Material and Methods

Study population

The data for this study were derived from the NHANES, a nationwide survey designed by the National Center for Health Statistics (NCHS) to assess the health and nutritional status of the U.S. population. The NHANES employs a complex, stratified, multistage probability sampling method to ensure that its sample reflects the diversity of the U.S. population. The survey integrates questionnaire responses, physical examinations, and laboratory data to provide comprehensive health information. The data used in this study are publicly available [https://www.cdc.gov/nchs/nhanes/], and all participants provided informed consent prior to participation. Additionally, ethical approval was obtained for the survey. The current study analyzed four cycles of NHANES data from 2017 to 2020. During this period, a total of 15,560 participants were surveyed. Because the current study focused on gallstones, participants under 20 years old (n=6,328) were excluded, leaving 9,232 participants. Additionally, individuals with missing data regarding urinary incontinence (n=22), weight from one year prior (n=163), current weight (n=793), waist circumference and height (n=350), monocyte counts (n=321) or HDL cholesterol (n=185) were excluded. Ultimately, 7,398 participants were included for analysis (Figure S1).

Prevalence of Gallstones

In this study, the prevalence of gallstones was used as the outcome variable. The prevalence of gallstones was based on the following the NHANES item: “Has a doctor ever told you that you had

gallstones?" (variable name: MCQ550). This question was administered by trained interviewers during home visits using the Computer-Assisted Personal Interview (CAPI) system.

Definition of Weight Gain Percentage

The weight gain percentage was calculated as the difference between the participant's current weight and their self-reported weight one year prior, divided by their weight one year prior, and then multiplied by 100. Based on this calculation, participants were categorized into the following groups: weight gain percentage -5 to 5% (control group), -10 to -5.1%, 5.1 to 10%, and >10%.

Definition of the Monocyte-to-High-Density Lipoprotein Cholesterol Ratio (MHR)

The MHR was defined as the ratio of monocyte count to the high-density lipoprotein cholesterol concentration. Monocyte counts were measured via whole blood samples analyzed at NHANES mobile examination centers (MECs) with a Beckman Coulter DxH 800 analyzer. This method is based on cell volume, conductivity, and scatter technology. HDL-C levels were directly measured via a specific endpoint reaction, in which PEG-modified cholesterol esterase and oxidase were employed to produce a purple–blue product, which was quantified photometrically at a wavelength of 600 nm.

Definition of Covariates

On the basis of previous studies (1-4) and an estimated effect size change of more than 10%, this study identified the following potential confounding factors: age, sex, race, educational level, marital status, family poverty-income ratio, smoking and drinking status, sleep duration, weight loss attempts in the past year, diabetes, hypertension, coronary heart disease, cancer, and asthma. Smoking status was defined on the basis of participants' responses to the following question: "Have you smoked at

least 100 cigarettes in your lifetime?" Drinking status was determined by whether participants reported consuming more than 12 alcoholic drinks in the past year. Diabetes, hypertension, cancer, and asthma were identified based on participants' self-reported medical history in the survey.

Statistical Analysis

All analyses were conducted via R version 4.2.1 (http://www.Rproject.org; The R Foundation, Vienna, Austria) and Free Statistics software (version 2.0; Beijing FreeClinical Medical Technology Co., Ltd., Beijing, China). The baseline characteristics of the study population were initially analyzed. Continuous variables that followed a normal distribution are expressed as the means ± standard deviations and were compared between groups using t tests or one-way analysis of variance (ANOVA). In contrast, continuous variables that did not follow a normal distribution are expressed as medians (interquartile ranges) and were compared between groups using the Mann–Whitney U test or the Kruskal–Wallis test. Categorical variables are expressed as frequencies and percentages, and between-group differences were assessed via χ^2 tests or Fisher's exact tests. Owing to the nonnormal distribution of MHR values, logarithmic transformation (Log2) was applied to meet the assumptions of subsequent statistical models. The Log2-transformed MHR was categorized into quartiles (Q1–Q4) based on its distribution in the study population, with Q1 representing the lowest range of values and Q4 representing the highest. To explore the associations among weight gain percentage, gallstone risk, and the MHR, multivariable logistic regression and linear regression models were employed, adjusting for potential confounders. The results from logistic regression are reported as odds ratios (ORs) with 95% confidence intervals (CIs), whereas linear regression results are presented as regression coefficients (β) with standard errors. Nonlinear relationships between weight gain percentage and gallstone risk were

assessed via threshold analysis to identify potential inflection points through segmented model fitting. Propensity score matching (PSM) was employed to balance baseline confounders and mimic randomized controlled trial conditions, thereby enhancing the reliability of the results. Bootstrapping was applied for mediation analysis to examine the mediating role of the MHR in the relationship between weight gain percentage and gallstone risk. Indirect effects and their CIs were calculated through repeated resampling to determine whether the MHR was a significant mediator this relationship. All analyses were two-sided, and a P value < 0.05 was considered statistically significant.

References

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