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The association between the metabolic syndrome and alanine amino transferase is mediated by insulin resistance via related metabolic intermediates (the Cohort on Diabetes and Atherosclerosis Maastricht [CODAM] study)

Marjon Jacobs[a,b], *Marleen M.J. van Greevenbroek*[a,b,*], *Carla J.H. van der Kallen*[a,b], *Isabel Ferreira*[a,b,c,d], *Edith J.M. Feskens*[e], *Eugene H.J.M. Jansen*[f], *Casper G. Schalkwijk*[a,b], *Coen Stehouwer*[a,b]

[a] *Cardiovascular Research Institute Maastricht (CARIM), Maastricht University, Maastricht, The Netherlands*
[b] *Department of Internal Medicine, Maastricht University Medical Centre, Maastricht, The Netherlands*
[c] *Department of Clinical Epidemiology and Medical Technology Assessment (KEMTA), Maastricht University Medical Centre, Maastricht, The Netherlands*
[d] *Care and Public Health Research Institute (CAPHRI), Maastricht University, Maastricht, The Netherlands*
[e] *Division of Human Nutrition, Wageningen University, Wageningen, The Netherlands*
[f] *Laboratory for Health Protection Research, National Institute for Public Health and the Environment, Bilthoven, The Netherlands*

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ABSTRACT

The metabolic syndrome is associated with nonalcoholic fatty liver disease (NAFLD) as well as with insulin resistance, inflammatory adipokines, endothelial dysfunction, and higher plasma levels of nonesterified fatty acids (NEFA), all of which may also affect the development of NAFLD. Therefore, we investigated to what extent the association between the metabolic syndrome and alanine aminotransferase (ALT, as a surrogate of NAFLD) can be explained by different metabolic intermediates of the metabolic syndrome. Cross-sectional analyses were performed in 434 subjects from the Cohort on Diabetes and Atherosclerosis Maastricht study (264 men; mean age, 59.5 ± 7.1 years). We used multiple linear regression analyses to investigate the association between the metabolic syndrome and ALT and the mediation role of potential mediators herein. The mediators considered were insulin resistance (homeostasis model assessment), an inflammatory adipokine score (based on interleukin-6, serum amyloid A, intercellular adhesion molecule, adiponectin, and leptin), an endothelial dysfunction score (based on E-selectin, vascular cell adhesion molecule, and von Willebrand factor), and plasma levels of NEFA. All analyses were adjusted for age, sex, smoking, alcohol consumption, and use of medication. Subjects with the metabolic syndrome (53.7%) had significantly higher levels of ALT (β = 0.67 SD [95% confidence interval, 0.49-0.85], $P < .001$). Adjustment for insulin resistance attenuated this difference by 77.3% (to 0.15 SD [–0.04 to 0.35]). Attenuation by adipose tissue–associated inflammation, endothelial dysfunction, and NEFA was more modest (20.7%, 13.1%, and 9.5%, respectively). Part of the attenuation by NEFA, but not of the other mediators, was additional to that of insulin resistance. Insulin resistance constitutes a key pathophysiological

* *Corresponding author*. Laboratory for Metabolism and Vascular Medicine, Internal Medicine (UNS 50/box 14), Maastricht University, PO Box 616, 6200 MD, Maastricht, The Netherlands. Tel.: +31 43 3882135; fax +31 43 3670916.
E-mail address: m.vangreevenbroek@intmed.unimaas.nl (M.M.J. van Greevenbroek).

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mechanism in the association between the metabolic syndrome and NAFLD (measured as ALT), which may operate through adipose tissue–associated inflammation and endothelial dysfunction and to a lesser extent through NEFA, which may have an independent role in the development of NAFLD in subjects with the metabolic syndrome.

1. Introduction

The increasing prevalence of the metabolic syndrome draws a heavy burden on public health. Nonalcoholic fatty liver disease (NAFLD) is a common liver disorder that affects up to 20% to 30% of the population in the developed world [1,2] and is a very prevalent finding in subjects with the metabolic syndrome [3]. Given the frequency of NAFLD in obesity and type 2 diabetes mellitus (T2DM), the prevalence of NAFLD in the metabolic syndrome can be estimated to be at least 70% to 80% [4,5].

