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One-carbon metabolism markers are associated with cardiometabolic risk factors

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Abstract *Background and aims:* Alterations to one-carbon metabolism, especially elevated plasma homocysteine (Hcy), have been suggested to be both a cause and a consequence of the metabolic syndrome (MS). A deeper understanding of the role of other one-carbon metabolites in MS, including *s*-adenosylmethionine (SAM), *s*-adenosylhomocysteine (SAH), and the methylation capacity index (SAM:SAH ratio) is required.

Methods and results: 118 men and women with MS-risk factors were included in this cross-sectional study and cardiometabolic outcomes along with markers of one-carbon metabolism, including fasting plasma SAM, SAH, Hcy and vitamin B_{12} concentrations, were analysed. Multiple linear regression models were also used to examine the association between plasma one-carbon metabolites and cardiometabolic health features.

We found that fasting plasma concentrations of Hcy, SAM and SAH were all positively correlated with markers of adiposity, including BMI (increase in BMI per 1-SD increase in one-carbon metabolite: 0.92 kg/m^2 95% CI (0.28; 1.56), $p = 0.005$; 0.81 (0.15; 1.47), $p = 0.02$; 0.67 (−0.01; 1.36), $p = 0.05$, respectively). Hcy, but not SAM, SAH or SAM:SAH ratio was associated with BMI and body fat percentage after mutual adjustments. SAM concentrations were associated with higher fasting insulin (9.5% 95% CI (0.3; 19.5) per SD increase in SAM, $p = 0.04$), HOMA-IR (10.8% (0.8; 21.9), $p = 0.03$) and TNF-α (11.8% (5.0; 19.0), $p < 0.001$).

Conclusion: We found little evidence for associations between SAM:SAH ratio and cardiometabolic variables, but higher plasma concentrations of SAM, SAH and Hcy are related to an overall higher risk of metabolic dysfunctions.

The studies were registered at www.clinicaltrials.gov (NCT01719913 & NCT01731366).

Abbreviations: 3G, Gut, Grain and Greens; ALAT, alanine aminotransferase; ASAT, aspartate aminotransferase; Hcy, Homocysteine; SAH, s-adenosylhomocysteine; SAM, s-adenosylmethionine.

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Introduction

Metabolic syndrome, a cluster of several risk markers including abdominal obesity, dyslipidemia, hypertension and hyperglycemia, is an important public health challenge worldwide due to the increase in risk of developing type 2 diabetes (T2D) and cardiovascular diseases (CVD) and all-cause mortality [1]. Metabolic syndrome is also associated with chronic low-grade inflammation, insulin resistance and non-alcoholic fatty liver disease (NAFLD) [1]. Another marker that is frequently related to CVD and all-cause mortality is homocysteine (Hcy) [2]. It is suggested that Hcy and metabolic syndrome markers often go together and that Hcy could be an additional marker for metabolic syndrome [3–5].

Elevated plasma levels of Hcy may be both a cause and/or a consequence of the metabolic syndrome as Hcy has been linked with insulin resistance because hyperglycemia and hyperinsulinemia might affect the activity of enzymes involved in Hcy turnover [6,7]. Additionally, Hcy has been linked to impaired pancreatic β-cell function and might affect insulin signaling in peripheral tissue [8–11]. Another link between plasma concentrations of Hcy and metabolic syndrome is through its role in one-carbon metabolism and epigenetics [12]. Hcy is linked to one-carbon metabolism and epigenetics through the s-adenosylmethionine (SAM) dependent methylation of DNA which results in s-adenosylhomocysteine (SAH), which can be metabolized further to Hcy. Hcy can then be remethylated to methinonine which can be converted to SAM thus completing the methyl donor cycle. Due to this closely linked metabolism, SAM, SAH and the SAM:SAH ratio (sometimes referred to as the methylation potential or capacity index [13]) have been suggested as markers of CVD risk [14,15].

Even though our understanding of the role of Hcy, one-carbon metabolism, epigenetic regulation and the development of metabolic syndrome has improved, few studies have examined this in cohorts with detailed cardiometabolic phenotyping. There is a need for improving our understanding of the role of SAM, SAH and methylation capacity in metabolic syndrome and their link to clinical metabolic characteristics. Therefore, the aim of the present study was to investigate the relationship between plasma one-carbon metabolites including fasting plasma levels of Hcy and the metabolic syndrome.

Methods

Study design

This cross-sectional study is based on baseline data from two dietary intervention studies [16]. Data from a total of 118 participants were used for these analyses. The studies were registered at http://www.clinicaltrials.gov (NCT01719913 & NCT01731366) and approved by the Research Ethics Committees of the Capital Region of Denmark in accordance with Helsinki declaration (H-2-2012-064 & H-2-2012-065) and the Danish Data Protection Agency (2012-54-0170 & 2007-54-0269). The study design has been described in detail by Ibrügger et al. [16].

Participants

The study participants for both studies comprised of men and women aged 20-65 years residing in the Greater Copenhagen area. Participants had to be weight stable and exhibit a metabolic risk profile [16]. The inclusion criteria were a BMI of 25-35 kg/m^2 and/or waist circumference ≥94 cm for men and ≥80 cm for women and furthermore at least one metabolic syndrome risk marker. Further details on inclusion and exclusion criteria and metabolic syndrome score is described in detail in supplemental method material.

Anthropometric, laboratory and analytical procedures

Standard anthropometric measurements were conducted for weight, height, waist circumference (WC), sagittal abdominal diameter (SAD) and blood pressure as well as body composition with bio electrical impedance (Supplemental methods section).

Blood samples were drawn after a ≥10 h overnight fast. Prior to blood sampling subjects were lying down for 10 min. The blood was drawn via an intravenous cannula in the elbow crease and separated into plasma and cells within 30 min after collection. Standard clinical measurements of serum alanine aminotransferase (ALAT), aspartate aminotransferase (ASAT), total-, LDL- and HDL-cholesterol, triglycerides, C-reactive protein (CRP), free-fatty acids (FFA), insulin, C-peptide, plasma glucose and whole blood glycated hemoglobin (HbA1c) were performed as well as measurements of serum inflammatory markers IL-6 and TNF-α(Supplemental methods section).

Plasma vitamin B_{12}, total homocysteine, SAM and SAH measurements

Plasma vitamin B_{12} was determined by a chemiluminescent assay (Architect, Abbott Laboratories, USA). Plasma total Hcy was quantified by a competitive immunoassay (Architect, Abbott Laboratories, USA). A tandem mass spectrometry was used for determining SAM and SAH in plasma, as described previously with only minor differences [17]: 100 μL was used, and a more sensitive tandem mass spectrometer was used (API5000 instead of API3000). CV% for quality control samples was between 2 and 5%.

Statistical analysis

Baseline descriptive population data are expressed as means ± SD or median (25th;75th percentile). All statistical tests were performed using the R statistical environment (http://cran.r-project.org/, version 3.1.3). Results were considered significantly different at $P < 0.05$, and trends reported if $P < 0.1$–0.05.

Using a ANCOVA model we tested whether fasting concentrations of SAM, SAH, Hcy, and SAM:SAH ratio and fasting plasma vitamin B_{12} concentration differed according

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