhep.2014.12.012 [PubMed: 25920090]
12. Kantartzis K, Machann J, Schick F, Fritsche A, Häring H-U, Stefan N. The impact of liver fat vs visceral fat in determining categories of prediabetes. Diabetologia. 2010;53(5):882–889. doi:10.1007/s00125-010-1663-6 [PubMed: 20099057]
13. Targher G, Byrne CD. Nonalcoholic Fatty Liver Disease: A Novel Cardiometabolic Risk Factor for Type 2 Diabetes and Its Complications. J Clin Endocrinol Metab. 2013;98(2):483–495. doi:10.1210/jc.2012-3093 [PubMed: 23293330]
14. Meex RCR, Watt MJ. Hepatokines: linking nonalcoholic fatty liver disease and insulin resistance. Nat Rev Endocrinol. 2017;13(9):509–520. doi:10.1038/nrendo.2017.56 [PubMed: 28621339]
15. Fabbrini E, Sullivan S, Klein S. Obesity and nonalcoholic fatty liver disease: Biochemical, metabolic, and clinical implications. Hepatology. 2010;51(2):679–689. doi:10.1002/hep.23280 [PubMed: 20041406]
16. Yilmaz Y, Younossi ZM. Obesity-Associated Nonalcoholic Fatty Liver Disease. Clin Liver Dis. 2014;18(1):19–31. doi:10.1016/j.cld.2013.09.018 [PubMed: 24274862]
17. Zelber-Sagi S, Lotan R, Shlomai A, et al. Predictors for incidence and remission of NAFLD in the general population during a seven-year prospective follow-up. J Hepatol. 2012;56(5):1145–1151. doi:10.1016/j.jhep.2011.12.011 [PubMed: 22245895]
18. Kim D, Chung GE, Kwak M-S, et al. Body Fat Distribution and Risk of Incident and Regressed Nonalcoholic Fatty Liver Disease. Clin Gastroenterol Hepatol. 2016;14(1):132–138.e4. doi:10.1016/j.cgh.2015.07.024 [PubMed: 26226099]
19. Chang Y, Ryu S, Sung E, et al. Weight gain within the normal weight range predicts ultrasonographically detected fatty liver in healthy Korean men. Gut. 2009;58(10):1419–1425. doi:10.1136/gut.2008.161885 [PubMed: 19505882]
20. Xu C, Yu C, Ma H, Xu L, Miao M, Li Y. Prevalence and Risk Factors for the Development of Nonalcoholic Fatty Liver Disease in a Nonobese Chinese Population: the Zhejiang Zhenhai Study. Am J Gastroenterol. 2013;108(8):1299–1304. doi:10.1038/ajg.2013.104 [PubMed: 23567356]

