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MEDICAL FACULTY

The Final thesis

Liver Enzymes and Function in Patients with Metabolic Syndrome. Literature review

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CONTENT

1. SUMMARY

This literature review highlights the existing association between liver function markers and metabolic syndrome. It presents the results of three studies conducted in Asia within the past decade with different focuses and approaches, but all converging on this correlation. Special emphasis was given to the correlation between single liver enzymes as alanine transferase, aspartate transferase and gamma-glutamyl transferase with metabolic abnormalities. The objective is therefore, to find out more about the particular role of each of these biomarkers in the pathophysiology of metabolic syndrome. These results are subsequently compared with those of other recent studies and concludes that the association between liver function markers and metabolic syndrome can be confirmed.

2. KEYWORDS AND ABBREVIATIONS

metabolic syndrome, liver enzymes, biomarker, ALT, AST, AST/ALT ratio, GGT, NAFLD, type 2 diabetes mellitus

Table 1 - Abbreviations

Abbreviation	*meaning*
AACE	American Association of Clinical Endocrinology
AHA/NHLBI	American Heart Association/National Heart, Lung, and Blood Institute
ALP	alkaline phosphatase
ALT	alanine aminotransferase
AST	aspartate aminotransferase
CI	Confidence interval
CVDs	cardiovascular diseases
DBP	diastolic blood pressure
EGIR	European Group for the study of Insulin Resistance
FBG	fasting blood glucose
FFA	Free fatty acids
GGT	gamma-glutamyl transferase
HDL	High density lipids

IDF	International Diabetes Federation
LDL	low density lipoproteins
MA	metabolic abnormality
MetS	Metabolic syndrome
NAFLD	nonalcoholic fatty liver disease
NASH	non-alcoholic steatohepatitis
NCEP ATP III	Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) final report (1)
OR	Odds ratio
S.D	Standart deviation
SBP	Systolic blood pressure
T2DM	type II diabetes mellitus
TGL	triglycerides
VLDL	very low density lipoproteins
WHO	World Health Organization

3. INTRODUCTION

Metabolic syndrome (MetS) is a global health problem that significantly rises in its occurrence worldwide. In Europe impressive numbers of around of 25-30% affected adults are reported (2). Yet more alarming statistics can be observed in Asia. "[The numbers are said to have risen] from 6.6% to 25.6% in the Chinese population, while the latest report showed that this figure jumped to 33.9%". (3)

Diagram 1 illustrates the uneven prevalence of MetS in 15 different ethnic groups around the world. For example, in urban populations in India 8% suffer from MetS, whereas 24% of men in the USA are affected (4). Comparing the prevalence in the USA among people of different ethnic origins was particularly interesting. Native American women have almost 40% higher prevalence than non-Hispanic white women living in the same location with ~20% prevalence. This highlights the influence of not only acquired entities, but also genetic and epigenetic factors. (5)

