Metabolic Signatures of Insulin Resistance in 7,098 Young Adults

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Metabolic associations with insulin resistance were studied in 7,098 young Finns (age 31 ± 3 years; 52% women) to elucidate underlying metabolic pathways. Insulin resistance was assessed by the homeostasis model (HOMA-IR) and circulating metabolites quantified by high-throughput nuclear magnetic resonance spectroscopy in two population-based cohorts. Associations were analyzed using regression models adjusted for age, waist, and standard lipids. Branched-chain and aromatic amino acids, gluconeogenic intermediates, ketone bodies, and fatty acid composition and saturation were associated with HOMA-IR (P < 0.0005 for 20 metabolite measures). Leu, Ile, Val, and Tyr displayed sex- and obesity-dependent interactions, with associations being significant for women only if they were abdominally obese. Origins of fasting metabolite levels were studied with dietary and physical activity data. Here, protein energy intake was associated with Val, Phe, Tyr, and Glu but not insulin resistance index. We further tested if 12 genetic variants regulating the metabolites also contributed to insulin resistance. The genetic determinants of metabolites were not associated with HOMA-IR, with the exception of a variant in GCKR associated with 12 metabolites, including amino acids (P < 0.0005). Nonetheless, metabolic signatures extending beyond obesity and lipid abnormalities reflected the degree of insulin resistance evidenced in young, normoglycemic adults with sex-specific fingerprints. Diabetes 61:1372–1380, 2012

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RESEARCH DESIGN AND METHODS

Study population. The study comprised two population-based Finnish cohorts, the Northern Finland Birth Cohort 1966 (NFBC) (16) and the Cardiovascular Risk in Young Finns Study (YFS) (17). Out of 7,718 individuals with metabolite data, 170 individuals missing glucose or insulin measurements or with concentrations outside the boundaries of the homeostasis model assessment of insulin resistance (HOMA-IR) index were therefore excluded. In addition, individuals diagnosed with type 1 or type 2 diabetes (n = 56), those taking lipid medication (n = 7) or antihypertensive medication (n = 150), and pregnant women (n = 237) were removed, leaving 7,098 individuals for analyses. Participants gave written informed consent, and the study protocols were approved by the local ethics committees.

The NFBC was initiated to study factors affecting preterm birth and subsequent morbidity (http://kelo.oulu.fi/NFBC). Data collection included clinical examination and 12-h fasting blood sampling at age 31 for 6,007 individuals, of which 5,471 had a fasting metabolite profile measured. Data from this time point were used for the current study. Attendees in the 31-year study field were representative of the original birth cohort. Plasma glucose concentrations were measured by glucose dehydrogenase (Granatest 250; Diagnostica Merck), and insulin concentrations were measured by radioimmunoassay (Pharmacia Diagnostics). Physical activity was assessed for 4,538 individuals by the metabolic equivalent of task (MET) index based on questionnaire data on frequency, intensity, and duration of physical activity as described previously (18).

The YFS was designed to study associations of childhood risk factors to cardiovascular disease in adulthood (http://med.utu.fi/cardio/youngfinnsstudy/). The baseline study in 1980 included 5,998 children aged 3 to 18. Data used in the current study were generated from a follow-up in 2001 that included 3,596 participants who provided fasting plasma samples. Clinical characteristics for men and women were compared using t tests. Clinical characteristics for men and women were compared using t tests.

Results

Clinical characteristics. The study comprised 7,098 young adults (mean age 31 years, range 24–39). Clinical characteristics of the population are shown in Table 1. The study population represents metabolically healthy individuals from the general Finnish population; only 11% had impaired fasting glucose (≥5.6 mmol/L), and the 80th percentile of HOMA-IR index was 1.3, corresponding to 77% HOMA insulin sensitivity (24).