21. Chang Y, Jung H-S, Cho J, et al. Metabolically Healthy Obesity and the Development of Nonalcoholic Fatty Liver Disease. Am J Gastroenterol. Published online May 17, 2016. doi:10.1038/ajg.2016.178
22. Yun KE, Nam GE, Lim J, et al. Waist Gain Is Associated with a Higher Incidence of Nonalcoholic Fatty Liver Disease in Korean Adults: A Cohort Study. PloS One. 2016;11(7):e0158710. [PubMed: 27420035]
23. Bruno S Incidence and risk factors for non-alcoholic steatohepatitis: prospective study of 5408 women enrolled in Italian tamoxifen chemoprevention trial. BMJ. 2005;330(7497):932–0. doi:10.1136/bmj.38391.663287.E0 [PubMed: 15746106]
24. Mantovani A, Byrne CD, Bonora E, Targher G. Nonalcoholic Fatty Liver Disease and Risk of Incident Type 2 Diabetes: A Meta-analysis. Diabetes Care. 2018;41(2):372–382. doi:10.2337/dc17-1902 [PubMed: 29358469]
25. Targher G, Marchesini G, Byrne CD. Risk of type 2 diabetes in patients with non-alcoholic fatty liver disease: Causal association or epiphenomenon? Diabetes Metab. 2016;42(3):142–156. doi:10.1016/j.diabet.2016.04.002 [PubMed: 27142870]
26. Shah RV, Allison MA, Lima JAC, et al. Liver fat, statin use, and incident diabetes: The Multi-Ethnic Study of Atherosclerosis. Atherosclerosis. 2015;242(1):211–217. doi:10.1016/j.atherosclerosis.2015.07.018 [PubMed: 26209814]
27. Ballestri S, Zona S, Targher G, et al. Nonalcoholic fatty liver disease is associated with an almost twofold increased risk of incident type 2 diabetes and metabolic syndrome. Evidence from a systematic review and meta-analysis. J Gastroenterol Hepatol. 2016;31(5):936–944. doi:10.1111/jgh.13264 [PubMed: 26667191]
28. Musso G, Gambino R, Cassader M, Pagano G. Meta-analysis: Natural history of non-alcoholic fatty liver disease (NAFLD) and diagnostic accuracy of non-invasive tests for liver disease severity. Ann Med. 2011;43(8):617–649. doi:10.3109/07853890.2010.518623 [PubMed: 21039302]
29. Sung K-C, Wild SH, Byrne CD. Resolution of Fatty Liver and Risk of Incident Diabetes. J Clin Endocrinol Metab. 2013;98(9):3637–3643. doi:10.1210/jc.2013-1519 [PubMed: 23873989]
30. Shibata M, Kihara Y, Taguchi M, Tashiro M, Otsuki M. Nonalcoholic Fatty Liver Disease Is a Risk Factor for Type 2 Diabetes in Middle-Aged Japanese Men. Diabetes Care. 2007;30(11):2940–2944. doi:10.2337/dc07-0792 [PubMed: 17666460]
31. Yamada T, Fukatsu M, Suzuki S, Wada T, Yoshida T, Joh T. Fatty liver predicts impaired fasting glucose and type 2 diabetes mellitus in Japanese undergoing a health checkup. J Gastroenterol Hepatol. 2010;25(2):352–356. doi:10.1111/j.1440-1746.2009.05998.x [PubMed: 19817963]
32. Fukuda T, Hamaguchi M, Kojima T, et al. The impact of non-alcoholic fatty liver disease on incident type 2 diabetes mellitus in non-overweight individuals. Liver Int. 2016;36(2):275–283. doi:10.1111/liv.12912 [PubMed: 26176710]
33. Gautier A, Balkau B, Lange C, Tichet J, Bonnet F, Group for the DS. Risk factors for incident type 2 diabetes in individuals with a BMI of <27 kg/m2: the role of γ-glutamyltransferase. Data from an Epidemiological Study on the Insulin Resistance Syndrome (DESIR). Diabetologia. 2010;53(2):247. doi:10.1007/s00125-009-1602-6 [PubMed: 19936701]
34. Fan J-G, Li F, Cai X-B, Peng Y-D, Ao Q-H, Gao Y. Effects of nonalcoholic fatty liver disease on the development of metabolic disorders. J Gastroenterol Hepatol. 2007;22(7):1086–1091. doi:10.1111/j.1440-1746.2006.04781.x [PubMed: 17608855]
35. Bae JC, Rhee EJ, Lee WY, et al. Combined Effect of Nonalcoholic Fatty Liver Disease and Impaired Fasting Glucose on the Development of Type 2 Diabetes. Diabetes Care. 2011;34(3):727–729. doi:10.2337/dc10-1991 [PubMed: 21278140]
36. Kasturiratne A, Weerasinghe S, Dassanayake AS, et al. Influence of nonalcoholic fatty liver disease on the development of diabetes mellitus: Influence of NAFLD on incident diabetes. J Gastroenterol Hepatol. 2013;28(1):142–147. doi:10.1111/j.1440-1746.2012.07264.x [PubMed: 22989165]
37. Chang Y, Jung H-S, Yun KE, Cho J, Cho YK, Ryu S. Cohort Study of Nonalcoholic Fatty Liver Disease, NAFLD fibrosis score, and the Risk of Incident Diabetes in a Korean population. Am J Gastroenterol. 2013;108(12):1861–1868. doi:10.1038/ajg.2013.349 [PubMed: 24100261]