Nonalcoholic fatty liver disease has a wide histologic spectrum ranging from simple steatosis and nonalcoholic steatohepatitis to more progressive forms of the disease such as fibrosis and eventually cirrhosis. Patients with simple steatosis are, among others, characterized by the accumulation of triglycerides in hepatocytes, whereas in nonalcoholic steatohepatitis, the accumulation of fat is accompanied by hepatic inflammation [1]. The "criterion standard" to diagnose NAFLD is liver biopsy [1,6]. However, this is an invasive procedure with risk of postinterventional bleeding and is therefore not acceptable without clinical indication. Alternative methods to detect hepatic fat accumulation are used in epidemiological studies including imaging techniques and measurement of circulating markers such as alanine aminotransferase (ALT) [6-8].

Several unfavorable metabolic syndrome–associated processes may affect the development of NAFLD. Insulin resistance is most likely involved in the initiation of hepatic steatosis, but NAFLD may in turn contribute to further progression of insulin resistance [9]. In adipose tissue, loss of insulin sensitivity can lead to increased release of nonesterified fatty acids (NEFA) in the circulation, which may be incorporated into hepatic triglycerides [10,11]. In combination with insufficient elimination of triglycerides, probably caused by hepatic insulin resistance, this can contribute to the development of NAFLD. Inflammation of adipose tissue, as commonly seen in the metabolic syndrome [12], may also contribute to the development of NAFLD. This is firstly because adipose tissue–associated inflammation yields insulin-resistant adipocytes by activating JNK and IKKβ pathways [13,14], and secondly because inflammatory adipokines contribute to systemic low-grade inflammation that may trigger hepatic inflammation and hepatic insulin resistance and thereby predispose to NAFLD [15]. Another metabolic syndrome–associated process that may contribute to the development of NAFLD is (local) endothelial dysfunction. The liver is a highly perfused organ, and disturbance of liver perfusion may disturb the delicate balance between the supply and removal of nutrients and metabolites [16]. Insulin resistance as well as the concomitant increase in NEFA may disturb microvascular function [17] and thereby possibly contribute to the development and/or progression of NAFLD [18]. The aforementioned pathways are not necessarily fully independent; they may also affect one another.

Taken together, the evidence so far suggests that insulin resistance, inflammatory adipokines, endothelial dysfunction, and NEFA may each explain, at least in part, the association between the metabolic syndrome and NAFLD. To gain more insight into these issues, we have determined the association between the metabolic syndrome and plasma ALT (as a systemic marker of NAFLD) and investigated the extent to which this association could be explained by insulin resistance, inflammatory adipokines, endothelial dysfunction, and/or NEFA.

2. Materials and methods

2.1. Subjects and study design

Cross-sectional analyses were performed on data from the Cohort on Diabetes and Atherosclerosis Maastricht study, which includes 574 subjects with an elevated risk for T2DM and cardiovascular disease as described in detail elsewhere [19]. In short, all subjects were Caucasian, were older than 40 years, and met at least one of the following criteria: body mass index greater than 25 kg/m^2, a positive family history for T2DM, a history of gestational diabetes, use of antihypertensive medication, a postprandial glucose greater than 6.0 mmol/L, and/or glycosuria. The study was approved by the Medical Ethical Committee of the Maastricht University, and all subjects gave written informed consent.

Subjects were excluded if they had missing data on one or more of the markers for insulin resistance, inflammatory adipokines, endothelial dysfunction, and/or NEFA (n = 44). In addition, subjects were excluded if they used insulin therapy (n = 11) because in those subjects insulin resistance cannot be estimated by homeostasis model assessment (HOMA2IR). Next, those with self-reported liver disease (n = 6), or alcohol consumption of more than 20 g/d (women) or 40 g/d (men) (n = 79) were excluded because this is the maximal amount of alcohol consumption that has been accepted in NAFLD [20]. The present study population therefore consisted of a total of 434 individuals.