Metabolite associations with HOMA-IR. To elucidate metabolic pathways characterizing or contributing to insulin resistance, 39 circulating metabolites and lipid measures from high-throughput profiling were studied. A list of analyzed metabolites is given in Supplementary Table 1. In total, 20 metabolite measures were found to be associated with HOMA-IR at P < 0.005 for either men or women and nominally significant in both cohorts as listed in Table 2. The metabolites include amino acids, intermediates of glycolysis and gluconeogenesis, and fatty acid composition and saturation measures. Gln and ketone bodies (3-hydroxybutyrate and acetoacetate) exhibited inverse associations, as did the average number of double bonds per fatty acid chain. Branched-chain amino acids, Tyr, Ala, and ketone bodies displayed sex-dependent effects (P < 0.001 for metabolite × sex interaction) with stronger associations observed for men. Furthermore, association magnitudes (e.g., β = 0.24 SD HOMA-IR per 1 SD Ala concentration, corresponding to 1.0 IU/L higher insulin

**TABLE 1**

| Characteristic | Men Mean (SD) | Women Mean (SD) |
|---------------|--------------|-----------------|
| Age (years)   | 31.2 (2.6)   | 31.2 (2.8)      |
| Waist (cm)    | 89 (10)      | 79 (12)         |
| Blood pressure (mmHg) | 128 (13) | 118 (13) |
| Total cholesterol (mmol/L) | 5.2 (1.0) | 4.9 (0.9) |
| HDL cholesterol (mmol/L) | 1.4 (0.3) | 1.6 (0.4) |
| Triglycerides (mmol/L) | 1.1 [0.8–1.7] | 0.9 [0.7–1.2] |
| Plasma glucose (mmol/L) | 5.1 [4.9–5.4] | 4.9 [4.6–5.1] |
| Insulin (IU/L) | 7.5 [6.0–9.7] | 7.0 [5.7–9.0] |
| HOMA-IR       | 0.98 [0.78–1.3] | 0.92 [0.73–1.2] |

Data are mean (SD) and median [interquartile range]. P < 0.005 for all comparisons of men and women with two-tailed t test except for age (P = 0.54).
**TABLE 2**
Associations of circulating metabolites with HOMA-IR