38. Mantovani A, Petracca G, Beatrice G, Tilg H, Byrne CD, Targher G. Nonalcoholic fatty liver disease and risk of incident diabetes mellitus: an updated meta-analysis of 501 022 adult individuals. Gut. Published online September 16, 2020:gutjnl-2020–322572. doi:10.1136/gutjnl-2020-322572
39. Dongiovanni P, Stender S, Pietrelli A, et al. Causal relationship of hepatic fat with liver damage and insulin resistance in nonalcoholic fatty liver. J Intern Med. 2018;283(4):356–370. doi:10.1111/joim.12719 [PubMed: 29280273]
40. Fuchsberger C, Flannick J, Teslovich TM, et al. The genetic architecture of type 2 diabetes. Nature. 2016;536(7614):41–47. doi:10.1038/nature18642 [PubMed: 27398621]
41. Fruci B, Giuliano S, Mazza A, Malaguarnera R, Belfiore A. Nonalcoholic Fatty Liver: A Possible New Target for Type 2 Diabetes Prevention and Treatment. Int J Mol Sci. 2013;14(11):22933–22966. doi:10.3390/ijms141122933 [PubMed: 24264040]
42. Bild DE, Bluemke DA, Burke GL, et al. Multi-Ethnic Study of Atherosclerosis: Objectives and Design. Am J Epidemiol. 2002;156(9):871–881. doi:10.1093/aje/kwf113 [PubMed: 12397006]
43. Tison GH, Blaha MJ, Nasir K, et al. Relation of Anthropometric Obesity and Computed Tomography Measured Nonalcoholic Fatty Liver Disease (from the Multiethnic Study of Atherosclerosis). Am J Cardiol. 2015;116(4):541–546. doi:10.1016/j.amjcard.2015.05.012 [PubMed: 26070222]
44. Golden SH, Lee HB, Schreiner PJ, et al. Depression and Type 2 Diabetes Mellitus: The Multiethnic Study of Atherosclerosis: Psychosom Med. 2007;69(6):529–536. doi:10.1097/PSY.0b013e3180f61c5c [PubMed: 17636146]
45. consultation W expert. Appropriate body-mass index for Asian populations and its implications for policy and intervention strategies. The Lancet. 2004;363(9403):157–163. doi:10.1016/S0140-6736(03)15268-3
46. Wilkins LW&. Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) Final Report. Circulation. 2002;106(25):3143–3143. [PubMed: 12485966]
47. Alberti KGMM, Zimmet P, Shaw J. Metabolic syndrome—a new world-wide definition. A Consensus Statement from the International Diabetes Federation. Diabet Med. 2006;23(5):469–480. doi:10.1111/j.1464-5491.2006.01858.x [PubMed: 16681555]
48. Bydder GM, Chapman RWG, Harry D, Bassan L, Sherlock S, Kreel L. Computed tomography attenuation values in fatty liver. J Comput Tomogr. 1981;5(1):33–35. doi:10.1016/0149-936X(81)90054-0 [PubMed: 7273824]
49. Kodama Y, Ng CS, Wu TT, et al. Comparison of CT Methods for Determining the Fat Content of the Liver. Am J Roentgenol. 2007;188(5):1307–1312. doi:10.2214/AJR.06.0992 [PubMed: 17449775]
50. McAuley PA, Hsu F-C, Loman KK, et al. Liver Attenuation, Pericardial Adipose Tissue, Obesity, and Insulin Resistance: The Multi-Ethnic Study of Atherosclerosis (MESA). Obesity. 2011;19(9):1855–1860. doi:10.1038/oby.2011.191 [PubMed: 21720430]
51. Greenland S, Pearl J, Robins JM. Causal diagrams for epidemiologic research. Epidemiology. Published online 1999:37–48. [PubMed: 9888278]
52. Mayer-Davis EJ, Vitolins MZ, Carmichael SL, et al. Validity and Reproducibility of a Food Frequency Interview in a Multi-Cultural Epidemiologic Study. Ann Epidemiol. 1999;9(5):314–324. doi:10.1016/S1047-2797(98)00070-2 [PubMed: 10976858]
53. Rodriguez LA, Jin Y, Talegawkar SA, et al. Differences in Diet Quality among Multiple US Racial/Ethnic Groups from the Mediators of Atherosclerosis in South Asians Living in America (MASALA) Study and the Multi-Ethnic Study of Atherosclerosis (MESA). J Nutr. Published online 2020. doi:10.1093/jn/nxaa050
54. Baron RM, Kenny DA. The Moderator-Mediator Variable Distinction in Social Psychological Research: Conceptual, Strategic, and Statistical Considerations. :10.
55. Vanderweele TJ, Vansteelandt S. Conceptual issues concerning mediation, interventions and composition. Stat Interface. 2009;2(4):457–468. doi:10.4310/SII.2009.v2.n4.a7

