Preventive Medicine 101 (2017) 72–76

Contents lists available at ScienceDirect

Preventive Medicine

journal homepage: www.elsevier.com/locate/ypmed

Healthy lifestyle and glucagon-like peptide-1 in young and healthy adults: A population-based study

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ARTICLE INFO

Article history:
Received 2 March 2017
Received in revised form 25 May 2017
Accepted 27 May 2017
Available online 01 June 2017

Keywords:
Glucagon-like peptide-1
Lifestyle
Hypertension
Diabetes mellitus
Epidemiology
Insulin resistance

ABSTRACT

A healthy lifestyle is associated with a lower risk of cardiovascular events and mortality, but underlying mechanisms are not fully understood. The aim of our study was to investigate the relationships between a healthy lifestyle and glucagon-like peptide-1 (GLP-1), an incretin hormone with both glycemic and cardiovascular properties. Healthy participants aged 25–41 years without cardiovascular disease, diabetes or a body mass index (BMI) >35 kg/m^2 were enrolled in a population-based study. The following metrics were used to build a lifestyle score ranging from 0 to 7 (a higher score indicating a healthier lifestyle): blood pressure (BP) (<120/80 mm Hg), plasma levels of glycated hemoglobin (<5.7%), total cholesterol levels (<200 mg/dl), BMI (<25 kg/m^2), not smoking cigarettes, moderate (≥150 min/week) or vigorous (≥75 min/week) physical activity and a healthy diet. Among 2133 participants median age was 36.7 years and 53.3% were female. GLP-1 levels decreased significantly from 39.5 to 30.9 ng/l ($p < 0.0001$) across increasing lifestyle score categories. This linear relationship persisted in multivariable adjusted linear regression models (B for GLP-1 per 1-unit increase of the lifestyle score −0.06; 95% confidence intervals −0.07, −0.04; $p < 0.0001$). Individual health metrics that were significantly associated with GLP-1 were a normal BMI (−0.07; −0.12, −0.03; $p = 0.001$), low total cholesterol levels (−0.07; −0.12, −0.03; $p = 0.001$), normal BP (−0.05; −0.10, −0.00; $p = 0.047$) and not smoking (−0.06; −0.10, −0.01; $p = 0.01$). A healthy lifestyle is strongly associated with lower GLP-1 levels in young and healthy adults.

1. Introduction

Cardiovascular diseases are a leading cause of morbidity and mortality worldwide (GBD 2013 Mortality and Causes of Death Collaborators, 2015; Mozaffarian et al., 2016). A healthy lifestyle is strongly associated with decreased cardiovascular disease, cardiovascular mortality and all-cause mortality (Folsom et al., 2011; Ford et al., 2012; Yang et al., 2012), however underlying pathophysiological mechanisms leading to these effects are not fully understood.

Glucagon-like peptide-1 (GLP-1), an incretin hormone, mediates its main effects through GLP-1 receptors, which are found ubiquitously in the cardiovascular and renal system (Ban et al., 2008; Crajoinas et al., 2011; Wei and Mojsov, 1995). Its main effects are the regulation of gastric emptying and improvement of insulin resistance (IR) (Holz et al., 1993; Willms et al., 1996), thereby improving HbA1c levels (Monami et al., 2014). Additionally GLP-1 and its agonists were shown to interact with other cardiovascular risk factors including blood pressure (BP) (Garber et al., 2009; Krisai et al., 2015; Robinson et al., 2013; Yamamoto et al., 2002), lipid metabolism and bodyweight (Monami et al., 2014; Robinson et al., 2013; van der Stouwe et al., 2015) and directly influence endothelial function (Basu et al., 2007; Gaspari et al., 2011). Thus, the GLP-1 pathway could be influenced by lifestyle habits which are related to cardiovascular risk and incident cardiovascular events, and furthermore play a mechanistic role in these relations. However the relation between lifestyle habits and GLP-1 is currently not known. We further hypothesized that markers of inflammation,

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http://dx.doi.org/10.1016/j.ypmed.2017.05.025
0091-7435/

endothelial dysfunction and IR could play an important role in this relation, as GLP-1 is known to be associated with all of them (Basu et al., 2007; Gaspari et al., 2011; Holz et al., 1993; van der Stouwe et al., 2015).

Therefore the aim of our study was to assess the relations between lifestyle habits and fasting GLP-1 levels in a large population based sample of young and healthy adults.

2. Methods

2.1. Study participants

All study participants included in the current analysis were participants of the ongoing prospective genetic and phenotypic determinants of blood pressure and other cardiovascular risk factors (GAPP) study. Detailed information on the study design has been published previously (Conen et al., 2013). From 2010 to 2013, all 6887 inhabitants of the Principality of Liechtenstein aged between 25 and 41 years were invited to participate in the study. Of these, 5775 individuals could be contacted and were eligible. 2170 participants (38% participation rate) were ultimately enrolled. Main exclusion criteria were current intake of antidiabetic drugs, a body mass index (BMI) >35 kg/m^2, established cardiovascular disease or any other severe illness. For the current analysis cross-sectional data from the baseline measurements were used. All participants with invalid or missing GLP-1 levels (n = 15) or missing health metrics (n = 30) were excluded, leaving 2133 (98.3%) subjects. The study protocol was approved by the local ethics committee and informed written consent was obtained from each participant.

