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Serum homocysteine is not independently associated with an atherogenic lipid profile: The Very Large Database of Lipids (VLDL-21) study

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ABSTRACT

Background and aims: Hyperhomocysteinemia is an independent risk factor for cardiovascular disease, but the mechanism for this risk remains unclear. While reducing serum total homocysteine (tHcy) has been shown to decrease strokes, there is no evidence for an effect on myocardial infarctions in randomized controlled trials. This study aims to examine the relationship between tHcy and several lipid measures.

Methods: Our analyses included 18,297 U.S. adults from the Very Large Database of Lipids who had an extended lipid panel including direct measurement of triglycerides (TG), and the cholesterol concentration of low-density lipoprotein (LDL-C), high-density lipoprotein (HDL-C), non-HDL-C, very low-density lipoprotein (VLDL-C), and remnant-lipoprotein cholesterol (RLP-C: IDL-C + $VLDL_3$-C). Additional measurements were tHcy, hemoglobin A1c (HbA1c), insulin, creatinine, and blood urea nitrogen (BUN). Subjects were categorized into tHcy quartiles. Linear regression models were performed using lipids and tHcy as dependent and independent variables respectively, and further adjusted with age, sex, HbA1c, insulin, creatinine, and BUN levels in multivariable regression.

Results: In unadjusted analysis, levels of LDL-C ($p < 0.001$), non-HDL-C ($p < 0.001$) and HDL-C ($p < 0.001$) were 7–10% lower whereas levels of TG ($p < 0.001$), VLDL-C ($p = 0.016$) and RLP-C ($p < 0.001$) were 2–6% higher in the highest tHcy quartile. These associations between tHcy levels and lipids were eliminated (p-value range: 0.101–0.750) when controlling for age, sex, HbA1c, insulin, creatinine, and BUN.

Conclusions: Although high levels of tHcy were associated with 2–6% higher TG-rich lipoproteins in unadjusted analysis, after adjustment for confounders our findings do not support the hypothesis that hyperhomocysteinemia is associated with an atherogenic lipid profile.

1. Introduction

High serum total homocysteine (tHcy) [1] has been implicated as an independent risk factor for cardiovascular disease (CVD) in numerous observational studies [2] with more pronounced risk among elderly persons [3], but the reason for this observed increased risk remains unclear. Concentrations of serum tHcy, an amino acid breakdown product readily detectable in blood, are increased by protein-rich diets and in persons with B vitamin deficiencies. From studies with animal and cell models, tHcy is thought to increase risk for cardiovascular disease through oxidative stress and endothelial cell dysfunction [4,5] leading to atherosclerosis [6–9]; by inducing secretion of cholesterol and apolipoprotein B100 [10–12]; and by inhibiting apolipoprotein A-I synthesis, and thus, high-density lipoprotein cholesterol (HDL-C) [13].

While studies examining the association between hyperhomocysteinemia and lipid changes in humans have had mixed conclusions [14–16], the most consistent findings indicate that higher tHcy is associated with decreased serum HDL-C [13,17–20]. Despite the biological plausibility and disease associations in cross-

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sectional and case-control studies, there is no consensus evidence from *meta*-analyses of randomized controlled trials in humans that reducing serum tHcy levels through B vitamin supplementation reduces the risk of CVD [21–24]. Though the most recent meta-analysis concluded no benefit to tHcy lowering interventions [21], subgroup analyses of individual trials have shown reductions in risk of stroke with interventions that lower tHcy [25–28], as has a recent large clinical trial in China [29]. It is plausible that the benefit to CVD risk is exclusive to stroke [30], and with new data [29], existing *meta*-analyses must be interpreted with caution until updated. Additionally, primary outcomes may be obscured in trials that do not exclude those with reduced renal function or an inability to metabolically process B12 [31]. Thus, with inconsistent conclusions from trials it is not surprising that observational studies continue to find evidence for a link between high tHcy and CVD risk [32,33].

This discrepancy between randomized controlled trials and observational tHcy research may also be due to confounding variables that were unaccounted for in observational studies. There have been several studies finding an association between serum tHcy levels and glycemic status and kidney function [34–37]. As type II diabetes and poor glycemic control are risk factors for CVD [38], a strong association between hemoglobin A1c (HbA1c) or insulin resistance and tHcy may in part explain the observed link of tHcy with CVD risk. Similar confounding by kidney function may also exist, as tHcy is associated with higher serum creatinine [39], and CVD risk is notably increased in individuals with chronic kidney disease [40]. Thus, it may be critical when interpreting observational associations between elevated serum tHcy and an atherogenic lipid profile or CVD risk to adjust for glycemic status and kidney function.