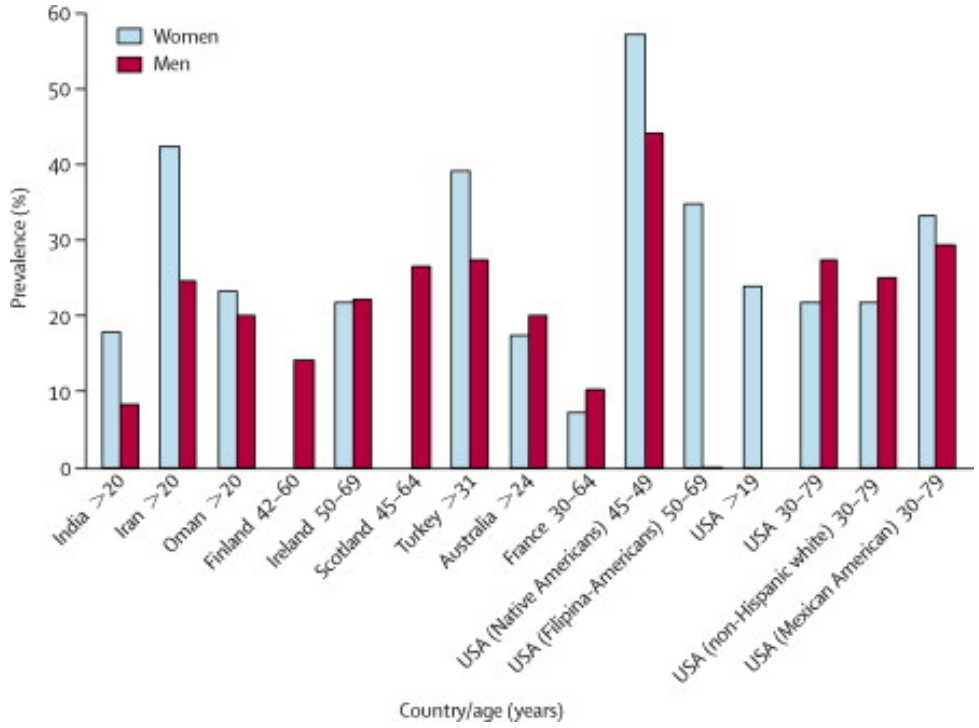

Diagram 1 - Prevalence of the metabolic syndrome from ATPIII definition, published 2005 (4)

Asian countries may not yet have the highest MetS prevalence worldwide, but the population has undergone major lifestyle transitions in the past decades. The numbers of MetS patients are increasing rapidly. Additionally, the contribution of genetic factors, such as certain associated genetic variants, to an increased risk of type II diabetes is being discussed. Ronald C. W. Ma writing in The New York Acadamy of Sience, states that “There is an epidemic of diabetes in Asia”. (6)

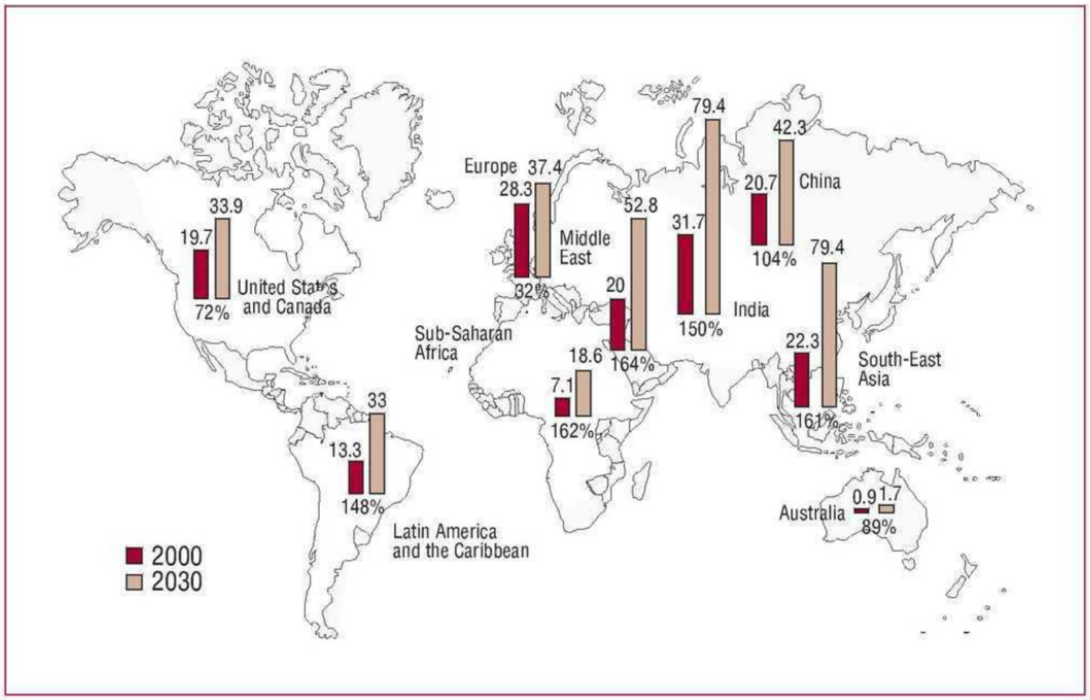

Figure 1 - Diabetes cases (in millions of individuals) in 2000 and predicted for 2030 (7)

Figure 1 underlines this statement drastically, by estimating diabetes cases worldwide for 2023 based on the current trends. Today, Europe is the leading continent with 28.3 million people diagnosed, but with the rapidly increasing trend in Asia, the prevalence will soon be significantly higher. With an estimated rise of 150% in India in 30 years, 79.4 million people will be diagnosed with diabetes. Both the Middle East and South-East Asia share this trend with a positive trend of more than 160%. In Europe, however, the increase is estimated to be only 32%, to a total of 37.4 million people. Considering MetS as a precursor to diabetes, the development in Asia is leading to several local clinical trials, such as community-based health-check investigations. (8)(9)(10) These large cohorts allow for representative research, which is not carried out to such an extent in Europe.