2.2. Laboratory measurements

The metabolic syndrome was defined according to the updated definition of the American Heart Association and the National Heart, Lung, and Blood Institute (2005) [21]. According to the results of a standard 75-g oral glucose tolerance test, 146 subjects had T2DM [22]. Of these T2DM subjects, 55% were not aware of their diabetes status before the screening. Use of medication, smoking behavior, and alcohol consumption were assessed in extensive interview

sessions and research-assistant–administered questionnaires. Waist circumference was measured at the level midway between the lateral lower rib margin and the spina iliaca anterior superior. Blood pressure was measured twice after 5 minutes of rest with an oscillometric precision blood pressure instrument (Maxi stable 3; Speidel & Keller; currently Welch Allyn, Skaneateles Falls, NY) on the right arm in the supine position.

Subjects were asked to stop their lipid-lowering medication 14 days before the visit, and all other medication was stopped the day before the visit (>80% adherence). Blood samples were obtained by venipuncture to determine glucose, cholesterol, triglycerides, interleukin-6 (IL6), serum amyloid A (SAA), soluble intercellular adhesion molecule (sICAM-1), and soluble vascular cell adhesion molecule (sVCAM-1) as described previously [23]. Adiponectin and leptin were measured by enzyme-linked immunosorbent assay in EDTA plasma (BioVendor Laboratory Medicine, Brno, Czech Republic). Soluble E-selectin (sE-selectin) was measured in EDTA plasma with a CD62-Elipair enzyme-linked immunosorbent assay (Diaclone; Tepnel, Besancon Cedex, France). Von Willebrand factor (vWF) was measured in citrate plasma as described previously [24]. Nonesterified fatty acids were measured in EDTA plasma using an enzymatic colorimetric method (NEFA-C; Wako Chemicals, Neuss, Germany), and ALT was measured in EDTA plasma as described before and used as a measure of hepatic fat accumulation [7]. Insulin was measured in EDTA plasma using a 2-sided radioimmunoassay (Medgenix Diagnostics, Brussels, Belgium). Insulin resistance was derived from the HOMA2IR and was computed using software downloaded at http://www.dtu.ox.ac.uk.

2.3. *Statistical analysis*

Variables with a skewed distribution, that is, triglycerides, ALT, HOMA2IR, IL6, SAA, sICAM-1, adiponectin, leptin, NEFA, sE-selectin, sVCAM-1, and vWF, were log-transformed before further analyses. Differences between the subjects with and without the metabolic syndrome were assessed by means of Student *t* tests for continuous variables and by χ^2 tests for categorical variables.

Two composite measures (average z scores) were calculated to reduce the problem of multiple testing. First was an inflammatory adipokines score, which was the average of the z scores of IL6, SAA, sICAM-1, leptin, and the z score of adiponectin multiplied by –1. These 5 markers have each been reported to be secreted by adipose tissue in relevant amounts and have inflammatory (IL6, SAA, sICAM-1, leptin) or anti-inflammatory (adiponectin) properties [25-28]. We therefore considered these markers good representatives of adipose tissue–associated inflammation as often seen in the metabolic syndrome. The second was an endothelial dysfunction score, which was the average of the z scores of sE-selectin, sVCAM-1, and vWF. To enable direct comparison of the strengths of all associations, z scores were also calculated for HOMA2IR, NEFA, and ALT.

We used linear regression analyses to investigate the following associations: first, between the metabolic syndrome (main independent variable) and ALT (main dependent variable); second, between the metabolic syndrome and the potential mediators, that is, insulin resistance, inflammatory adipokines, endothelial dysfunction, or NEFA; and third, between the potential mediators and ALT. Finally, we examined the extent to which the association between the metabolic syndrome and ALT was explained, ie, potentially mediated, by the potential mediators considered. This was done by quantifying, in percentage, the attenuations in the magnitude of the linear regression coefficient reflecting the association between the metabolic syndrome and ALT after adjustment for those mediators. Because there was no interaction between sex and the metabolic syndrome in the association with ALT, analyses were conducted in the whole population. All analyses were adjusted for age, sex, smoking, alcohol consumption, and the use of lipid-lowering and antihypertensive medication.

