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Research Paper

Elevated Plasma Levels of 3-Hydroxyisobutyric Acid Are Associated With Incident Type 2 Diabetes

Adil Mardinoglu [a,b,*], Silvia Gogg [c], Luca A. Lotta [d], Alena Stančáková [e], Annika Nerstedt [c], Jan Boren [c], Matthias Blüher [f], Ele Ferrannini [g], Claudia Langenberg [d], Nicholas J. Wareham [d], Markku Laakso [e], Ulf Smith [c,**]

[a] Science for Life Laboratory, KTH - Royal Institute of Technology, Stockholm, Sweden
[b] Department of Biology and Biological Engineering, Chalmers University of Technology, Gothenburg, Sweden
[c] Department of Molecular and Clinical Medicine, University of Gothenburg, Sahlgrenska University Hospital, Gothenburg, Sweden
[d] MRC Epidemiology Unit, University of Cambridge, Cambridge, UK
[e] Institute of Clinical Medicine, Internal Medicine, University of Eastern Finland, Kuopio University Hospital, Kuopio, Finland
[f] University of Leipzig, Department of Medicine, Leipzig, Germany
[g] CNR Institute of Clinical Physiology, Pisa, Italy

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ABSTRACT

Branched-chain amino acids (BCAAs) metabolite, 3-Hydroxyisobutyric acid (3-HIB) has been identified as a secreted mediator of endothelial cell fatty acid transport and insulin resistance (IR) using animal models. To identify if 3-HIB is a marker of human IR and future risk of developing Type 2 diabetes (T2D), we measured plasma levels of 3-HIB and associated metabolites in around 10,000 extensively phenotyped individuals. The levels of 3-HIB were increased in obesity but not robustly associated with degree of IR after adjusting for BMI. Nevertheless, also after adjusting for obesity and plasma BCAA, 3-HIB levels were associated with future risk of incident T2D. We also examined the effect of 3-HIB on fatty acid uptake in human cells and found that both HUVEC and human cardiac endothelial cells respond to 3-HIB whereas human adipose tissue-derived endothelial cells do not respond to 3-HIB. In conclusion, we found that increased plasma level of 3-HIB is a marker of future risk of T2D and 3-HIB may be important for the regulation of metabolic flexibility in heart and muscles.

1. Introduction

Insulin resistance (IR) is a common consequence of obesity and the major driver of the current global epidemic of type 2 diabetes (T2D) and associated diseases. However, not all obese individuals develop IR or T2D. Thus, we need reliable and easily measured biomarkers of IR and risk of future disease in order to initiate early clinical interventions (Mardinoglu and Nielsen, 2012). IR does not only include fat and carbohydrate metabolism but is also associated with amino acid metabolism. Circulating levels of branched-chain amino acids (BCAAs), including valine, leucine and isoleucine, are elevated in obese subjects and a distinctive metabolic signature related to BCAA catabolism has been revealed through metabolomics profiling of obese versus lean subjects (Newgard et al., 2009). Levels of BCAAs as well as aromatic amino acids, including tyrosine and phenylalanine, also predict future development of T2D independent of age, sex, body mass index (BMI) and family history (Wang et al., 2011). However, mechanistic explanations for how increased BCAAs can cause insulin resistant states remain unclear.

A previous study by Jang et al. (2016) has identified 3-hydroxyisobutyrate (3-HIB), a valine metabolite and derived from 3-hydroxyisobutyryl-coenzyme A by HIBC hydrolase (encoded by *Hibch*) (Fig. 1A) as a paracrine regulator of trans-endothelial fatty acid transport in the skeletal muscle in mice. The authors showed that 3-HIB, secreted from muscle cells, enhances their fatty acid uptake leading to increased lipid accumulation and induction of whole-body IR. Inhibiting synthesis of 3-HIB reduced fatty acid uptake by skeletal muscle microvascular endothelial cells and prevented IR.

However, before concluding that 3-HIB also can contribute to human disease, the findings need to be validated in man. The human translation of the experimental data in mice was less convincing. 3-HIB levels were elevated in the muscle of diabetic db/db mice but only in three of the

* Correspondence to: A. Mardinoglu, Science for Life Laboratory, KTH - Royal Institute of Technology, Stockholm, Sweden.
** Correspondence to: U. Smith, Department of Molecular and Clinical Medicine, University of Gothenburg, Sahlgrenska University Hospital, Gothenburg, Sweden.
E-mail addresses: adilm@scilifelab.se (A. Mardinoglu), silvia.gogg@medic.gu.se (S. Gogg), luca.lotta@mrc-epid.cam.ac.uk (L.A. Lotta), alena.yaluri@uef.fi (A. Stančáková), annika.nerstedt@gu.se (A. Nerstedt), Jan.Boren@wlab.gu.se (J. Boren), Matthias.Blueher@medizin.uni-leipzig.de (M. Blüher), ferranni@ifc.cnr.it (E. Ferrannini), Claudia.Langenberg@mrc-epid.cam.ac.uk (C. Langenberg), nick.wareham@mrc-epid.cam.ac.uk (N.J. Wareham), markku.laakso@uef.fi (M. Laakso), ulf.smith@medic.gu.se (U. Smith).