56. Pearl J. Direct and Indirect Effects. In: Proceedings of the Seventeenth Conference on Uncertainty in Artificial Intelligence. UAI'01. Morgan Kaufmann Publishers Inc.; 2001:411–420. Accessed December 1, 2016. http://dl.acm.org/citation.cfm?id=2074022.2074073
57. Lange T, Vansteelandt S, Bekaert M. A Simple Unified Approach for Estimating Natural Direct and Indirect Effects. Am J Epidemiol. 2012;176(3):190–195. doi:10.1093/aje/kwr525 [PubMed: 22781427]
58. Cole SR, Hernan MA. Constructing Inverse Probability Weights for Marginal Structural Models. Am J Epidemiol. 2008;168(6):656–664. doi:10.1093/aje/kwn164 [PubMed: 18682488]
59. Sparling YH. Parametric survival models for interval-censored data with time-dependent covariates. Biostatistics. 2006;7(4):599–614. doi:10.1093/biostatistics/kxj028 [PubMed: 16597670]
60. Hosmer DW, Lemeshow S, May S. Applied Survival Analysis: Regression Modeling of Time-to-Event Data. Second Edition. John Wiley & Sons, Inc.; 2008. 10.1002/9780470258019.fmatter
61. Lindsey JC, Ryan LM. Tutorial in biostatistics methods for interval-censored data. Stat Med. 1998;17(2):219–238. [PubMed: 9483730]
62. VanderWeele TJ. Explanation in Causal Inference: Methods for Mediation and Interaction. Oxford University Press; 2015.
63. Perito ER, Rodriguez LA, Lustig RH. Dietary treatment of nonalcoholic steatohepatitis. Curr Opin Gastroenterol. 2013;29(2):170–176. doi:10.1097/MOG.0b013e32835ca11d [PubMed: 23283181]
64. Roden M. Mechanisms of Disease: hepatic steatosis in type 2 diabetes--pathogenesis and clinical relevance. Nat Clin Pract Endocrinol Metab. 2006;2(6):335–348. doi:10.1038/ncpendmet0190 [PubMed: 16932311]
65. Hernán MA, Taubman SL. Does obesity shorten life? The importance of well-defined interventions to answer causal questions. Int J Obes. 2008;32(S3):S8–S14. doi:10.1038/ijo.2008.82
66. Holland PW. Statistics and Causal Inference. J Am Stat Assoc. 1986;81(396):945–960. doi:10.1080/01621459.1986.10478354
67. Dowman JK, Tomlinson JW, Newsome PN. Systematic review: the diagnosis and staging of non-alcoholic fatty liver disease and non-alcoholic steatohepatitis. Aliment Pharmacol Ther. 2011;33(5):525–540. doi:10.1111/j.1365-2036.2010.04556.x [PubMed: 21198708]
68. Lattuada G, Ragogna F, Perseghin G. Why Does NAFLD Predict Type 2 Diabetes? Curr Diab Rep. 2011;11(3):167–172. doi:10.1007/s11892-011-0190-2 [PubMed: 21431854]
69. Valenti L, Bugianesi E, Pajvani U, Targher G. Nonalcoholic fatty liver disease: cause or consequence of type 2 diabetes? Liver Int. 2016;36(11):1563–1579. doi:10.1111/liv.13185 [PubMed: 27276701]
70. Tarantino G, Citro V, Capone D. Nonalcoholic Fatty Liver Disease: A Challenge from Mechanisms to Therapy. J Clin Med. 2019;9(1):15. doi:10.3390/jcm9010015