| Metabolite                          | Men                                  | Women                                | Molecular link to insulin resistance                                                                 |
|-------------------------------------|--------------------------------------|--------------------------------------|-------------------------------------------------------------------------------------------------------|
| Branched-chain amino acids          |                                      |                                      | Essential amino acids; phosphorylation of insulin receptor substrate-1 and the mammalian target of rapamycin complex (8,10,27,31–33) |
| Leu                                 | $0.14 \text{ (0.015)}$ $9 \times 10^{-21}$ | $0.082 \text{ (0.015)}$ $5 \times 10^{-8}$ | $6 \times 10^{-4}$                                                                                   |
| Ile                                 | $0.22 \text{ (0.019)}$ $2 \times 10^{-31}$ | $0.11 \text{ (0.017)}$ $5 \times 10^{-12}$ | 0.006                                                                                               |
| Val                                 | $0.14 \text{ (0.015)}$ $4 \times 10^{-21}$ | $0.097 \text{ (0.015)}$ $5 \times 10^{-11}$ | 0.001                                                                                               |
| Aromatic amino acids                |                                      |                                      | Precursors for neurotransmitters; thyroid function (10,27,28)                                        |
| Phe                                 | $0.17 \text{ (0.017)}$ $5 \times 10^{-24}$ | $0.18 \text{ (0.016)}$ $5 \times 10^{-29}$ | 0.28                                                                                               |
| Tyr                                 | $0.21 \text{ (0.016)}$ $1 \times 10^{-37}$ | $0.044 \text{ (0.015)}$ $0.003^\dagger$  | $2 \times 10^{-14}$                                                                                        |
| Glycolysis and gluconeogenesis      |                                      |                                      | Gluconeogenesis and glycolysis; nitric oxide; transamination (28,43,44)                                |
| intermediates                        |                                      |                                      | Ketogenesis; fatty acid oxidation (45,46)                                                               |
| Ala†                                | $0.24 \text{ (0.016)}$ $9 \times 10^{-56}$ | $0.16 \text{ (0.015)}$ $1 \times 10^{-24}$ | $6 \times 10^{-5}$                                                                                   |
| Gln                                 | $-0.082 \text{ (0.017)}$ $1 \times 10^{-63}$ | $-0.11 \text{ (0.015)}$ $2 \times 10^{-13}$ | 0.86                                                                                               |
| Lactate                             | $0.17 \text{ (0.016)}$ $7 \times 10^{-27}$ | $0.13 \text{ (0.014)}$ $2 \times 10^{-20}$ | 0.24                                                                                               |
| Pyruvate                            | $0.21 \text{ (0.015)}$ $2 \times 10^{-44}$ | $0.21 \text{ (0.014)}$ $4 \times 10^{-50}$ | 0.97                                                                                               |
| Ketone bodies                       |                                      |                                      | Low-grade inflammation (47)                                                                          |
| Acetoacetate                        | $-0.17 \text{ (0.014)}$ $4 \times 10^{-32}$ | $-0.10 \text{ (0.014)}$ $1 \times 10^{-13}$ | $1 \times 10^{-4}$                                                                                   |
| 3-hydroxybutyrate                   | $-0.13 \text{ (0.015)}$ $2 \times 10^{-18}$ | $-0.070 \text{ (0.014)}$ $6 \times 10^{-7}$ | $6 \times 10^{-5}$                                                                                   |
| Glycoproteins                       |                                      |                                      | Energy storage; lipid transport; precursors of prostaglandins and endocannabinoids; free fatty acid–mediated insulin resistance; impairment of fatty acid oxidation (5,6,7) |
| α1-acid glycoprotein                | $0.14 \text{ (0.02)}$ $7 \times 10^{-13}$ | $0.15 \text{ (0.018)}$ $8 \times 10^{-18}$ | 0.66                                                                                               |
| Fatty acids                          |                                      |                                      | Membrane and lipoprotein composition; cellular signaling (48)                                        |
| Phospholipids                        |                                      |                                      | Fatty acid saturation and chain length (11)                                                           |
| Phosphocholines                     | $0.17 \text{ (0.039)}$ $2 \times 10^{-5}$ | $0.17 \text{ (0.038)}$ $8 \times 10^{-6}^\dagger$ | 0.84                                                                                               |
| Phosphoglycerides                   | $0.15 \text{ (0.034)}$ $5 \times 10^{-6}$ | $0.15 \text{ (0.034)}$ $7 \times 10^{-6}^\dagger$ | 0.92                                                                                               |
| Fatty acid saturation measures      |                                      |                                      |                                                                                                       |
| Average number of methylene groups per double bond | $0.11 \text{ (0.018)}$ $1 \times 10^{-9}$ | $0.073 \text{ (0.016)}$ $7 \times 10^{-6}^\dagger$ | 0.60                                                                                               |
| Average number of double bonds per fatty acid chain | $-0.096 \text{ (0.018)}$ $1 \times 10^{-7}$ | $-0.078 \text{ (0.016)}$ $2 \times 10^{-6}^\dagger$ | 0.72                                                                                               |

β-Regression coefficients (SE) are in units of 1 SD HOMA-IR per 1-SD change in metabolite concentration. The associations were adjusted for age, waist circumference, total cholesterol, HDL cholesterol, and triglycerides. Associations were meta-analyzed for the NFBC and the YFS ($n = 7,098$). Metabolites were denoted significant at $P < 0.005$. †Nominally significant in one of the cohorts only.

per 1 SD Ala) in men were similar for amino acids and total fatty acids, whereas in women, fatty acid associations tended to be stronger. The metabolites explained 44 and 38% of variance in HOMA-IR for men and women, respectively, in combination with established risk factors (age, waist, standard lipids, and physical activity), and added an additional respective 12 and 8% to the variance explained by established risk factors alone. In a stepwise model, most amino acids and gluconeogenesis intermediates remained significant, while several fatty acid composition measures were not independently associated with HOMA-IR (Supplementary Table 3).