All statistical analyses were performed using the SPSS package version 15.0 (SPSS, Chicago, IL), and statistical significance was set at $P < .05$.

3. Results

The basic characteristics of the study population are shown in Table 1. The prevalence of the metabolic syndrome was 53.7%. Subjects with the metabolic syndrome had higher levels of plasma of ALT, NEFA, and inflammatory adipokines; had worse endothelial function; and were more insulin resistant compared with those without the metabolic syndrome.

After adjustment for age, sex, smoking, alcohol consumption, and use of lipid-lowering and antihypertensive medication, subjects with the metabolic syndrome had significantly higher levels of ALT than those without (linear regression coefficient β [95% confidence interval] = 0.67 SD [0.49-0.85], $P < .001$). The metabolic syndrome was also positively associated with insulin resistance, inflammatory adipokines, endothelial dysfunction, and NEFA (Table 2); and all these 4 potential mediators were also associated with ALT (Table 3, model 1), even after additional adjustment for the metabolic syndrome (Table 3, model 2).

The differences in ALT levels between subjects with and without the metabolic syndrome were attenuated by adjustment for insulin resistance (77.3%; Table 4, model 2) and, to a lesser extent, by adjustment for inflammatory adipokines (20.7%, model 3a), endothelial dysfunction (13.1%, model 4a), or NEFA (9.5%, model 5a). Of note, mediation by endothelial dysfunction (1.8%, model 3b) and inflammatory adipokines (–0.4%, model 4b) did not add to that by insulin resistance. This was different for NEFA, which by themselves mediated 9.5% of the association and added 4.4% to the mediation of insulin resistance (model 5b).

When the metabolic syndrome, insulin resistance, inflammatory adipokines, endothelial dysfunction, and NEFA were added in a full model including also age, sex, smoking, alcohol consumption, and use of lipid-lowering and antihypertensive medication, insulin resistance (0.41 [0.30-0.51], $P < .001$), endothelial dysfunction (0.21 [0.06-0.036], $P = .005$), and NEFA (0.13 [0.05-0.21], $P = .002$), but not the metabolic syndrome or inflammatory adipokines, were significantly associated with plasma ALT.

Table 1 – Characteristics of the Cohort on Diabetes and Atherosclerosis Maastricht according to the absence or presence of the metabolic syndrome

	Without MetS (n = 201)	With MetS (n = 233)	P value
Men/women (n)	119/82	145/88	.519
Age (y)	58.7 ± 7.5	60.2 ± 6.7	.034
Waist (cm)	93.0 ± 9.8	104.5 ± 10.8	<.001
HDL cholesterol (mmol/L)	1.36 ± 0.31	1.01 ± 0.25	<.001
Triglycerides (mmol/L)	1.1 (0.8-1.4)	1.8 (1.4-2.2)	<.001
Systolic blood pressure (mm Hg)	134 ± 19	145 ± 18	<.001
Diastolic blood pressure (mm Hg)	79 ± 9	84 ± 9	<.001
Fasting plasma glucose (mmol/L)	5.4 ± 0.6	6.5 ± 1.6	<.001
ALT (mmol/L)	18.5 (14.6-24.2)	23.8 (19.4-31.4)	<.001
Use of antihypertensive medication (%)	23.9	50.2	<.001
Use of lipid-lowering medication (%)	12.9	23.2	.006
T2DM (%)	6.0	36.5	<.001
HOMA2IR	0.89 (0.73-1.09)	1.5 (1.2-2.2)	<.001
sE-selectin (ng/mL)	68 (48-89)	91 (68-120)	<.001
sVCAM-1 (ng/mL)	447 (386-512)	474 (387-566)	.021
vWF (% of criterion standard)	116 (92-159)	122 (93-163)	.596
Endothelial dysfunction score	−0.17 ± 0.58	0.14 ± 0.62	<.001
IL6 (pg/mL)	1.1 (0.8-1.7)	1.6 (1.1-2.4)	<.001
SAA (μg/mL)	6.3 (4.0-15.0)	8.0 (4.6-14.6)	.320
sICAM-1 (ng/mL)	315 (272-367)	356 (307-411)	<.001
Adiponectin (μg/mL)	8.3 (6.4-11.5)	6.4 (4.3-8.2)	<.001
Leptin (ng/mL)	7.2 (3.7-16.8)	13.0 (8.1-24.4)	<.001
Inflammatory adipokine score	−0.24 ± 0.52	0.21 ± 0.51	<.001
NEFA (mmol/L)	0.47 (0.36-0.57)	0.52 (0.43-0.61)	<.001