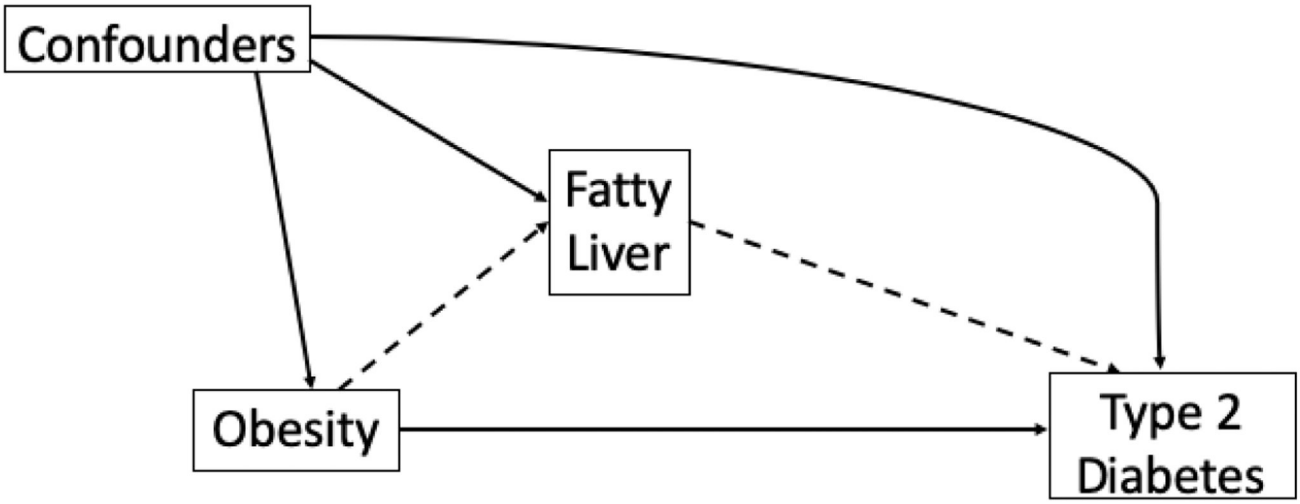

Figure 1.

Hypothesized causal diagram of the association between obesity and type 2 diabetes where "confounders" denote the same set of socio-demographic and lifestyle confounders (i.e. age, sex, race/ethnicity, education, diet and exercise) of the associations between obesity and fatty liver, obesity and type 2 diabetes, and fatty liver and type 2 diabetes. The solid arrow between obesity and type 2 diabetes represents the direct association and dashed arrows represent the indirect association via fatty liver.

Table 1

Baseline Characteristics Overall, and by BMI Category, Among 4,522 Men and Women participating in the Multi-Ethnic Study of Atherosclerosis, United States, 2000–2012

		BMI Category[a]		
Characteristic	**Total (*n* = 4,522; 100%)**	**Normal (*n* = 1,221; 27%)**	**Overweight (*n* = 1,913; 42%)**	**Obese (*n* = 1,388; 31%)**
Incident diabetes cases. n (%)	557 (12)	54 (4.4)	207 (11)	296 (21)
Diabetes incidence rate per	16 (15, 18)	5.5 (4.2, 7.2)	14 (13, 16)	30 (27, 33)
1.000 person-years (95% CI)				
Liver fat HU units, n (%)				
Quartile 1 (70 to 110) (lowest fat)	1,138 (25)	395 (32)	491 (26)	252 (18)
Quartile 2 (64 to <70)	1,136 (25)	392 (32)	484 (25)	260 (19)
Quartile 3 (57 to <64)	1,144 (25)	323 (26)	487 (25)	334 (24)
Quartile 4 (−27 to <57) (highest fat)	1,104 (25)	111 (9.1)	451 (24)	542 (39)
Age, mean ± SD	62 ± 10	62 ± 11	62 ± 10	61 ± 10
Sex. n (% female)	2,417 (54)	692 (57)	898 (47)	827 (60)
Race/Ethnicity, n (%)				
White	1,853 (41)	612 (50)	753 (39)	488 (35)
African American	1,159 (26)	219 (18)	451 (24)	489 (35)
Hispanic	973 (22)	178 (15)	456 (24)	339 (24)
Chinese American	537 (12)	212 (17)	253 (13)	72 (5.2)
Education, n (%)				
Less than high school	734 (16)	168 (14)	340 (18)	226 (16)
Completed high school	797 (18)	203 (17)	335 (18)	259 (19)
Some college	1,277 (28)	331 (27)	509 (27)	437 (31)
≥Bachelor's degree	1,714 (38)	519 (43)	729 (38)	466 (34)
Diet Quality, AHEI, mean ± SD	55 ± 10	57 ± 10	55 ± 10	52 ± 10
Kcal/day, median	1522	1427	1518	1627
[interquartile range]	[1116–2067]	[1044–1926]	[1116–2029]	[1175–2201]
Intentional Exercise,	840	1043	975	630
MET-min/week, median [interquartile range]	[165–2100]	[315–2363]	[210–2130]	[0–1713]

