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ARTICLE

Molecular Biology

Metabolic profiling of tissue-specific insulin resistance in human obesity: results from the Diogenes study and the Maastricht Study

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Abstract

Background Recent evidence indicates that insulin resistance (IR) in obesity may develop independently in different organs, representing different etiologies toward type 2 diabetes and other cardiometabolic diseases. The aim of this study was to investigate whether IR in the liver and IR in skeletal muscle are associated with distinct metabolic profiles.

Methods This study includes baseline data from 634 adults with overweight or obesity (BMI ≥ 27 kg/m^2) (≤65 years; 63% women) without diabetes of the European Diogenes Study. Hepatic insulin resistance index (HIRI) and muscle insulin sensitivity index (MISI), were derived from a five-point OGTT. At baseline 17 serum metabolites were identified and quantified by nuclear-magnetic-resonance spectroscopy. Linear mixed model analyses (adjusting for center, sex, body mass index (BMI), waist-to-hip ratio) were used to associate HIRI and MISI with these metabolites. In an independent sample of 540 participants without diabetes (BMI ≥ 27 kg/m^2; 40–65 years; 46% women) of the Maastricht Study, an observational prospective population-based cohort study, 11 plasma metabolites and a seven-point OGTT were available for validation.

Results Both HIRI and MISI were associated with higher levels of valine, isoleucine, oxo-isovaleric acid, alanine, lactate, and triglycerides, and lower levels of glycine (all $p < 0.05$). HIRI was also associated with higher levels of leucine, hydroxyisobutyrate, tyrosine, proline, creatine, and n-acetyl and lower levels of acetoacetate and 3-OH-butyrate (all $p < 0.05$). Except for valine, these results were replicated for all available metabolites in the Maastricht Study.

Conclusions In persons with obesity without diabetes, both liver and muscle IR show a circulating metabolic profile of elevated (branched-chain) amino acids, lactate, and triglycerides, and lower glycine levels, but only liver IR associates with lower ketone body levels and elevated ketogenic amino acids in circulation, suggestive of decreased ketogenesis. This knowledge might enhance developments of more targeted tissue-specific interventions to prevent progression to more severe disease stages.

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Introduction

The obesity pandemic has reached alarming proportions with an expected 3.3 billion overweight or obese persons worldwide (i.e., 58% of the world's adult population) by 2030 [1]. Overweight and obesity are key risk factors for the development of cardiometabolic disorders, such as type 2 diabetes and cardiovascular disease [2]. However, there is considerable heterogeneity in cardiometabolic risk among obese persons. In the past decade it has become increasingly clear that persons with similar fat mass may present with completely distinct clinical metabolic profiles (e.g., either low or high triglycerides, systemic inflammation, ectopic fat, and/or insulin sensitivity) [3].

Insulin resistance (IR) plays a major role in the pathogenesis of cardiometabolic disorders and is a common consequence of excess body fat, likely through the increased circulation of nonesterified fatty acids and the accumulation of ectopic lipids [4–6]. IR can manifest within different tissues (e.g., liver, skeletal muscle, adipose tissue) and impaired insulin signaling in the affected tissues leads to a multitude of cardiometabolic effects. For instance, impaired insulin signaling in the liver causes increased or less-suppressed hepatic glucose production, while IR in skeletal muscle results in less efficient muscle glucose uptake [4, 7]. IR may develop at a different rate or to a different degree in different tissues, thereby introducing heterogeneity within the cardiometabolic profile. In line with this, a recent small study was able to identify individuals with overweight, obesity or nonsevere type 2 diabetes who had impairments in glucose metabolism either predominantly localized in the liver or in the muscle [8]. Furthermore, some evidence exists that metabolic tissues differ in their likelihood to respond to interventions aimed at increasing insulin sensitivity. For example, metformin and a low-fat, high-complex-carbohydrate diet might be more efficient in treating liver IR, while physical activity and a Mediterranean or Paleolithic diet might more potently improve muscle insulin sensitivity [9–11].

The characterization of the metabolic profile of persons with obesity and IR has undergone a boost by the development of new metabolomics techniques. Metabolomics is the comprehensive characterization of metabolites, including small-molecule intermediates and products of metabolism. Several metabolomics studies have reported that higher levels of branched-chain amino acids (BCAA), aromatic amino acids, acylcarnitines, lactate, glycolytic intermediates, triglycerides, and long-chain fatty acids, as well as lower levels of glycine, betaine, tricarboxylic acid cycle intermediates, ketone bodies, and lysophosphatidylcholines are associated with or predict the development of IR and (pre)diabetes [12–15]. Notably, although many metabolites have been identified in relation to whole-body IR, the metabolic profile related to tissue-specific IR has not yet been investigated. More knowledge on the metabolic profiles of distinct tissue-specific IR or prediabetic phenotypes, may give leads for more targeted interventions in the early stages of cardiometabolic disease development.

Therefore, the aim of the current study was to examine in individuals without diabetes, but with overweight or obesity, whom are at risk for cardiometabolic disease, whether liver IR and muscle insulin sensitivity are associated with distinct metabolic profiles.

Subjects and methods

Study design and sample selection

The Diogenes Study is a multicenter, randomized, controlled dietary intervention study involving eight European cities (ClinicalTrials.gov: NCT00390637). In total, 938 caucasian adults (18–65 years) with overweight or obesity (body mass index (BMI ≥ 27 kg/m^2)) were recruited and completed the baseline examination between February 2006 and December 2007. Subjects were free of diabetes (blood fasting glucose <6.1 mmol/L) and cardiovascular disease. More details on recruitment, inclusion and exclusion criteria, design and study procedures have been described previously [16]. Local ethics committees approved the study and all participants gave written informed consent. For the current study we used baseline data from individuals with available information on metabolomics ($n = 752$) and tissue-specific IR, as estimated from OGTT ($n = 634$).