2.2. Blood and urine samples

Venous blood samples were drawn from each participant after overnight fasting and immediately stored at −80 °C after centrifugation. Morning spot urine samples were collected from all participants and immediately stored at −80 °C. Plasma levels of GLP-1 and endothelin-1 (ET-1) were assayed from frozen EDTA plasma samples using a single-molecule counting technology assay (Erenna Immunoassay System, Singulex, Alameda, CA, USA) (Todd et al., 2007). The remaining biomarkers were assayed from fresh samples. Hemoglobin A_{1c} (HbA1c) was measured by high-performance liquid chromatography (Biorad D10, Pratteln, Switzerland). High-sensitivity C-reactive protein (Hs-CRP) and cholesterol levels were assayed using a Roche Cobas 6000 analyzer (F. Hoffmann – La Roche, Switzerland) (Conen et al., 2013).

Urinary sodium excretion over 24 h as a proxy for sodium consumption was estimated from fasting morning urinary samples using the formula by Kawasaki et al. (1993). Homeostasis model assessment (HOMA) was calculated using the formula for HOMA-IR (Matthews et al., 1985), and a value ≥2.0 was interpreted as indicating IR.

2.3. Assessment of other study variables

Standardized questionnaires of the Swiss Federal Office of Public Health were used to assess personal, medical, lifestyle and nutritional factors (Swiss Health Survey, 2007). Education status was also determined with the official questionnaire of the Swiss Federal Office of Public Health (Swiss Health Survey, 2007). Physical activity was assessed with the validated individual physical activity questionnaire and used as a continuous variable (Craig et al., 2003). A healthy diet score was adapted from the current AHA recommendations with a possible score from 0 to 3 points, whereas 1 point was given for consumption of ≥5 units/day of fruits or vegetables, ≥2 units of fish/week or a 24 h sodium excretion <1500 mg/d. Alcohol consumption was self-reported and presented as grams of alcohol per day. Smoking status was graded as current, past or never by self-report. Weight was measured in underwear with a calibrated balance (Seca 877, Hamburg). Height was determined without shoes backwards in front of a wall with a calibrated measuring tape (Seca 202, Hamburg). BMI was calculated as body weight in kilogram divided by height in meters squared. Three consecutive BP recordings were performed after 5 min of rest using a validated oscillometric device (Microlife BP3AG1, Microlife AG, Switzerland) (Conen et al., 2013). The mean of the second and third reading was used for our analysis.

2.4. Lifestyle score

Healthy lifestyle was classified according to a validated cardiovascular health metric score, which has previously been shown to predict cardiovascular and all-cause mortality (Yang et al., 2012). One point was given for each of the following seven metrics: non-smoker or quit smoking >12 months, BMI <25 kg/m^2, >75 min/week vigorous or >-150 min/week moderate physical activity, healthy diet score ≥ 2 of the three elements making up this component, total cholesterol levels <200 mg/dl (5.17 mmol/l), BP <120/80 mm Hg in the absence of antihypertensive treatment and HbA1c levels <5.7%. The score ranged from 0 to 7, where 0 indicated the unhealthiest and 7 the healthiest lifestyle.

2.5. Statistical analysis

Baseline characteristics were shown non-stratified. The distribution of continuous variables was assessed using skewness, kurtosis and visual inspection of the histogram. Continuous data were presented as means ± standard deviations or medians (interquartile ranges), as appropriate Mean values over categories were compared using analysis of variance. Categorical variables were presented as counts (percentages) and compared using chi square tests.

Multivariable linear regression models were built to compare the B-coefficients and 95% confidence intervals (CI) of GLP-1 levels across the lifestyle-score categories with 0–1, 2, 3, 4, 5 and 6–7 points. Due to small numbers in the extreme categories of the lifestyle score, we opted to merge the participants with either 0–1 points or 6–7 points into single categories. Participants in the 4 point category were used as the reference group due to group size. GLP-1 was log-transformed throughout all analyses to improve the normality of its distribution. Crude models were adjusted for age, sex, alcohol intake and education status. As all observed relationships were approximately linear, we performed additional analyses using the lifestyle score as an ordinal variable. As alcohol consumption is difficult to assess we performed a sensitivity analysis for alcohol intake in the main model. Further sensitivity analyses of the main model were performed with a cumulative variable of moderate and vigorous physical activity and with the exclusion of all participants receiving antihypertensive medication. To assess the relationships of each individual lifestyle score component with GLP-1 levels, all individual lifestyle score components were mutually adjusted for in one combined, multivariable adjusted model. To test for the influence of plasma ET-1 levels, Hs-CRP levels, HOMA-IR and white blood count (WBC) as potential confounders of the observed relationships, the above mentioned multivariate models were further adjusted for these factors.

Pre-specified subgroup analyses included ordinal multivariable linear regression models for the relation between the lifestyle score and log-transformed GLP-1 stratified by sex, age (<35 versus ≥35 years), BMI (≤25 versus >25 kg/m^2) and prediabetes (HbA1c >5.7 and ≤6.4 versus ≤5.7%). Differences across subgroups were investigated by including multiplicative interaction terms, computed by multiplication of log-transformed GLP-1 and the respective, continuous subgroup variable, in the non-stratified models. All statistical analyses were performed using SAS version 9.4 (SAS Institute Inc., Cary, NC), and a p-value of <0.05 was pre-specified to indicate statistical significance. None of the funders had any influence in statistical analyses, drafting the manuscript or submitting the manuscript for publication.