Abbreviations: BMI, body mass index; HU, Hounsfield units.

[a]BMI categories: Chinese American normal <23, overweight 23–27.4, obese ≥27.5; other: normal <25, overweight 25–29.9, obese ≥30

Table 2

Direct and Indirect Effects of Generalized Obesity (BMI Category[a]) on Incident Type 2 Diabetes with Liver Fat Attenuation as a Mediator, Multi-Ethnic Study of Atherosclerosis, United States, 2000–2012

Exposure		aHR[b]	95% CI	% mediated
Overweight vs. normal BMI [a]	Direct Effect	1.89	1.35, 2.66	27 (18, 41)
	Indirect Effect	1.18	1.13, 1.26	
	Total Effect	2.22	1.49, 2.96	
Obese vs. normal BMI [a]	Direct Effect	3.25	2.30, 4.58	36 (27, 44)
	Indirect Effect	1.38	1.28, 1.49	
	Total Effect	4.48	3.02, 5.94	

Abbreviations: BMI, body mass index; HR, hazard ratio.

[a]BMI categories: Chinese American normal <23, overweight 23–27.4, obese ≥27.5; other: normal <25, overweight 25–29.9, obese ≥30

[b]Model adjusted for age, sex, race/ethnicity, education, exercise, dietary quality and total caloric intake.

Table 3

Direct and Indirect Effects of Central Obesity (Elevated Waist Circumference[a]) on Incident Type 2 Diabetes with Liver Fat Attenuation as a Mediator, Multi-Ethnic Study of Atherosclerosis, United States, 2000–2012

Exposure		aHR[b]	95% CI	% mediated
Elevated vs. normal waist circumference [a]	Direct Effect	2.16	1.76, 2.65	
	Indirect Effect	1.24	1.19, 1.30	32 (24, 40)
	Total Effect	2.69	2.15, 3.23	

Abbreviations: HR, hazard ratio.

[a]Elevated waist circumference according to the Third Report of the National Cholesterol Education Program[46]: >102cm for men and >88cm for women

[b]Model adjusted for age, sex, race/ethnicity, education, exercise, dietary quality and total caloric intake.

Table 4

Direct and Indirect Effects of Central Obesity (Elevated Waist Circumference[a]) on Incident Type 2 Diabetes with Liver Fat Attenuation as a Mediator, Multi-Ethnic Study of Atherosclerosis, United States, 2000–2012

Exposure		aHR[b]	95% CI	% mediated
Elevated vs. normal waist circumference [a]	Direct Effect	2.55	1.89, 3.46	
	Indirect Effect	1.22	1.17, 1.27	27 (21, 33)
	Total Effect	3.11	2.19, 4.03	

Abbreviations: HR, hazard ratio.

[a]Elevated waist circumference according to the International Diabetes Federation metabolic syndrome classification[47]: >94cm for White and African American men, >90cm for Chinese American and Hispanic men and >80cm for all women

[b]Model adjusted for age, sex, race/ethnicity, education, exercise, dietary quality and total caloric intake.