To validate our findings we used data from the Maastricht Study, an observational prospective population-based cohort study with extensive phenotyping. Eligible for participation were all individuals (40–75 years) living in the southern part of the Netherlands, with an oversampling of individuals with type 2 diabetes. The study has been approved by the institutional medical ethical committee (NL31329.068.10) and the Minister of Health, Welfare and Sports of the Netherlands (Permit 131088-105234-PG). All participants gave written informed consent. More details on recruitment, inclusion and exclusion criteria, design and study procedures have been described previously [17]. Currently, cross-sectional data from the first 3451 participants who completed the baseline examination between November 2010 and September 2013 are available. For the purpose of the current paper, to be comparable to the Diogenes Study population, we only selected participants with a BMI ≥ 27 kg/m^2, an age ≤65 years, without diabetes or cardiovascular disease ($n = 663$) and with available data on metabolomics and tissue-specific IR ($n = 540$).

Tissue-specific insulin resistance

Participants of the Diogenes Study underwent a standard five-time point OGTT (0, 30, 60, 90, 120 min), while in the Maastricht Study a seven-time point OGTT was performed (0, 15, 30, 45, 60, 90, 120 min). After an overnight fast, participants ingested a load of 75 g glucose dissolved in 300 ml water within a 5-min period. Venous blood samples were obtained at the different time-points and plasma and serum were stored at −80 °C until analysis of glucose and insulin concentrations. Detailed descriptions of blood collection and laboratory analyses of both studies are given in the Supplementary Information.

The magnitude of the rise in blood glucose and insulin concentrations immediately (0–30 min) following the glucose load is thought to reflect the (in)ability of insulin to suppress hepatic endogenous glucose production. Therefore, the hepatic insulin resistance index (HIRI) was calculated as the product of the area under curves (AUCs) for glucose and insulin during the first 30 min of the OGTT—i.e., glucose0–30[AUC in mmol/L h] × insulin0–30[AUC in pmol/L h]. HIRI has been developed and validated against the product of fasting plasma insulin and endogenous glucose production in clamp studies [18].

The decline in blood glucose concentration after 60 min primarily reflects glucose uptake by peripheral tissues, mainly skeletal muscle. Therefore, the muscle insulin sensitivity index (MISI) was calculated as the rate of decay of glucose concentration during the OGTT divided by the mean insulin concentration during the OGTT in umol/L/min/pmol/L. The rate of decay was calculated as the slope of the least square fit to the decline in glucose concentration from peak to nadir. MISI was developed and validated against the rate of peripheral insulin-mediated glucose disposal measured with the euglycemic insulin clamp [18].

For descriptive purposes, participants were classified into groups according to the presence or absence of liver and/or muscle IR by creating tertiles of HIRI and MISI, as done before [10, 15]. The highest tertile of HIRI represented individuals with liver IR; the lowest tertile of MISI represented individuals with muscle IR. Accordingly, participants were categorized in one of four groups: (1) no IR; (2) IR in liver only; (3) IR in muscle only; (4) IR in both muscle and liver.

Limited information was available on adipose tissue IR, which might be associated with a specific metabolite profile as well. In the Diogenes Study, the adipose tissue IR index (ATIRI) was calculated as fasting insulin × fasting NEFA (available for $n = 552$). No information on adipose tissue IR was available in the Maastricht Study.

NMR-based metabolite profiling

In the Diogenes Study, 18 low-molecular-weight metabolites in serum were quantified from nuclear-magnetic-resonance (NMR) spectra, including the BCAA valine, isoleucine, leucine, and their metabolites oxo-isovaleric acid and hydroxyisobutyrate; other amino acids alanine, tyrosine, proline, and glycine; glycolysis-related metabolites glucose and lactate; ketone bodies acetoacetate and 3-OH-butyrate; and other metabolites creatinine, creatine, n-acetyl, acetate, and triglycerides. Glucose was not further analyzed because of the incorporation of glucose in our IR outcome measures. The NMR acquisition and quantification protocols are given in the Supplementary Information.

In the Maastricht Study metabolites were quantified in EDTA plasma samples using the Nightingale Health Ltd (Helsinki, Finland) targeted high-throughput NMR metabolomics platform. Out of the 17 circulating metabolites measured in the Diogenes Study, the following 11 circulating metabolites were available for validation: valine, isoleucine, leucine, alanine, tyrosine, lactate, acetoacetate, 3-OH-butyrate, creatinine, acetate, and triglycerides. Details of the experimentation and epidemiological applications of the NMR metabolomics platform have been described previously [19].

Covariates

Covariates in both studies included sex, BMI, waist-to-hip ratio (WHR), and in the Diogenes Study also study center. Weight and height were measured without shoes and only wearing light clothing. Waist circumference was measured midway between the lower rib and iliac crest following normal expiration. Hip circumference was measured as the largest circumference in the area around the buttocks. BMI was calculated as weight in kilograms divided by height in meters squared. WHR was calculated as waist circumference divided by hip circumference. Measurements of other descriptive variables can be found in the Supplementary Information.