Data are expressed as number or frequency (percentage), mean ± standard deviation, or median (interquartile range). MetS indicates metabolic syndrome; HDL, high-density lipoprotein.

3.1. *Additional analyses*

In the above-described analyses, we used an average z score for inflammatory adipokines assuming that these markers represent adipose tissue–associated inflammation as often seen in the metabolic syndrome. To exclude that our current results merely represent general low-grade inflammation, the analyses were repeated with C-reactive protein as potential mediator. C-reactive protein did not essentially mediate the association of the metabolic syndrome with ALT; mediation by C-reactive protein was 4.2% as compared with the 20.7% that was observed for the inflammatory adipokine score.

It has been reported that plasma ALT levels may underestimate liver fat content in T2DM patients [29]. The analyses reported above were therefore repeated without subjects with T2DM, and the results of these analyses did not materially differ from what was reported above. The analyses were also repeated with a more stringent cutoff value for alcohol consumption, that is, by excluding all subjects who consume more than 20 g alcohol daily because this is the proposed threshold to distinguish between nonalcoholic and alcoholic FLD [20]. Again, the results of these analyses did not materially differ from what was reported above (data not shown).

Table 2 – Associations of the metabolic syndrome with insulin resistance, endothelial dysfunction, inflammatory adipokines, and NEFA

Dependent variables	Independent variable: metabolic syndrome	
	β (95% CI)	P value
HOMA2IR	1.094 (0.932; 1.255)	<.001
Inflammatory adipokines	0.391 (0.291; 0.490)	<.001
Endothelial dysfunction	0.212 (0.099; 0.324)	<.001
NEFA	0.363 (0.169; 0.558)	<.001

All analyses were adjusted for age, sex, smoking, alcohol consumption, and use of lipid-lowering and antihypertensive medication. β indicates difference in dependent variable (expressed in SD) between subjects with vs without the metabolic syndrome; CI, confidence interval; NEFA, non-esterified fatty acids.

4. Discussion

In this study, we showed that, among the several metabolic syndrome–associated processes that were investigated, insulin resistance was the strongest mediator of the association between the metabolic syndrome and ALT, although inflammatory adipokines, endothelial dysfunction, and NEFA also explained a part of the association between the metabolic syndrome and ALT, but to a lesser extent. Inflammatory adipokines and endothelial dysfunction, however, did not add to the mediation by insulin resistance, whereas approximately half of the mediation by NEFA was additional to that by insulin resistance.

Because of well-known mutual relations between the metabolic syndrome, insulin resistance, and steatosis [30], the strong mediation by insulin resistance was largely anticipated. A more interesting finding is that although inflammatory adipokines were by themselves moderate mediators of the association between the metabolic syndrome and ALT, this effect was abolished when insulin resistance was also included in the model. This suggests that these processes, at least partly, represent the same mechanism(s). The overlap between mediation by inflammatory adipokines and insulin resistance may be explained by the fact that compromised insulin signaling and/or hyperinsulinemia coincides with an altered production of these adipokines. Data in the literature show that the production of leptin by human adipocytes is regulated by hyperinsulinemia and/or insulin signaling [31], as is the production of adiponectin [32,33] and IL6 [34,35]. Insulin signaling affects ICAM expression in leukocytes [36], and high insulin concentrations increase the expression of ICAM on endothelial cells [37]. Some of these adipokines may subsequently or perhaps concomitantly worsen (adipose) insulin resistance via activation of inflammatory pathways [38]. Thus, our data suggest that inflammatory adipokines may be responsible for a part of the mediating effect of insulin resistance. Our finding that it is inflammation of adipose tissue rather than general inflammation accentuates the importance of adipose tissue dysfunction in the