With one exception [17], prior cross-sectional studies showing an association between high tHcy and a more atherogenic lipid profile, mainly lower HDL-C and higher low-density lipoprotein cholesterol (LDL-C), did not control for both glycemic status and kidney function [14–16,18–20,41–44]. Moreover, these studies used a standard lipid panel, without analyzing associations of serum tHcy and other important lipid measurements, such as remnant lipoprotein cholesterol (RLP-C) or LDL particle density. To better determine the relationship between high tHcy and dyslipidemia, our study sought to examine the distribution of several directly measured lipids by tHcy quartiles while controlling for age, sex, glycemic status, and kidney function.

2. Materials and methods

2.1. Database

We used the Very Large Database of Lipids (VLDL), a database collected from 1,340,614 adults in the United States who were referred for Vertical Auto Profile (VAP) ultracentrifugation analysis of their lipid profile from 2009 to 2011. The distribution of lipid values contained in the dataset closely matches the distribution in the National Health and Nutrition Examination Survey 2007 to 2008 [45]. For the primary analysis, we used a subset of 18,297 individuals from the VLDL database who had measurements of lipid fractions and tHcy in addition to age, sex, HbA1c, insulin, creatinine, and blood urea nitrogen (BUN). All laboratory measures were performed at Atherotech Diagnostics Laboratory in Birmingham, AL. Fasting status was not available.

2.2. Lipid measurements

Direct measurements of LDL-C, intermediate-density lipoprotein cholesterol (IDL-C), very low-density lipoprotein cholesterol (VLDL-C), and HDL-C were conducted using inverted rate zonal, single vertical spin, density gradient ultracentrifugation by the VAP technique [46]. A high level of accuracy in VAP testing was confirmed through split sample comparisons conducted yearly (2007–2012) with beta quantification at Washington University's Core Laboratory for Clinical Studies reference laboratory for lipoprotein analysis, St. Louis, MO. Triglycerides were measured with the Abbott ARCHITECT C-8000 system (Abbott Park, IL). LDL particle density was determined using the Logarithmic LDL Density Ratio (LLDR), which gives a ratio of dense-to-buoyant LDL subclasses. LLDR was calculated as described previously as $\ln[(LDL_3\text{-}C + LDL_4\text{-}C)/(LDL_1\text{-}C + LDL_2\text{-}C)]$ [47]. Higher values of LLDR indicate denser LDL, which is potentially more atherogenic. To convert lipoprotein values from mg/dL to mmol/L, multiply by 0.0259. To convert TG values from mg/dL to mmol/L, multiply by 0.0113. RLP-C was defined as IDL-C plus $VLDL_3$-C.

2.3. Homocysteine measurements

Total homocysteine in serum was analyzed using an enzymatic assay. The assay utilizes the Diazyme Homocysteine 2 Reagent (Diazyme Laboratories, Poway, CA) and Architect analyzer (Abbott Laboratories, Abbott Park, IL). In this assay, oxidized homocysteine (Hcy) is first reduced to free Hcy which then reacts with a co-substrate, S-adenosylmethionine (SAM), catalyzed by a Hcy S-methyltransferase to form methionine (the Hcy conversion product of Hcy) and S-adenosylhomocysteine (SAH, the co-substrate conversion product). SAH is assessed by coupled enzyme reactions including SAH hydrolase, adenosine (Ado) deaminase and glutamate dehydrogenase, wherein SAH is hydrolyzed into adenosine and Hcy by SAH hydrolase. The formed Hcy that is originated from the co-substrate SAM is cycled into the Hcy conversion reaction by Hcy S-methyltransferase. This forms a co-substrate conversion product based enzyme cycling reaction system with significant amplification of detection signals. The formed Ado is immediately hydrolyzed into inosine and ammonia. In the last step, the enzyme glutamate dehydrogenase (GLDH) catalyzes the reaction of ammonia with 2-oxoglutarate and NADH to form NAD^+. The concentration of Hcy in the sample is directly proportional to the amount of NADH converted to NAD^+ ($\Delta A_{340\ nm}$).

2.4. Hemoglobin A1c measurements

HbA1c was measured using two different methodologies. In the initial stage of data collection, an assay based on the AxSym chemistry analyzer and an immunoassay reagent (catalog # 3L93-20) both made by Abbott Diagnostics was used. However, methodology was changed on 07/20/2010 to one based on the Tosoh Automated Glycohemoglobin Analyzer and G8 Variant Elution Buffer No. 1 (S) (Ref# 0021956), No. 2 (S) (Ref#0021957), and No. 3 (S) (Ref# 021958). The analyzer and the buffers were both manufactured by the Tosoh Corporation. The Tosoh assay is traceable to the Diabetes Control and Complication Trial (DCCT) reference method. To convert HbA1c from % to mmol/mol multiply by 10.93 and subtract 23.5.

2.5. Insulin measurements

Insulin was measured using a chemiluminescent microparticle immunoassay (CMIA) principle. The ARCHITECT c System chemistry analyzer and Architect Insulin Reagent Kit Ref: 8K41, both made by Abbott Diagnostic Laboratory, were used for testing insulin. To convert insulin from μIU/L to pmol/L, multiply by 6.945.