Statistical analyses

Baseline characteristics were compared, for men and women separately, across tissue-specific IR groups using analysis of variance for normally distributed variables and Kruskal–Wallis test for non-normally distributed variables. Subsequently, all metabolites, HIRI, and MISI were ln-transformed to normalize distributions and standardized to be able to compare effect sizes across metabolites. Separate per-metabolite linear mixed model analyses with metabolite as dependent variable, HIRI or MISI as independent variable (=fixed effect) and study center as random effect were

performed. Next, analyses were adjusted for sex, BMI, and WHR (included as fixed effects). Subsequently, analyses on HIRI were additionally adjusted for MISI and analyses on MISI were adjusted for HIRI to assess the independent associations of HIRI/MISI with metabolites. Sex interactions in the association of MISI or HIRI with each of the metabolites were also tested by including HIRI × sex and MISI × sex interaction terms as fixed effects in the models. For validation purposes, comparable linear regression models were conducted in the Maastricht study.

Results

Participant characteristics

Diogenes study

Clinical characteristics of the participants are shown in Table 1. In total, 333 persons showed no IR, 88 persons presented with muscle IR only, 103 persons showed liver IR only, and 110 persons were in the group of combined muscle and liver IR. Persons with liver IR were most often men (58.3% men), while persons with muscle IR were most often women (76.1% women, $p < 0.001$). HIRI was higher in men (567 ± 383) compared with women (406 ± 312, $p < 0.001$), while MISI did not differ between men (0.15 ± 0.13) and women (0.14 ± 0.13, $p = 0.47$). Correlations between HIRI and MISI were moderate and comparable in men (Spearmen $r = -0.53$) and women (Spearman $r = -0.47$). Most metabolite levels differed between men and women (Supplementary Table S1). Spearman correlations between HIRI, MISI, HOMA2-IR, and all circulating metabolites can be found in the Supplementary Fig. S1.

Characteristics across tissue-specific IR groups, separated by sex, are shown in Table 2. Women with liver IR were more (abdominally) obese compared with women with no IR or muscle IR. Women with both liver and muscle IR had the lowest high-density lipoprotein cholesterol, highest triglyceride, and C-reactive protein levels. In contrast, groups did not differ on any of these characteristics in men, except for higher WHR in liver IR. In both men and women, those with liver IR showed the highest levels of fasting glucose, fasting insulin, 30-min insulin, HOMA-IR, and HIRI, while men and women with muscle IR showed the highest 2-h glucose levels and the lowest MISI.

The Maastricht study

Clinical characteristics of the selected Maastricht Study participants are also shown in Table 1. HIRI was higher in men (605 ± 389) than in women (529 ± 375, $p < 0.001$), while MISI did not differ between men (0.14 ± 0.10) and women (0.14 ± 0.12, $p = 0.61$). HIRI and MISI levels, as well as Spearman correlations between HIRI and MISI were comparable to those found in the Diogenes Study ($r = -0.57$ in men and $r = -0.45$ in women).

Table 1 Clinical characteristics of the Diogenes Study and the Maastricht Study participants.

	Diogenes Study ($N = 634$)	The Maastricht Study ($N = 540$)
Age (years)	41.6 (6.2)	55.7 (6.5)
Women	392 (61.8)	238 (44.1)
Body mass index (kg/m^2)	34.5 (4.9)	29.7 (3.0)
Obesity (BMI ≥ 30 kg/m^2)	513 (80.9)	182 (33.8)
Waist-to-hip ratio	0.93 (0.09)	0.95 (0.08)
Central obesity[a]	569 (89.7)	393 (72.9)
Triglycerides (mmol/L)	1.3 (0.9–1.7)	1.3 (1.0–1.8)
HDL cholesterol (mmol/L)	1.2 (0.3)	1.4 (0.4)
LDL cholesterol (mmol/L)	3.1 (0.9)	3.5 (1.0)
Total cholesterol (mmol/L)	4.9 (1.0)	5.6 (1.0)
Lipid-modifying medication (%)	1 (0.2)	107 (19.9)
Systolic blood pressure (mmHg)	126 (15)	136 (15)
Diastolic blood pressure (mmHg)	78 (11)	80 (9)
Antihypertensive medication (%)	4 (0.6)	160 (29.7)
Fasting glucose (mmol/L)	5.1 (0.6)	5.5 (0.5)
2-h glucose (mmol/L)	6.6 (2.1)	6.2 (1.7)
Fasting insulin (pmol/L)	68 (45–101)	77 (53–107)
HOMA2-insulin resistance	1.3 (0.9–1.9)	1.7 (1.2–2.4)
Hepatic insulin resistance index ([mmol/L] h × [pmol/L] h)	374 (254–562)	479 (317–698)
Muscle insulin sensitivity index (umol/L/min/pmol/L)	0.12 (0.07–0.19)	0.11 (0.07–0.18)

Data are presented as N (%) for dichotomous variables, mean (SD) for normally distributed continuous variables or as median (IQR) for non-normally distributed continuous variables.

[a]Central obesity is defined as a waist circumference ≥102 cm in men or ≥88 cm in women.

HIRI is associated with profound metabolic alterations, including low ketone body levels

In the Diogenes Study, HIRI was associated with elevated serum levels of valine, leucine, isoleucine, oxo-isovaleric acid, hydroxyisobutyrate, alanine, tyrosine, proline, lactate, creatine, and triglycerides, and with lower serum levels of glycine, acetoacetate, and 3-OH-butyrate. Adjustment for sex, BMI, and WHR attenuated associations, but all remained significant (Fig. 1; Supplementary Table S3). Additional adjustment for MISI hardly affected the strength

Table 2 Sex-stratified clinical characteristics of the Diogenes Study participants across tissue-specific insulin resistance groups.