Table 3 – Associations of insulin resistance, endothelial dysfunction, inflammatory adipokines, and NEFA with ALT

Independent variables	Dependent variable: log-ALT			
	Model 1: adjusted for age, sex, smoking, alcohol consumption, medication		Model 2: adjusted for age, sex, smoking, alcohol consumption, medication, metabolic syndrome	
	β (95% CI)	P value	β (95% CI)	P value
HOMA2IR	0.514 (0.432-0.595)	<.001	0.472 (0.375-0.569)	<.001
Inflammatory adipokines	0.523 (0.358-0.688)	<.001	0.355 (0.184-0.525)	<.001
Endothelial dysfunction	0.499 (0.347-0.652)	<.001	0.413 (0.265-0.561)	<.001
NEFA	0.229 (0.139-0.319)	<.001	0.178 (0.092-0.265)	<.001

β indicates difference in log-ALT (expressed in SD [1 SD = 0.16 log(mmol/L)]) per 1-SD increase in each independent variable; NEFA, non-esterified fatty acids; ALT, alanine aminotransferase; HOMA2IR, homeostasis model assessment of insulin resistance.

association of the metabolic syndrome with ALT and supports the use of the inflammatory adipokine score.

The observed (modest) mediation by endothelial dysfunction in the association between the metabolic syndrome and ALT also disappeared in the presence of concomitant adjustments for insulin resistance. This may be explained by direct effects of hyperinsulinemia and/or insulin signaling on endothelial dysfunction [39] and additionally by effects of inflammatory adipokines on endothelial dysfunction [40]. Because we have only systemic markers of endothelial dysfunction available and because of the cross-sectional design of our study, we currently cannot distinguish between the possibility that local insulin resistance in the liver is causal in the generation of fatty liver and the alternative explanation that an overall status of endothelial dysfunction is merely a reflection of the insulin-resistant state. Although this was not the primary aim of our analyses, we did observe that endothelial dysfunction was associated with ALT, independently of insulin resistance and the other potential mediators considered, suggesting that it can also be an operative pathophysiological mechanism linking risk factors other than the metabolic syndrome to ALT. Taken together, although these data must be interpreted with caution, our findings support the possibility that insulin resistance–associated adipose tissue inflammation and hepatic microvascular dysfunction might actually contribute to the development and/or progression of ALT/NAFLD in the metabolic syndrome.

Increased plasma NEFA concentrations are a hallmark of the metabolic syndrome, and it is generally accepted that plasma NEFA contribute to hepatic triglyceride accumulation and the development of NAFLD [10]. We had hypothesized that mediation of the association between the metabolic syndrome and ALT by NEFA would be similar to that by inflammatory adipokines because both adipose insulin resistance and adipose inflammation are known to induce fatty acid release from adipose tissue. However, NEFA mediated the association more modestly than inflammatory adipokines (9.5% vs 20.7%); but despite this and in contrast to the latter, mediation by NEFA was not completely abolished in the presence of concomitant mediation by insulin resistance. Our data suggest that half of the mediation attributable to NEFA may occur via pathways included in the process of adipose tissue insulin resistance and inflammation, whereas the remaining half most likely occurs via an insulin resistance–independent mechanism. Data in the literature report that in upper-body obese women, upper-body subcutaneous, not visceral, fat was main source of plasma NEFA [41]; and systemic NEFA have been reported to be a major source of the extrahepatic fatty acids that accumulate in NAFLD [10].

