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A minireview on the diagnostic role of serum alanine aminotransferase levels in insulin resistant states

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INTRODUCTION

Non alcoholic steatohepatitis (NASH) is a hepatic insulin resistant state very much prevalent in both developed and developing countries.

NAFLD causes asymptomatic elevation of the level of liver enzymes alanine aminotransferase (ALT), aspartate aminotransferase (AST) and γ -glutamyltransferase (GGT)^[2]. Of these liver enzymes, ALT is most closely related to liver fat accumulation^[3] and consequently ALT has been used as marker of NAFLD. GGT may also be elevated in patients with NAFLD, but only limited data on its occurrence and degree of elevation are available. Furthermore, the correlation of liver fat measured by proton-MR spectroscopy with GGT is markedly lower than with ALT, possibly because GGT is also produced in other tissues^[4,5]. Finally, to the best of our knowledge no direct validation of GGT with fat content in liver biopsy specimen has been reported. Several cross-sectional studies have found associations of ALT with several features of the metabolic syndrome. At present, ALT is often used in epidemiological studies as a surrogate marker for NAFLD. In addition, recent prospective epidemiological studies have demonstrated that ALT is associated with future risk of type 2 diabetes mellitus and the metabolic syndrome^[5-12].

This review discusses the biochemical and meta-

bolic properties of ALT, its applicability as a marker of NAFLD and describes its possible role in the pathogenesis of the metabolic syndrome and type 2 diabetes mellitus and subsequent cardiovascular disease (CVD).

ALT catalyses the reversible transamination between L-alanine and α -ketoglutarate to form pyruvate and L-glutamate and as such has an important role in gluconeogenesis and amino-acid metabolism. The reaction is reversible, but the equilibrium of the ALT reaction favours the formation of L-alanine. The vitamin B6 vitamers pyridoxal-5'-phosphate (PLP) and its amino analogue pyridoxamine-5'-phosphate function as co-enzymes in the amino transfer reaction. ALT enzyme activity is primarily found in the liver, but its activity, although much lower, is also found in many other organs including muscle, heart, kidney, brain and adipose tissue^[13-15].

In 1957, Wroblewski and Cabaud described a colorimetric ALT assay based on measurement of glutamate by paper chromatography^[16]. In later assays, the transamination reaction was coupled to a second reaction involving the reduction of pyruvate to lactate by lactate dehydrogenase^[17]. In this reaction nicotinamide adenine dinucleotide (NADH) is oxidised to NAD⁺ and the decrease in NADH is assessed by measuring the absorption at 340 or 334 nm. In the past, comparison between ALT activities reported by differ-

ent laboratories was hampered by the fact that assay temperatures ranging from 25°C to 37°C were used, leading to large inter-laboratory differences in reported ALT activities and reference values. The International Federation of Clinical Chemistry and Laboratory Medicine established in 2002 a reference system for the measurement of enzyme activity of clinically important enzymes, including ALT, to be measured at 37°C^[18]. Levels of 10-45 U/l are considered as normal, although reference values may still vary among laboratories^[24]. ALT activity is stable for 24 h in whole blood, with a marked decrease thereafter. In serum, ALT enzyme activity is stable for 3 days at room temperature and for 3 weeks in the refrigerator, but a marked decrease is observed after repeated freezing and thawing^[19-21]. It is therefore strongly advisable not to determine ALT activity in frozen samples, as a falsely decreased ALT activity may be found. Day-to-day variations of 10-30% have been observed^[22]. Moreover, significant diurnal variations have been reported in both healthy subjects and in patients with chronic liver disease (CLD), necessitating standardised blood sampling times when comparing enzyme values in clinical practice and research^[23]. The AST/ALT ratio is however greater than 1 in alcoholic liver disease and less than 1 in non-alcoholic steatohepatitis.