	Men ($N = 242$)					Women ($N = 392$)				
	No IR	Liver IR, no muscle IR	Muscle IR, no liver IR	Muscle + liver IR	p^a	No IR	Liver IR, no muscle IR	Muscle IR, no liver IR	Muscle + liver IR	p^a
N (%)	108 (44.6)	60 (24.8)	21 (8.7)	53 (21.9)		225 (57.4)	43 (11.0)	67 (17.1)	57 (14.5)	
Age (years)	43.1 (5.7)	41.4 (5.7)	44.2 (4.8)	42.8 (6.2)	0.18	41.0 (6.3)	41.0 (7.3)	40.8 (6.1)	40.8 (6.1)	0.99
Body mass index (kg/m^2)	34.0 (4.9)	34.4 (4.2)	35.4 (5.6)	35.0 (4.5)	0.40	34.1 (5.0)	35.6 (5.3)	34.5 (4.6)	35.7 (5.2)	0.07
Waist circumference (cm)	112.8 (12.9)	114.6 (10.5)	117.3 (13.9)	116.6 (12.4)	0.19	102.2 (11.3)	108.4 (11.8)	104.4 (11.7)	110.1 (13.7)	<0.001
Hip circumference (cm)	112.4 (10.2)	113.5 (8.7)	114.2 (9.5)	113.5 (9.9)	0.78	118.1 (10.7)	121.2 (12.0)	119.2 (11.1)	119.3 (12.2)	0.38
Waist-to-hip ratio	1.00 (0.06)	1.01 (0.05)	1.03 (0.06)	1.03 (0.05)	0.04	0.87 (0.07)	0.90 (0.06)	0.88 (0.06)	0.92 (0.08)	<0.001
Sagittal diameter (cm)	26.6 (3.9)	26.9 (3.5)	27.5 (5.0)	26.8 (3.8)	0.74	24.0 (3.4)	25.5 (3.6)	24.4 (3.1)	25.3 (4.1)	0.02
Fat-free mass (kg)	73.8 (9.7)	71.4 (10.4)	74.6 (8.2)	71.7 (9.4)	0.34	53.0 (7.4)	56.2 (9.6)	52.4 (7.0)	54.4 (8.6)	0.08
Fat mass (kg)	36.1 (13.1)	36.9 (12.4)	40.4 (12.6)	37.7 (10.3)	0.59	41.7 (10.3)	43.9 (9.9)	42.2 (10.7)	45.2 (12.0)	0.17
Body fat (%)	32.1 (7.1)	33.6 (8.0)	34.5 (6.5)	34.0 (5.3)	0.33	43.6 (4.9)	43.7 (5.1)	44.1 (4.9)	44.9 (4.9)	0.44
Triglycerides (mmol/L)	1.4 (1.0–1.9)	1.5 (1.0–2.2)	1.7 (1.1–2.1)	1.6 (1.2–2.1)	0.43	1.1 (0.8–1.4)	1.3 (1.0–1.9)	1.3 (0.9–1.6)	1.4 (0.9–1.9)	<0.001
HDL cholesterol (mmol/L)	1.1 (0.3)	1.1 (0.3)	1.0 (0.3)	1.0 (0.2)	0.11	1.3 (0.3)	1.2 (0.3)	1.2 (0.3)	1.1 (0.4)	0.001
LDL cholesterol (mmol/L)	3.2 (1.0)	3.3 (1.0)	3.3 (0.9)	3.1 (0.9)	0.73	2.9 (0.8)	3.1 (0.6)	2.9 (0.9)	3.0 (0.8)	0.46
Total cholesterol (mmol/L)	5.0 (1.1)	5.2 (1.1)	5.1 (1.1)	4.9 (1.1)	0.40	4.7 (0.9)	5.0 (0.8)	4.8 (1.0)	4.8 (0.9)	0.25
Free fatty acid (mmol/L)	0.5 (0.4–0.6)	0.5 (0.4–0.6)	0.5 (0.4–0.6)	0.5 (0.4–0.6)	0.97	0.7 (0.5–0.8)	0.6 (0.5–0.9)	0.6 (0.5–0.8)	0.6 (0.5–0.8)	0.93
C-reactive protein (mg/L)	2.1 (1.3–4.2)	2.3 (1.3–3.9)	3.2 (1.8–5.5)	2.4 (1.3–4.2)	0.59	2.7 (1.3–5.5)	4.4 (2.3–7.1)	4.2 (2.0–7.2)	5.5 (3.4–9.8)	<0.001
Systolic blood pressure (mmHg)	133 (13)	132 (12)	131 (12)	132 (14)	0.99	121 (15)	125 (16)	123 (13)	126 (12)	0.10
Diastolic blood pressure (mmHg)	81 (10)	81 (11)	83 (10)	82 (12)	0.86	75 (12)	79 (10)	77 (10)	76 (9)	0.18
Fasting glucose (mmol/L)	5.2 (0.5)	5.5 (0.8)	5.1 (0.4)	5.2 (0.6)	0.001	5.0 (0.7)	5.2 (0.6)	5.0 (0.6)	5.2 (0.6)	0.007
2-h glucose (mmol/L)	6.2 (1.8)	6.3 (2.4)	8.0 (2.5)	7.2 (2.8)	0.001	6.2 (1.8)	6.0 (1.8)	7.6 (1.9)	7.8 (2.2)	<0.001
Fasting insulin (pmol/L)	62 (45–81)	99 (71–132)	93 (61–131)	119 (85–147)	<0.001	45 (34–63)	92 (65–112)	65 (53–89)	109 (83–159)	<0.001
30-min insulin (pmol/L)	303 (221–373)	628 (495–908)	406 (312–442)	819 (625–1090)	<0.001	265 (191–360)	626 (504–743)	333 (287–415)	708 (612–1000)	<0.001
HOMA2-insulin resistance	1.2 (0.8–1.5)	1.9 (1.3–2.5)	1.8 (1.1–2.5)	2.2 (1.6–2.8)	<0.001	0.8 (0.6–1.2)	1.6 (1.2–2.1)	1.2 (1.0–1.6)	2.0 (1.5–3.0)	<0.001
HOMA2-beta cell function (%)	94 (79–118)	119 (95–147)	134 (102–165)	153 (118–178)	<0.001	87 (70–111)	118 (98–157)	112 (93–131)	149 (123–187)	<0.001
Hepatic insulin resistance index ([mmol/L] h × [pmol/L] h)	299 (243–383)	651(562–921)	401 (311–437)	813 (597–1044)	<0.001	256 (168–343)	598 (539–693)	327 (263–388)	715 (576–933)	<0.001
Muscle insulin sensitivity index (umol/L/min/pmol/L)	0.18 (0.13–0.28)	0.13 (0.09–0.16)	0.05 (0.03–0.07)	0.05 (0.04–0.06)	<0.001	0.16 (0.11–0.23)	0.12 (0.10–0.18)	0.05 (0.04–0.07)	0.04 (0.03–0.06)	<0.001
Adipose tissue IR index (pmol/L × mmol L)	30.9 (20.3–41.8)	54.1 (31.5–83.8)	56.6 (29.9–66.5)	55.8 (37.3–80.2)	<0.001	28.8 (19.6–43.9)	61.1 (38.8–78.1)	39.0 (33.1–57.2)	76.6 (50.4–109.9)	<0.001