Table 4 – Associations of the metabolic syndrome with ALT and the mediation by insulin resistance, endothelial dysfunction, inflammatory adipokines, and NEFA

Model	Adjustments	Independent variable: metabolic syndrome			
		β (95% CI)	P value	Mediation [a] (%)	Additional mediation [b] (%)
1	Age, sex, smoking, alcohol consumption, medication	0.671 (0.490 to 0.851)	<.001		
2	Model 1 + HOMA2IR	0.152 (–0.043 to 0.347)	.127	77.3	
3a	Model 1 + inflammatory adipokines	0.532 (0.343 to 0.721)	<.001	20.7	
3b	Model 1 + HOMA2IR + inflammatory adipokines	0.140 (–0.056 to 0.337)	.161	79.1	1.8
4a	Model 1 + endothelial dysfunction	0.583 (0.406 to 0.760)	<.001	13.1	
4b	Model 1 + HOMA2IR + endothelial dysfunction	0.155 (–0.038 to 0.348)	.115	76.9	–0.4
5a	Model 1 + NEFA	0.607 (0.427 to 0.786)	<.001	9.5	
5b	Model 1 + HOMA2IR + NEFA	0.123 (–0.070 to 0.317)	.211	81.7	4.4

β indicates difference in ALT (expressed in SD) between subjects with vs without the metabolic syndrome.

[a] Indicates change in the magnitude of the regression coefficient (β) as compared with model 1 (expressed in percentage).

[b] Indicates the difference in the percentage attenuation by HOMA2IR and inflammatory adipokines (model 3b), by HOMA2IR and endothelial dysfunction (model 4b), or by HOMA2IR and NEFA (model 5b) in comparison to the percentage mediation by HOMA2IR only (model 2). NEFA, non-esterified fatty acids; ALT, alanine aminotransferase; HOMA2IR, homeostasis model assessment of insulin resistance.

These NEFA may contribute to hepatic steatosis partly via increased insulin resistance and inflammation as described above and partly via direct uptake into hepatocytes. These latter associations of dietary fatty acids and direct uptake of subcutaneous adipose tissue–derived NEFA may also be reflected by the significant independent contribution of NEFA to plasma ALT levels in the full model (including all potential mediators).

In this study, we particularly focused on potential mediators that were not included in the metabolic syndrome because these may provide insight in the metabolic pathways that are intermediate between the metabolic syndrome and ALT levels. We did not investigate the extent to which each of the individual components of the metabolic syndrome contributed to the relation between the metabolic syndrome and ALT levels. Some prospective studies showed that plasma concentrations of ALT could predict the metabolic syndrome [42,43]. The latter also showed that a substantial part of this association was explained by insulin resistance, which is in line with our current findings.

A limitation of our study may be the use of ALT levels as a measure of hepatic fat accumulation. However, although ALT levels are not the ideal measure of hepatic fat accumulation, the associations of ALT with the metabolic syndrome and with insulin resistance, inflammation, NEFA, and endothelial dysfunction reported herein are in agreement with those reported by others when other measures for hepatic fat accumulation were used [9,16,44,45]. Other limitations of our study include its cross-sectional design, which prohibits conclusions about causality, and the fact that we studied middle-aged white persons, which excludes extrapolation of our results to other age categories or other ethnicities.

In conclusion, we showed that insulin resistance mediates up to 75% to 80% of the association of the metabolic syndrome with ALT. Our findings additionally suggest that insulin resistance and its associated adipose tissue inflammation and endothelial dysfunction may contribute to the progression of fatty liver disease (measured as ALT) via a common pathophysiological mechanism, whereas the effect of NEFA may be partly independent of insulin resistance. Treatment of insulin resistance, which may concomitantly ameliorate endothelial dysfunction and the amount of circulating inflammatory cytokines, thus remains a main target for interventions aiming at the prevention of NAFLD in these individuals. Circulating levels of NEFA may also constitute an additional treatment target.

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