Early biochemical studies have suggested the existence of two isoforms of ALT in humans. On the basis of the amino-acid sequence of liver ALT^[24]. Sohocki and colleagues have cloned the human ALT1 located on human chromosome 8q24.^[25] Recently, Yang and colleagues cloned the second isoform ALT2 that was mapped to the human chromosome 16q12.1 and demonstrated that this isoform was mainly expressed in muscle and adipose tissue^[26]. The expression of ALT2 in adipose tissue may indicate that this isoform may contribute to the homeostasis of fatty acid metabolism and storage. In fatty livers of obese mice, Jadhao and colleagues demonstrated that murine ALT2 gene expression was induced two-fold, whereas expression of murine ALT1 remained the same, and the total hepatic ALT enzyme activity was elevated by 30%^[27]. These observations indicate that ALT2 may be responsible for the increased ALT activity in hepatic steatosis. In the pathogenesis of NAFLD, the role of different isoforms in humans has not been studied yet. With the

standard ALT assay used in clinical practice, enzyme activity of both ALT isoforms is measured.

ALT and fatty liver

Fatty liver content can be assessed with different methods. Direct measurement of hepatic fat by means of liver biopsies, which is regarded as the 'gold standard'^[28], although questionable because of the unknown distribution of fatty infiltration in the liver, has only limited applicability in epidemiological and clinical studies, because of the elaborate and invasive nature and associated risks of this technique. Furthermore, no consensus exists on the standardization of diagnostic criteria based on liver histology, although only recently a scoring system for NAFLD has been proposed^[29] 36. Imaging techniques, such as ultrasound, computed tomography and proton magnetic resonance spectroscopy (¹H-MRS), are non-invasive and can be used rather than biopsies^[30,31]. Ultrasound of the liver is another non-invasive technique, which can be used to indicate the presence or absence of fatty infiltration of the liver; however, at present, this technique at its best can provide only semi-quantitative data with regard to NAFLD. However, non-invasive techniques cannot differentiate between fatty liver alone and steatohepatitis. ¹H-MRS has been validated against direct determination of triglyceride content in human liver biopsies^[32], and may be used for clinical purposes, but has limited applicability in larger populations because of logistics and high costs. In more recent epidemiological studies, ALT has been used as a surrogate marker for liver fat accumulation. This was first described by Nanji and colleagues in 1986, who reported an association of liver enzymes (i.e. the ALT to AST ratio) with fatty liver in obese patients^[33]. Studies assessing the specificity and sensitivity of ALT as a marker of NAFLD are limited^[34,35]. Prati and colleagues have suggested new cut-off values for ALT in order to facilitate the identification of subjects with NAFLD^[36]. However, since cut-off values depend on the assay used, this proposal has been questioned by others^[37]. One study has assessed the correlation of ALT with ¹H-MRS and found a modest, but significant, correlation ($r = 0.5$).

In order to avoid confounding by other factors that might cause ALT elevation, a detailed history on alcohol intake is mandatory to exclude those patients with

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ALT elevation caused by alcohol abuse, and to address alcohol intake as a possible confounder or effect modifier. Viral and auto-immune liver diseases and hepatotoxic medication should be considered as well. With these limitations in mind, ALT is an acceptable marker for hepatic steatosis in epidemiological studies; its use for diagnostic and monitoring purposes in clinical care needs to be further delineated.

ALT and incident metabolic syndrome and type 2 diabetes mellitus

The putative role of the liver in the pathogenesis of type 2 diabetes mellitus has gained much interest, and several cross-sectional and prospective studies have shown associations of ALT with type 2 diabetes mellitus and the metabolic syndrome. Several cross-sectional studies have demonstrated that ALT is related to features of the metabolic syndrome and type 2 diabetes mellitus^[38-40]. Clark and colleagues (23), for example, found in their analysis of the National Health and Nutrition Examination Survey (NHANES) that up to 31% of the elevated aminotransferase activity could be explained by high alcohol consumption, hepatitis B or C infection and/or high transferrin saturation. In the remaining 69%, the elevated ALT activity was significantly associated with higher body mass index, waist circumference, triglycerides, fasting insulin and lower HDL cholesterol. Additionally, in women this elevation was associated with type 2 diabetes mellitus and hypertension^[41]. Several studies addressed the prospective relation of ALT with the metabolic syndrome and type 2 diabetes mellitus. Nakanishi and co-workers found that of the liver enzymes studied, ALT was associated with future risk of metabolic syndrome in middle-aged Japanese men, but used body mass index instead of waist circumference to define the metabolic syndrome. Hanley and co-workers studied the relation of four different liver enzymes (including ALT) with the development of the metabolic syndrome in a multiethnic cohort and demonstrated that ALT was associated with an increased risk of incident metabolic syndrome^[42].