Data are presented as mean (SD) for normally distributed continuous variables or as median (IQR) for non-normally distributed continuous variables.

IR insulin resistance.

[a]Based on analyses of variance for normally distributed variables and on Kruskal–Wallis test for non-normally distributed variables.

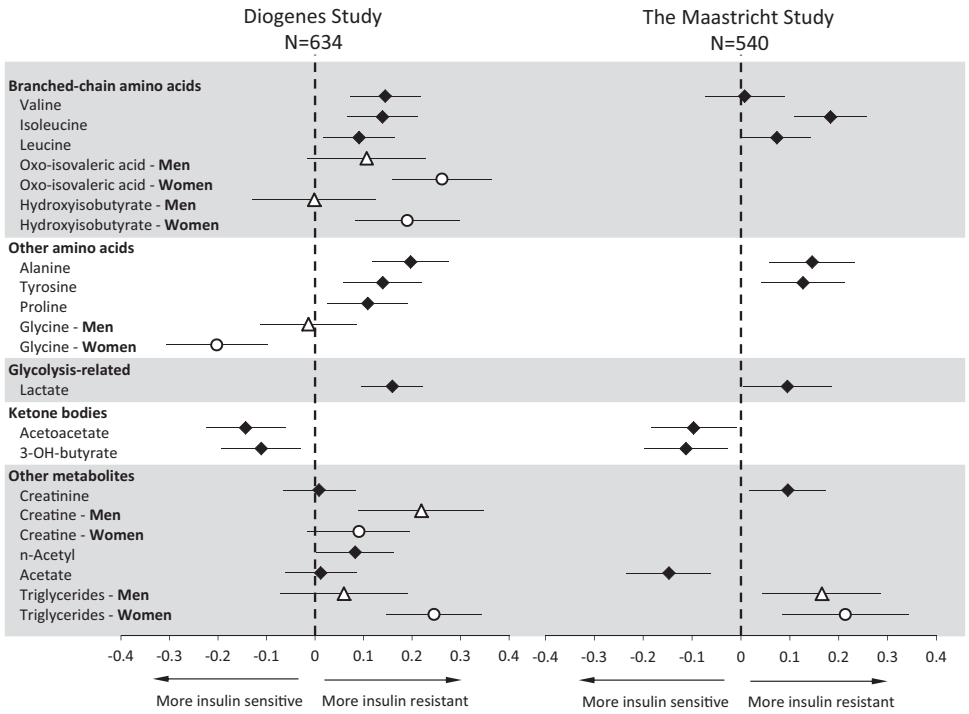

Fig. 1 Standardized beta's (95% CI) of associations between HIRI and metabolites. HIRI = hepatic insulin resistance index. Based on separate linear mixed model (Diogenes Study) or regression (the Maastricht Study) analyses with the individual metabolites as dependent variables, HIRI as independent variable, adjusted for sex, BMI, and WHR; study center was included as random effect in the Diogenes Study; HIRI and all metabolites were ln-transformed and standardized before inclusion in the analyses; in the Diogenes Study, several significant sex interactions ($p < 0.05$) were observed, for these metabolites associations are presented sex stratified (black diamonds represent total sample; open triangles represent men; open circles represent women); 11 of the 17 metabolites of the Diogenes Study were available for validation in the Maastricht Study.

of any of these associations, suggesting that associations of HIRI with these serum metabolites were independent of MISI. No consistent associations between HIRI and serum creatinine, n-acetyl, and acetate were found. Sex interactions with HIRI were found for serum oxo-isovaleric acid ($p = 0.02$), hydroxyisobutyrate ($p = 0.02$), glycine ($p = 0.03$), creatine ($p = 0.03$), and triglycerides ($p = 0.03$) (Supplementary Table S2). The associations between HIRI and serum oxo-isovaleric acid, hydroxyisobutyrate, glycine, and triglycerides were stronger and only statistically significant in women, while the association between HIRI and serum creatine was only significant in men (Fig. 1; Supplementary Table S3).

