Cell Metabolism, Volume *26*

Supplemental Information

Causes, Characteristics, and Consequences of Metabolically Unhealthy Normal Weight in Humans

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Supplemental Information

CONTACT FOR REAGENT AND RESOURCE SHARING

Detailed information and requests for the methods of phenotyping that were used should be directed to and will be fulfilled by the corresponding author Norbert Stefan **(norbert.stefan@med.uni-tuebingen.de).**

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Study cohort

Nine hundred and eighty one adults, years of age 45.0 ± 12.8 (mean ± SD), 613 females, 368 males. The study protocol was approved by the Medical Ethics Committee of the University of Tübingen, Germany. All subjects signed written informed consent forms.

Data of subjects who participated in the ongoing Tübingen Family Study and the Tübingen Lifestyle intervention Program (Stefan *et al*., 2008; Stefan *et al*. 2014-2; Stefan *et al*., 2015) were investigated. Subjects were included into these studies when their risk of cardiometabolic diseases was increased based on the following criteria: a family history of type 2 diabetes, a BMI >27 $kg \cdot m^{-2}$, previous gestational diabetes in women, prediabetes or NAFLD. Thus, more overweight and obese subjects were studied, resulting in that these data may not be representative for the general population. However, there is large agreement in the scientific community that more precise phenotyping methods of cardiometabolic risk should only be done in subjects who are at increased risk of cardiometabolic diseases. Participants were considered healthy according to results of a physical examination and routine laboratory tests.

METHOD DETAILS

Oral glucose tolerance test

All individuals underwent a 75-g oral glucose tolerance test (OGTT). We obtained venous plasma samples at 0, 30, 60, 90, and 120 minutes for determination of plasma glucose and insulin levels. We measured insulin sensitivity (IS) from the OGTT as proposed by Matsuda and DeFronzo ([10,000 / √(Insmean·Glucmean·Ins0·Gluc0)]) (Matsuda *et al*., 1999). Insulin secretion was determined by calculation of the insulinogenic index (IGI; insulin at 30 min-insulin at 0 min)/(glucose at 30 min-glucose at 0 min). The disposition index (DI), an estimate of beta cell function relative to insulin sensitivity, was calculated as the product of insulin sensitivity and insulin secretion.

Body fat mass and distribution and liver fat content

Measurements of body fat mass and distribution was performed by an axial T1-weighted fast spin echo technique with a 1.5 T whole-body magnetic resonance imager (Machann *et al.,* 2010; Stefan *et al.,* 2014-2). Whole-body imaging for the determination of adipose tissue distribution was performed by using an axial T1-weighted fast spin-echo technique with the following parameters: echo time of 12 msec, repetition time of 490 msec, 10-mm-thick sections, 10-mm gap between sections, 256 × 178 matrix, 450–530-mm field of view depending on the size of the subject, and five sections per acquisition. The acquisition time was 12 seconds, which enabled breath-hold examinations in the abdominal region. A body coil was used as the transmit-receive coil. Volunteers were imaged in the prone position with arms extended. The table shift after each acquisition was 10 cm, and images were recorded from fingers to toes with a total acquisition time of approximately 25 minutes. Liver fat content was measured by localized proton magnetic

resonance (^{1}H-MR) spectroscopy (Machann *et al.,* 2010; Stefan *et al.*, 2014-2). MR spectroscopy was performed in the posterior part of segment VII of the liver by using a single-voxel stimulated-echo acquisition mode localization technique with a short echo time (10 msec). Acquisition parameters were as follows: 15-msec mixing time, 4-second repetition time, 3 × 3 × 2-cm3 volume of interest, and 32 acquisitions without water suppression. Data from MR spectroscopy were acquired in expiration to minimize breathing effects by using a single element of the spine array coil. Water and lipid signal intensity (methylene and methyl signals) were integrated, and the ratio of lipid to water signal intensity was calculated without T2 correction owing to the short echo time and long repetition time. The complete liver protocol, including morphologic imaging, shimming, and spectroscopy, took about 10 minutes. NAFLD was defined as liver fat content >5.56 % (Szczepaniak *et al.*, 2005).

The carotid intima-media thickness

The carotid intima-media thickness (cIMT) was measured in the fasting state using a high-resolution ultrasound system (AU5 idea, Esaote Biomedica, Munich, Germany) with an integrated electrocardiography (ECG) package as previously described (Balletshofer *et al.*, 2005).

Cardiorespiratory fitness

For the measurement of cardiorespiratory fitness the maximal aerobic capacity (VO_{2max}) was determined. The individuals underwent a continuous, incremental exercise test to volitional exhaustion using a cycle ergometer. The cycle ergometer test was performed on an electromagnetically braked cycle ergometer (Ergometrics 800 S; Ergoline, Bitz, Germany).

Oxygen consumption was measured using a spiroergometer (MedGraphics System Breese Ex 3.02 A; MedGraphics) (Kantartzis *et al.*, 2009).

Biochemical analyses

Blood glucose was determined using a bedside glucose analyzer (YSI, Yellow Springs, CO). Plasma insulin was determined on an ADVIA Centaur XP and all other routine parameters on an ADVIA 1800 clinical chemistry system (Siemens Healthcare systems, Erlangen, Germany). Plasma triglycerides, total cholesterol, HDL and LDL cholesterol were measured using the ADVIA 1800 clinical chemical analyser.

QUANTIFICATION AND STATISTICAL ANALYSIS

Data that were not normally distributed (Shapiro-Wilk W test; e.g. cIMT, insulin sensitivity, body fat mass, liver fat, content) were logarithmically transformed. Differences between groups were tested using χ^2-test or linear correlations. To adjust the effects of covariates and identify independent relationships, stepwise and multivariate linear regression analyses were used. Principal component analyses were performed to visualize the complex phenotypic relationships and to identify common phenotypic patterns. The statistical software package JMP 11.0 (SAS Institute Inc, Cary, NC, USA) was used.

Table S1

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Body fat composition and liver fat content		
T1-weighted fast spin echo	Machann *et al.*, 2010	**N/A**
^{1}H-MR spectroscopy	Machann et al., 2010	**N/A**
Software and Algorithms		
JMP 11.0	SAS institute	https://www.jmp.com/en_us/home.html
Insulin sensitivity	Matsuda *et al.*, 1999	http://mmatsuda.diabetes-smc.jp/english.html
Other		
Measurements of outcome variables	This paper	Figures 2 and 3, Table

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