ALT and cardiovascular disease

Several studies have demonstrated associations between NAFLD or ALT and markers of atherosclerosis or inflammation. An association of ALT with endothe-

lial dysfunction was demonstrated, measured as flow-mediated vasodilatation^[43]. Kerner and colleagues found a relation of ALT with C-reactive protein^[44]. Only a limited number of studies have addressed the prospective relation of ALT with CVD and mortality. A recent study by Nakamura and co-workers found a positive association of ALT with all-cause mortality in Japanese men and women, but only for those with a body mass index below the median (22.7 kg/m²)^[45].

Pathogenic mechanisms

To date, an elevated ALT is considered a consequence of hepatocyte damage due to NAFLD. However, the measured plasma elevations of ALT may also be a consequence of high systemic ALT2 isoform levels that is largely derived from adipose tissue in obesity and insulin resistance, as has been observed in mice^[46]. Insulin resistance, increased pro-inflammatory cytokine production, oxidative stress and mitochondrial dysfunction leading to hepatocyte damage/destruction, have all been posed as important pathophysiological mechanisms. Indeed a recent study reported on the association of ALT and GGT with markers of inflammation and oxidative stress^[47].

Adiponectin, released by adipose tissue has been implied in the pathogenesis of NAFLD. Obese subjects and patients with type 2 diabetes mellitus have lower levels of adiponectin compared to healthy controls. Yokoyama and colleagues found an inverse association of ALT, AST and GGT with adiponectin levels in Japanese male workers. These associations were independent of age, body mass index, insulin resistance, serum triglycerides and total cholesterol^[48]. Aygun and co-workers found that adiponectin levels were lower in patients with biopsy-proven NAFLD and elevated liver enzymes, compared to patients with NAFLD and normal liver enzymes and to those without NAFLD^[49]. The exact mechanisms by which adiponectin is related to NAFLD are not fully elucidated and need further clarification.

Insulin resistance and visceral obesity have a major impact on regulatory processes of the (postprandial) lipoprotein and glucose metabolism. The normal suppression of lipolysis by insulin is impaired in the insulin-resistant state and this leads to an elevated release of free fatty acids from the adipose tissue and

subsequent accumulation in hepatocytes, further enhancing hepatic steatosis and contributing to increased risk of atherothrombotic disease in subjects with NAFLD and insulin resistance. Dietary fat composition also seems to influence the degree of hepatic steatosis, although the majority of triglycerides arise from free fatty acids derived from adipose tissue lipolysis^[50]. It has been demonstrated that a fatty liver is insulin resistant, resulting in an elevated glucose and very low-density lipoprotein production. Furthermore, cytokines released from the (visceral) adipose tissue, including interleukin-6 and tumour necrosis factor-alpha, both associated with decreased hepatic insulin sensitivity^[51], may further enhance fatty infiltration and decrease hepatocyte integrity.

Another explanation might be the up-regulation of ALT enzyme activity. Among the amino acids, alanine is the most effective precursor for gluconeogenesis^[52]. Gluconeogenesis is increased in subjects with type 2 diabetes mellitus^[53], owing to increased substrate delivery (e.g. alanine), and an increased conversion of alanine to glucose^[54]. This increased conversion might contribute to the increased ALT activity, as previously observed in alloxan-diabetic rats^[55].

ALT might thus be up-regulated as a compensatory response to the impaired hepatic insulin signalling or, alternatively, may leak more easily out of the hepatocytes as a consequence of fatty infiltrations and subsequent damage.

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