In the Maastricht Study, the sex-BMI-WHR-adjusted associations between HIRI and higher plasma levels of isoleucine, leucine, alanine, tyrosine, lactate, and triglycerides, as well as the inverse associations of HIRI with plasma acetoacetate and 3-OH-butyrate were replicated (Fig. 1; Supplementary Table S3). After additional adjustment for MISI, the associations with plasma isoleucine, tyrosine, and triglycerides were still significant, but with plasma leucine, alanine, and lactate were not. In addition, significant associations between HIRI and higher plasma creatinine levels and lower plasma acetate levels were found, although these lost significance after additional adjustment for MISI. The association of HIRI with circulating valine, as well as the sex interaction for the association between HIRI and circulating triglycerides ($p = 0.25$) was not replicated in the Maastricht Study. To test whether the lack of replication was possibly related to different age ranges in both studies (i.e., in the Diogenes Study 37% of adults were <40 years, while in the Maastricht Study all adults were ≥40 years), these nonreplicated associations were additionally tested separately in persons <40 and ≥40 years in the Diogenes Study. While HIRI was associated with serum valine in both age groups, the HIRI × sex interaction for serum triglycerides was only significant in adults <40 years ($N = 237$, $p = 0.04$), but not in persons ≥40 years ($N = 397$, $p = 0.43$). In both studies, the associations between HIRI and circulating metabolites did not change after additional adjustment for age. Finally, results of the Maastricht Study were similar when excluding persons with antihypertensive or lipid-lowering medication (data not shown). Other circulating metabolites (oxo-isovaleric acid, hydroxyisobutyrate, proline, glycine, creatine, n-acetyl) were not available for validation in the Maastricht Study.

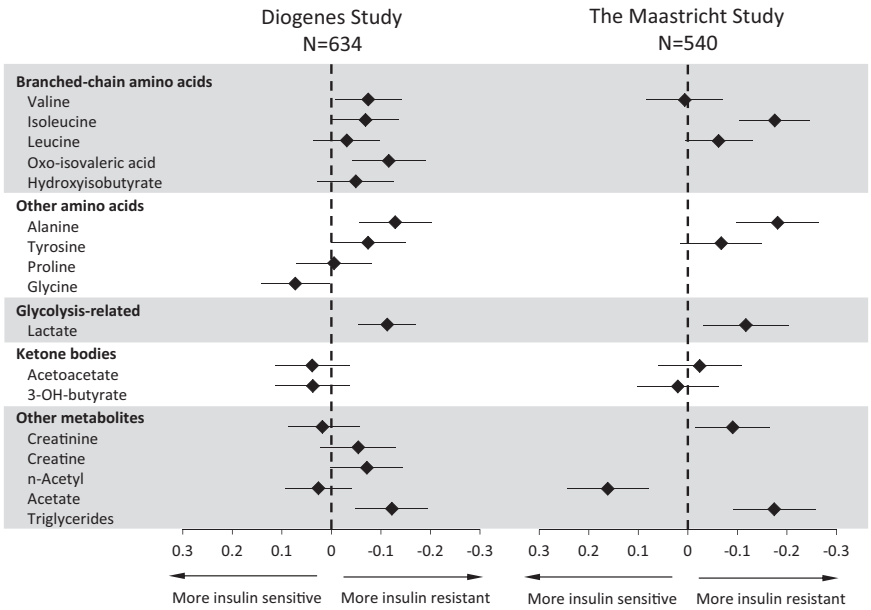

Fig. 2 Standardized beta's (95% CI) of associations between MISI and metabolites. MISI = muscle insulin sensitivity index. Based on separate linear mixed model (Diogenes Study) or regression (the Maastricht Study) analyses with the individual metabolites as dependent variables, MISI as independent variable, adjusted for sex, BMI, and WHR; study center was included as random effect in the Diogenes Study; MISI and all metabolites were ln-transformed and standardized before inclusion in the analyses (black diamonds represent total sample); 11 of the 17 metabolites of the Diogenes Study were available for validation in the Maastricht Study.

MISI is associated with several metabolic alterations, but not with ketone body levels

The associations of MISI with serum metabolites in the Diogenes Study were weaker compared with HIRI, but showed a partly similar pattern: after adjustment for sex, BMI, and WHR, MISI was associated with lower serum levels of valine, isoleucine, oxo-isovaleric acid, alanine, tyrosine, lactate, and triglycerides, and higher serum levels of glycine. However, no associations were found between MISI and serum leucine, hydroxyisobutyrate, proline, acetoacetate, 3-OH-butyrate, creatinine, creatine, n-acetyl, and acetate. After additional adjustment for HIRI, none of the associations remained significant (Fig. 2; Supplementary Table S4). No significant MISI × sex interactions were found (Supplementary Table S2).

In the Maastricht Study the associations between MISI and plasma isoleucine, alanine, lactate, and triglycerides were replicated and these associations were still significant after additional adjustment for HIRI. MISI was not associated with plasma valine, leucine, tyrosine, acetoacetate, or 3-OH-butyrate. In addition, MISI was associated with higher plasma creatinine levels and lower plasma acetate levels. After additional adjustment for HIRI, the association with plasma creatinine was no longer significant, but that with plasma acetate was (Fig. 2; Supplementary Table S4). Overall the pattern of associations found in the Maastricht Study for MISI was quite similar to the pattern for HIRI, with the exception of circulating leucine, tyrosine, and most clearly the ketone bodies. In both the Diogenes Study and the Maastricht Study, the associations between MISI and circulating metabolites did not change after additional adjustment for age.