Both insulin resistance and circulating metabolite levels are linked with abdominal obesity (1,6,7). Therefore, we assessed whether the metabolite associations were consistent across tertiles of waist circumference. Results for amino acids are illustrated in Fig. 1; for the remaining metabolites, results are shown in Supplementary Fig. 1.
These analyses revealed metabolite × waist interaction for most of the metabolites (14 and 12 associations with $P < 0.05$ for metabolite × waist tertile for men and women, respectively), with stronger associations observed for more abdominally obese individuals. It is notable that for women, associations of branched-chain amino acids and Tyr with HOMA-IR were significant only in the upper tertile of waist circumference. In contrast, men displayed associations for branched-chain and aromatic amino acids throughout the range of waist circumference; however, the sex interactions persisted even for obese individuals (data not shown). Correspondingly, Gln was inversely associated with HOMA-IR for abdominally obese men only, whereas the association was present across tertiles of waist circumference for women.

**Dietary composition, physical activity, and metabolites.**

Associations of relative dietary energy intake and physical activity (MET index) with metabolites linked with HOMA-IR are shown in Fig. 2. Dietary composition was associated with several fasting metabolite levels but not with insulin resistance index. Protein energy intake per total energy intake was directly associated with Val, Phe, and Tyr and inversely associated with Gln. Relative protein as well as fat energy intake were directly associated with less fatty acid saturation. Dietary associations with the metabolites were essentially unaltered when conditioned for HOMA-IR (data not shown). In contrast to dietary measures, physical activity was inversely associated with insulin resistance index as well as several metabolites, including Ile, Phe, Tyr, α1-acid glycoprotein, total fatty acids, and fatty acid saturation measures. The physical activity associations with metabolites were of smaller magnitude than with HOMA-IR and were attenuated or rendered nonsignificant upon conditioning on HOMA-IR.

**Genetic variants, metabolites, and insulin resistance.**

To gain insight into the direction of effect underlying the metabolite associations, we tested if genetic variants regulating the metabolite levels were also modifying HOMA-IR. Associations of the SNPs with HOMA-IR are shown in Fig. 3A. None of the SNPs affecting the metabolites were associated with HOMA-IR ($P > 0.05$), with the exception of a variant in GCKR previously associated with insulin resistance.
resistance and other metabolic traits (15,25,26). The lack of associations with HOMA-IR was despite the fact that the analyzed genetic variants were significant determinants of the metabolites (Fig. 3B). The SNP rs1260326 in GCKR was associated with insulin resistance index ($P = 0.001$) and additionally associated with 12 of the 20 metabolites ($P < 0.005$), as shown in Fig. 3C. Most pronounced associations were found for Ile, Ala, α1-acid glycoprotein, total fatty acids, and n-9 and saturated fatty acids ($P < 1 \times 10^{-7}$ for all). Of note, the insulin resistance–lowering
FIG. 3. Associations of genetic variants regulating metabolite levels with HOMA-IR (A) and the strongest circulating metabolite measure (B). Associations for rs1260326 in GCKR with the metabolites before (C) and after (D) adjustment for triglycerides. Error bars indicate 95% CIs and numbers indicate P values of association. All associations were adjusted for sex, age, waist, and population structure and meta-analyzed for the two cohorts (n = 6,343). Association magnitudes are in units of 1 SD HOMA-IR or metabolite concentration per allele copy. Av., average.
allele was associated with the metabolite levels in the opposite direction to those observed between the metabolite levels and HOMA-IR. The associations were essentially unaltered when adjusting for HOMA-IR (data not shown); however, upon conditioning on triglycerides, they were largely attenuated, while the association with HOMA-IR was enhanced (Fig. 3D).

**DISCUSSION**

This study demonstrates that the systemic metabolite profile strongly reflects the degree of insulin resistance evidenced in young, apparently healthy adults. The metabolic signatures of insulin resistance were different for men and women and modulated by obesity. Analyses of genetic and lifestyle determinants of the metabolite profile did not lend support to an etiological role of the metabolites in the pathogenesis of insulin resistance; however, the observed pleiotropy for insulin resistance, lipids, and amino acids for a variant in GCKR illustrates how altered glucose sensing may widely affect the metabolite profile.