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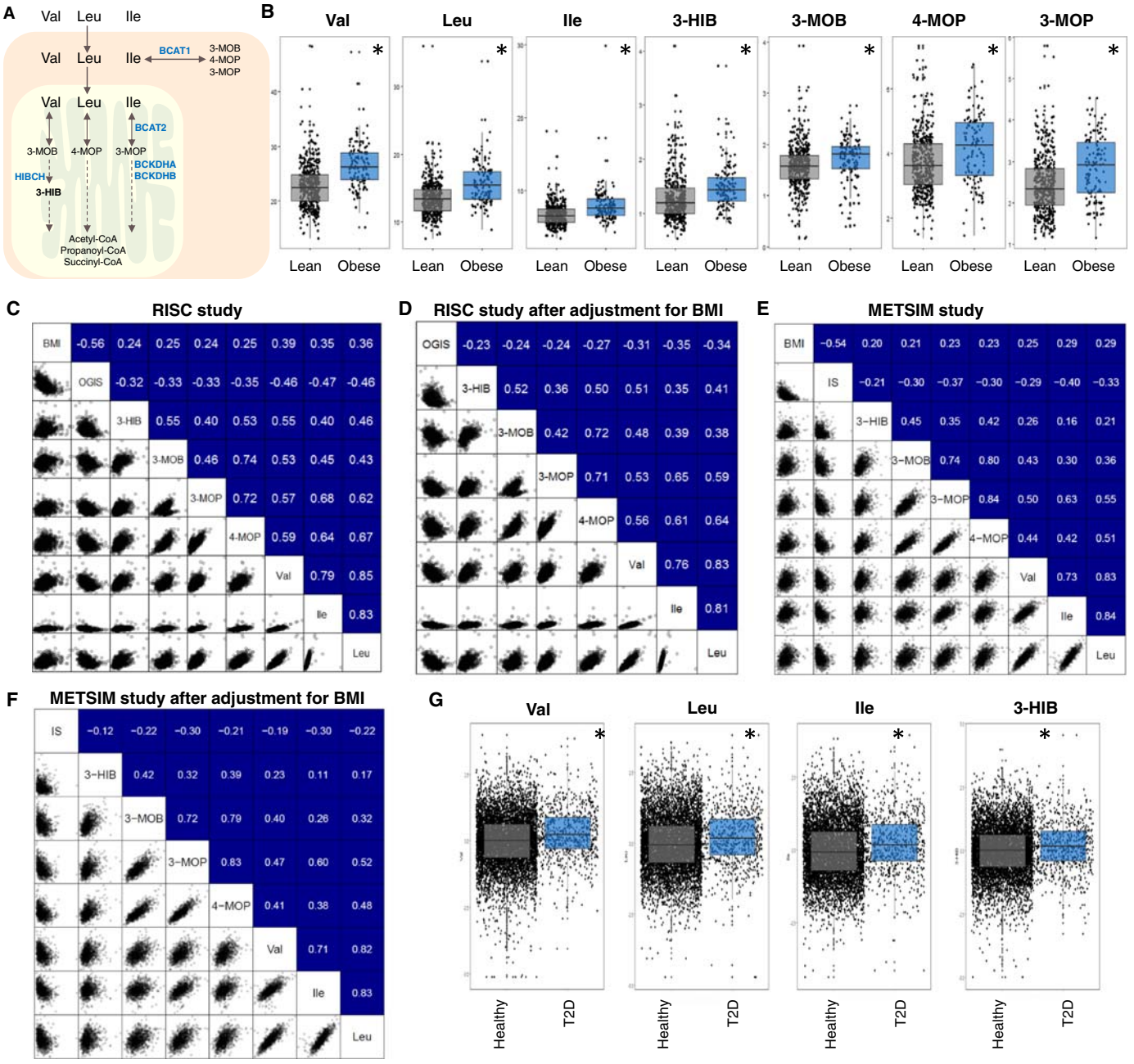

Fig. 1. Plasma levels of 3-HIB are strongly related to BMI. A) Catabolism of branched-chain amino acids (BCAAs) including valine (val), leucine (leu) and isoleucine (Ile). B) Plasma levels of metabolites involved in BCAA catabolism in lean and obese subjects in RISC study. The Spearmen correlation of BMI, OGIS and the plasma levels of metabolites involved in BCAA metabolism before (C) and after eliminating the effect of BMI (D) in the RISC study. Correlation of BMI, IS and the plasma levels of metabolites involved in BCAA metabolism before (E) and after eliminating the effect of BMI (F) in the METSIM study. G) The plasma level of the metabolites involved in BCAA metabolism in healthy subjects and T2D patients in the EPIC-Norfolk study. (*P-value < 0.05).

seventeen analyzed human T2D subjects compared to non-diabetic individuals (Jang et al., 2016). Furthermore, a positive effect of 3-HIB on fatty acid uptake in human cells was only shown in HUVEC cells which are of macrovascular, rather than tissue-related microvascular origin. Furthermore, to be of clinical predictive value we need to understand if plasma levels of 3-HIB can be used as a risk indicator of IR and future development of T2D in man. If so, *Hibch* can be a target for developing efficient treatment strategies for T2D. To examine this, we analyzed 3-HIB plasma levels in around 10,000 human subjects and their relation to future development of T2D. We also examined the effect of 3-HIB on fatty acid uptake by human microvascular endothelial cells from the adipose tissue and heart as well as by HUVEC macrovascular cells.

2. Materials and Methods

2.1. Plasma Metabolomics Data

The subjects included in this study have been described previously (Lee et al., 2016; Mardinoglu et al., 2017). Signed informed consent has been obtained from human subjects in all cohorts, and the study protocol has been approved by the relevant ethical committees. The studies were conducted according to standards indicated by the Declaration of Helsinki.

Measurement of plasma levels of metabolites involved in BCAA metabolism was performed using LC-MS. Briefly, the liquid chromatography-tandem mass spectrometry (LC-MS/MS) platform

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