ATIRI is associated with several metabolic alterations, including high ketone body levels

ATIRI (Diogenes Study only) showed moderate correlations with HIRI (Spearman $r = 0.58$) and MISI (Spearman $r = -0.39$). After adjustment for sex, BMI, WHR, HIRI, and MISI, ATIRI associated significantly and positively with serum valine, isoleucine, leucine, oxo-isovaleric acid, hydroxyisobutyrate, lactate, acetoacetate, 3-OH-butyrate, and triglycerides and negatively with serum glycine (Supplementary Table S5). No ATIRI × sex interactions were found (not shown).

Discussion

This study reports on the circulating metabolic profiles for liver and skeletal muscle IR in two independent studies. The combined results show that liver IR, but not muscle IR, is associated with lower circulating ketone body levels (acetoacetate, 3-OH-butyrate) and higher circulating levels of two ketogenic amino acids (leucine, tyrosine), and possibly

with higher circulating levels of hydroxyisobutyrate, proline, creatine, and n-acetyl. On the other hand, both liver IR and muscle IR are associated with higher circulating levels of isoleucine, alanine, lactate, and triglycerides, and possibly with higher oxo-isovaleric acid and lower glycine levels in circulation.

In two independent study samples of individuals with overweight or obesity, but no diabetes, we show that liver IR, but not muscle IR, is associated lower circulating ketone body levels. Ketone bodies are the result of ketogenesis, which takes place almost exclusively in the liver [20]. Ketone bodies are formed from acetyl-coA, available from β-oxidation of NEFA, in particular when there is an excess of NEFA and glucose levels are low [20]. Ketogenesis is suppressed by insulin which inhibits, via increased production of malonyl-CoA, acyl-CoAs entrance into the mitochondria [20]. Insulin sensitivity for ketogenesis suppression has been shown to be still intact in obese individuals with IR [21]. Previous publications have reported both higher and lower levels of ketone bodies in obesity and IR states [22–25]. Whether ketogenesis is increased or decreased in IR might depend on diet, but also on the stage and/or mechanism of IR development. A large study in men showed that subjects with isolated, nondiabetic impaired fasting glucose—as also seen in our subjects with liver IR—had lower circulating ketone body levels, while subjects with impaired glucose tolerance, type 2 diabetes, and impaired insulin secretion had elevated circulating ketone body levels [26]. Thus, in the case of nondiabetic liver IR, high levels of insulin could simultaneously inhibit ketogenesis and stimulate de novo lipogenesis from excess glucose formed by increased gluconeogenesis. In the case of nondiabetic adipose tissue IR, which results in elevated plasma NEFA levels, our results indicate that ketogenesis is increased. Ketogenesis might be even further enhanced when an obese individual starts fasting. Decreased versus increased ketogenesis within a subject might therefore putatively also reflect the relative presence of liver IR versus adipose tissue IR. Next to lower circulating ketone body levels in liver IR, our study also shows that circulating levels of the ketogenic amino acids leucine and tyrosine are high in liver IR. Leucine and tyrosine are, in contrast to isoleucine, broken down to acetoacetate without first converting to acetyl-CoA. High levels of leucine and tyrosine are possibly (partly) the result of the suppression of ketogenesis.

The results of both our study samples are in line with the growing number of studies that show that IR is associated with increased levels of BCAA (metabolites) and that plasma BCAA levels can predict the development of type 2 diabetes many years before onset (e.g., [12, 27–31]). In 2009, Newgard et al. [28] first suggested that increased BCAA consumption in combination with a high-fat diet causes BCAA to go into catabolism instead of protein synthesis, thereby saturating the capacity for mitochondrial fuel oxidation in the muscle, leading to accumulation of metabolic intermediates that may enhance muscle IR. In our analyses, elevated BCAA (metabolite) levels are only moderately associated with muscle IR, but more consistently associated with liver IR, and most strongly associated with adipose tissue IR. Previous research indeed suggests that the adipose tissue plays a central role in regulating BCAA metabolism in obesity [32]. Reduced expression and activity of mitochondrial branched-chain aminotransferase and the branched-chain keto acid dehydrogenase complex, the first two enzymes in BCAA catabolism, have repeatedly been observed in the adipose tissue of obese and/or insulin resistant rodents or humans, explaining increased BCAA levels [27, 33–36]. Evidence suggests that impaired mitochondrial oxidation of fatty acids, as well as BCAA in adipose tissue might be the result of adipose tissue remodeling and inflammation that is common in obesity [32, 37]. Notably, increased BCAA concentrations and decreased BCAA catabolism can already be observed in adipose tissue of individuals with obesity without whole-body IR [38]. Subsequently, increased circulating levels of both BCAA and BCAA-derived intermediates might lead to IR in adipose tissue, liver, and other tissues. Another mechanism that may contribute to the BCAA–IR relationship might involve the gut microbiome as a different gut microbiome composition in persons with IR has been found to increase BCAA absorption from the gut [39] and/or increase BCAA biosynthesis in the gut [30].