High-throughput metabolic profiling identified 20 metabolite measures associated with HOMA-IR. A variety of metabolites comprising amino acids, glycolysis intermediates, ketone bodies, and lipid constituents displayed pronounced associations independent of the established dyslipidemic pattern of insulin resistance (3,4). These findings provide hypotheses implicating known and novel metabolites as markers of early stage insulin resistance. Potential mechanisms underpinning the associations are given in Table 2. The prominent imprint of insulin resistance on the metabolite profile substantiates and extends findings from smaller profiling studies (8,9,27,28) by providing quantitative information on individual metabolites in population-based cohorts of young adults. The 12 and 8% of additional variance in HOMA-IR explained by the metabolites for men and women, respectively, emphasizes metabolic signatures beyond obesity and lipid abnormalities and further contrasts findings from genome-wide association studies where genetic variants have explained only a minor fraction of the variance in insulin resistance (26).

Men and women displayed significant differences in their metabolite profiles despite a similar range of HOMA-IR in this study (Supplementary Table 1 and Table 1). While sex differences in metabolite concentrations are well known (29), this study revealed novel sex-dependent associations for amino acids and ketone bodies with insulin resistance (Table 2). For instance, Tyr displayed five times higher magnitude of association for men, whereas Phe did not exhibit different effects for men and women. This could indicate involvement of sex hormones in the regulation of these precursors of thyroid hormones and neurotransmitters. The associations were also modified by obesity, with Tyr and branched-chain amino acids being significant for women only if they were abnormally overweight (Fig. 1). Sex differences in metabolite associations with insulin action have previously been observed in a small study with stronger associations of a cluster of large neutral amino acids for overweight men (9). Differences in adipose tissue composition and adipokine levels could underpin these observations; however, the molecular mechanisms remain to be investigated. Although the prevalence of diabetes is similar among men and women, there are differences in the development of insulin resistance, with young men being more insulin resistant after puberty in conjunction with an adverse lipid profile (3,4). In addition, some studies suggest greater protein turnover rates and lesser insulin sensitivity of protein anabolism for women (30). Several studies on amino acids in relation to insulin resistance have been conducted for men only (28,31,32); however, our results indicate that future studies should account for sex- and obesity-specific differences.

Assessment of lifestyle effectors and genetic determinants of metabolite levels may illuminate the etiology underpinning the metabolic signatures of insulin resistance. Both diet and physical activity were associated with the fasting metabolite profiles (Fig. 2). None of the dietary measures were linked with insulin resistance index; however, the elevation of several amino acids by increased protein energy intake could potentially be detrimental for insulin sensitivity, as suggested by several studies (8,10,31–33). On the other hand, high protein intake was also associated with an increase in n-3 fatty acids and a higher number of double bonds per fatty acid, thus pointing to a beneficial role of a protein-rich diet. Lipids of high double bond content were recently linked with decreased risk for incidence of diabetes (11), in accordance with the inverse association between average number of double bonds per fatty acid chain and insulin resistance index found in this study.

Physical activity was inversely associated with insulin resistance index as well as lipids and amino acids; however, the associations were less pronounced for the metabolites. Furthermore, the associations diminished when conditioning on HOMA-IR, indicating that the metabolite associations are not independent of insulin resistance. Because physical activity is known to improve insulin sensitivity (34), these results could indicate that physical activity is primarily affecting insulin resistance and the associations observed with the metabolite profile could be secondary hereto.

The question whether amino acids contribute to the pathogenesis of insulin resistance and type 2 diabetes in a functional manner remains unsettled (35). Experimental studies suggest that branched-chain amino acids may promote insulin resistance (8,31), yet direct evidence in humans is lacking. Dietary composition is likely to influence both metabolite levels and the development of insulin resistance in a causal manner (8,33), yet we did not find concomitant associations with dietary measures and HOMA-IR despite the observation that protein energy intake was associated with fasting amino acid levels. These results, therefore, do not suggest an etiological role of amino acids in the disease pathogenesis. The cross-sectional dietary associations, however, may potentially be confounded by other environmental factors. To circumvent such bias, we tested genetic variants determining metabolite levels. Such analyses may serve to elucidate direction of effect, since insulin resistance can be affected by genetic variants, whereas the genes are not influenced by insulin resistance (36). We found that 11 out of 12 genetic variants, including 6 regulating branched-chain and aromatic amino acids, were not associated with HOMA-IR (Fig. 3A). Thus, these analyses on the direction of effect do not lend support to the notion of a causal role of amino acids in the development of insulin resistance. On the other hand, the modest effects of each genetic variant and the limited range of HOMA-IR in the young study population may partly account for the lack of associations, and further studies are required to determine the etiological roles of amino acids in the pathogenesis.