Many studies show that not only BCAA levels, but also other amino acids levels are elevated in whole-body IR [29, 40, 41]. In our two study samples, circulating alanine levels are elevated in both liver and muscle IR, while circulating tyrosine levels are only higher in liver IR. Proline levels in circulation are also higher in liver IR in the Diogenes Study, but were not available for validation. Elevated circulating alanine levels in IR are possibly explained by increased transamination from pyruvate in the muscle, as a result of an accumulation of glutamate due to an elevated flux of BCAA into the first step of BCAA catabolism [28]. Alanine, in turn, might further contribute to increased gluconeogenesis in the liver, as it is a highly glucogenic amino acid. Tyrosine, next to being ketogenic, is an aromatic amino acid, and might be found at increased levels in the circulation as a result of impaired transport into the cell, due to an increased occupation of the large neutral amino acid transporter by BCAA [28]. In contrast to the other amino acids, circulating glycine levels are found to be lower in liver IR, muscle IR, as well as adipose tissue IR, suggesting that this amino acid is a more general marker for IR. Low glycine levels have repeatedly been observed in patients with obesity, IR, and type 2 diabetes [12, 42, 43].

Our study shows that liver IR, muscle IR, as well as adipose tissue IR are associated with high circulating triglyceride levels, suggesting that high triglyceride levels are more likely a reflection of whole-body IR. For liver IR we observed a significant sex interaction in adults <40 years, as we have reported previously [15]. Possibly, even though young women in general have lower triglyceride levels and show less liver IR than young men, under liver IR conditions, VLDL production might be more accelerated in these women than in men, thereby increasing circulating triglyceride levels [44–46].

The present study benefits from several important strengths. First, we were able to replicate most of our findings from the Diogenes Study in the Maastricht Study, an independent, somewhat older, more metabolically compromised cohort, using a different metabolomics method, which greatly enhances the generalizability of our findings. Second, OGTT data were available in both well-characterized cohorts, which enabled us to differentiate between IR in liver versus muscle by methods validated against the gold standard hyperinsulinemic-euglycemic clamp studies. Third, we included individuals with overweight or obesity without cardiometabolic diseases, allowing us to study early disturbances in the progression toward these diseases. Lastly, we were able to test for sex interactions, which indicated that metabolic profiles related to IR might partly be sex dependent. A limitation of our study is that data were cross-sectional, precluding any causal inferences. Longitudinal and experimental studies are necessary to further our understanding of the biological pathways underlying tissue-specific IR. Also, the tissue specificity of HIRI and MISI has been questioned [47], suggesting that circulating metabolites associated with these indices are actually metabolites associated with whole-body IR. While this may be true for those circulating metabolites that were found to be associated with both HIRI and MISI, our results clearly show that lower ketone body levels and elevated ketogenic amino acids in circulation are consistently only associated with HIRI and not with MISI. This suggests that the alterations in these circulating metabolites are specific for liver IR. Furthermore, circulating metabolites were only measured under fasting conditions. Possibly, muscle insulin sensitivity is more profoundly associated with altered postprandial or postexercise, instead of fasting, circulating metabolite levels. In addition, no information on adipose tissue IR was available in the Maastricht Study for validation, nor did we have NEFA measurements across the OGTT for a more adequate quantification of adipose tissue IR. Future studies should replicate our findings with respect to adipose tissue IR. Lastly, no information of family history of T2D was available in the Diogenes Study. Possibly this information could have provided additional insight into the associations between tissue-specific IR and circulating metabolites.

In conclusion, in the obese nondiabetic state, liver, and skeletal muscle IR are characterized by partly overlapping and partly distinct circulating metabolic profiles. Both liver and muscle IR, as well as adipose tissue IR, show a circulating metabolic profile of elevated (branched-chain) amino acids, lactate, triglycerides, and lower glycine levels, suggesting that these circulating metabolites are involved in whole-body IR. In contrast, only liver IR associates with lower ketone body levels and elevated ketogenic amino acids in circulation, suggestive of decreased ketogenesis which is specific to liver IR. Instead, the preliminary results suggest that circulating ketone body levels in individuals with a similar obesity level with adipose tissue IR seem to be increased. These observed distinctions in circulating metabolic profiles might enhance developments of more targeted tissue-specific interventions to prevent progression to more severe cardiometabolic diseases. Future studies should more intensively examine the role of adipose tissue IR to even better differentiate tissue-specific IR metabolic profiles.

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Compliance with ethical standards

Conflict of interest AV is full-time employee at Nestlé Institute of Health Sciences SA. WHMS reports having received research support from several food companies, such as Nestlé, DSM, Unilever, Nutrition et Sante, and Danone as well as Pharmaceutical companies, such as GSK, Novartis, and Novo Nordisk; he is an unpaid scientific advisor for the International Life Science Institute, ILSI Europe. AA reports grants and personal fees from McCain Foods, personal fees from Dutch Beer Knowledge Institute, the Netherlands, personal fees from Gelesis, personal fees from Novo Nordisk, Denmark, outside the submitted work, and royalties received for the book first published in Danish as *Verdens Bedste Kur* (Politiken; Copenhagen, Denmark), and subsequently published in Dutch as *Het beste dieet ter wereld* (Kosmos Uitgevers; Utrecht/Antwerpen, the Netherlands), in Spanish as *Plan DIOGENES para el control del peso. La dieta personalizada inteligente* (Editorial Evergra´ficas; Léon, Spain), and in English as

World's Best Diet (Penguin, Australia). EEB receives grant support from food industry, such as DSM, Danone, Friesland Campina, Avebe, and Sensus, partly within the context of public–private consortia and has received funding from pharmaceutical companies like Novartis. She is involved in several task forces/expert groups related to the International Life Science Institute, ILSI Europe. All other authors report no possible conflicts of interest.

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