A single genetic variant, rs1260326 in the glucokinase regulatory protein gene GCKR, was associated with insulin sensitivity, as suggested by several studies (8,10,31–33). On the other hand, high protein intake was also associated with an increase in n-3 fatty acids and a higher number of double bonds per fatty acid, thus pointing to a beneficial role of a protein-rich diet. Lipids of high double bond content were recently linked with decreased risk for incidence of diabetes (11), in accordance with the inverse association between average number of double bonds per fatty acid chain and insulin resistance index found in this study.

Physical activity was inversely associated with insulin resistance index as well as lipids and amino acids; how-
resistance index. This missense variant has previously been linked with insulin resistance, glycemia, and several other metabolic traits and, recently, amino acids (15,25,26,37,38). The insulin resistance–lowering allele was associated with elevated metabolite levels, in contrast to the direct correlations observed between metabolites and HOMA-IR (Fig. 3C). Theotypic variant has been suggested to mimic the consequences of glucokinase overexpression leading to increased glycolytic flux (25,39,40). Associations with Ala, lactate, pyruvate, and branched-chain amino acids suggest effects on the glucose-alanine cycle as well (38). Because the metabolite associations were largely attenuated upon adjustment for triglycerides (Fig. 3D), we propose a shared origin underlying the associations for amino acids and lipids with GCKR. The pleiotropy observed for GCKR indicates how perturbations in the glucose sensory mechanism may subsequently affect lipid and amino acid levels. Altered glucose utilization could also underlie the metabolite associations with insulin resistance established in this study, and the genetic analyses are therefore compatible with elevated amino acid and lipid levels being predominantly secondary rather than direct contributors to the development of insulin resistance.

The large population studied rendered direct measurement of insulin sensitivity infeasible, and insulin resistance was therefore approximated by the HOMA index (24). While the HOMA model largely reflects hepatic insulin resistance, studies in Finnish populations show that fasting measures are adequate surrogates of clamp test–derived insulin resistance (41). The limited sensitivity of high-throughput NMR confines quantification to highly abundant metabolites, and the wider coverage achieved by mass spectrometry, in particular for molecular lipid species, holds promise to provide further insight into the pathophysiology of insulin resistance (7,11,42). Our study was conducted in a homogenous population, yet the results confirm previous smaller studies in older populations including Asian men (27). Strengths of the study include metabolic profiling in population-based cohorts of young adults and the combination of additional phenotype and genotype data to elucidate origins of the associations.

The diversity of metabolic associations with HOMA-IR highlights metabolic signatures of insulin resistance beyond the characteristics of metabolic syndrome and suggests a strong relation between insulin resistance and the systemic metabolite profile already evidenced in early adulthood. A combination of amino acids, lipids, and intermediates of glycolysis formed sex-specific imprints of insulin resistance on the metabolite profile that warrant attention in future physiological studies. Genetic evidence did not provide support for a functional role of the metabolites in the pathogenesis of insulin resistance, yet irrespective of cause or effect, even modest insulin resistance was associated with an adverse cardiometabolic profile. Understanding the relation between insulin resistance and the systemic metabolite profile in young, normoglycemic adults may help to promote lifestyle habits for prevention of insulin resistance prior to development of hyperglycemia and overt type 2 diabetes.

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P.W. researched and interpreted data and wrote the manuscript. V.-P.M., T.Tu., J.K., S.R., and M.A.-K. contributed to interpretation of data and discussion. P.S. performed the NMR experiments. A.J.K. analyzed the NMR spectral data. M.J.S., T.Ta., J.S.V., T.R., M.K., T.L., O.T.R., and M.-R.J. provided clinical data and interpretations. All authors reviewed, commented on, and accepted the manuscript. P.W. is the guarantor of this work and, as such, had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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1380 DIABETES, VOL. 61, JUNE 2012