If left untreated, MetS significantly increases the risk of developing type II diabetes mellitus (T2DM), cardiovascular diseases (CVDs) and non-alcoholic fatty liver disease (NAFLD). Given that CVDs are the leading cause of morbidity and mortality worldwide, accounting for 32% of all global deaths according to the WHO, it is crucial to investigate the impact of MetS in this context. (11)(12) The prevalence of MetS can be attributed to several factors, such as ethnicity, alcohol consumption, smoking, diets that are high in refined carbohydrates and saturated fats, as well as a sedentary lifestyle (13).

Worldwide, there exist multiple definitions of MetS from various institutions. In general, the criteria share a number of similarities, including: increased blood pressure, insulin resistance, waist circumference and abnormal cholesterol levels. (14) When assessing patients suspected of having MetS, current blood chemistry tests are typically used to test for hyperglycemia, renal dysfunction, and lipid abnormalities, with liver function and enzymes play a minor role. Principally, they are used as indicators of primary liver disorders of hepatic or cholestatic origin (3). Standardized liver function tests include alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and gamma-glutamyl transferase (GGT). Currently, numerous studies are investigating the role of liver enzymes as potential predictors of non-liver-related diseases such as stroke, dementia, colorectal adenoma and more (13)(15)(16). This report grows on the hypothesis about a correlation between elevated serum aminotransferase levels and MetS. (13)

By gaining a better understanding of MetS, researchers and healthcare professionals can develop targeted strategies to improve patient outcomes and reduce the burden of this condition on individuals and society as a whole. This report aims to highlight the potential benefits of incorporating liver enzymes as a novel biomarker for MetS diagnosis. The inclusion of liver enzymes as a biomarker for MetS diagnosis has the potential to improve patient risk stratification and earlier detection of the syndrome. Furthermore, this approach may enhance comparability between studies, thus contributing to a better understanding of the disease. The objective is therefore, to find out more about the particular role of each of these biomarkers in the pathophysiology of metabolic syndrome.

4. LITERATURE SELECTION STRATEGY

To ensure comparability of the studies, inclusion and exclusion criteria needed to be set. Therefore, recent studies with similar approaches were chosen from the platform PubMed and THE LANCET. First, all available studies published in or translated to English, released in the past decade were assessed for their relevance to the research question. In view of the objective, a geographical limitation was made to studies in the Asian region. Furthermore, the materials and methods used were assessed for validity and reliability. Particular attention was paid to the diagnostic criteria for the MetS. Studies were included if they used Adult Treatment Panel III (ATP III) or "the International Diabetes Federation Task Force" diagnostic criteria.

Table 2 - Diagnostic criteria of metabolic syndrome according to "Adult Treatment Panel III (ATP III)" and "International Diabetes Federation Task Force"

Adult Treatment Panel III (ATP III)	International Diabetes Federation Task Force
1) WC ≥ 40 inches in men, 35 inches women) 2) SBP ≥ 130/85 mmHg 3) TG ≥ 150 mg/dl 4) HDL < 40 mg/dl (men) or 50 mg/dl (women) 5) FG ≥ 100 mg/dl	1) WC ≥ 90 cm in men, ≥ 80cm in women 2) TG ≥ 1.70 mmol/L 3) HDL < 1.03 mmol/L in men, < 1.29mmol/L in women 4) SBP ≥ 130 mmHg or DBP ≥ 85 mmHg 5) FG ≥ 5.60 mmol/L

Other inclusion criteria were the assessment of transaminases AST and ALT. The kinetic, ultraviolet and oxidase-peroxidase methods were acceptable. Waist circumference and the prevalence of diabetes also had to be determined. On the other hand, exclusion criteria were studies including participants with a history of liver diseases, alcohol intake or hepatotoxic drug intake.

It was then necessary to convert the main patient parameters into homogeneous units, as shown in Table 2. because standardized units allow a more accurate and understandable comparison. Meaning that the values of free blood glucose (FBG), total cholesterol (TC), triglycerides (TGL) and high-density lipids (HDL) are presented in the unit mg/dl. The cross-sectional studies ("*Study 1*" and "*Study 2*") help to assess the prevalence of elevated transaminases in MetS, by including significantly more participants than the case control study. Whereas "*Study 3*" is designed to determine whether elevated transaminases are associated with MetS. Certainly, the case control study comprises matched participants, explaining the equal

gender ratio. In contrast, both cross-sectional studies show an uneven allocation of male and female participants. This imbalance is perceived particularly in "*Study 2*", which poses a focus on the disease expression in different gender.

Table 3 - Descriptive statistics in " Study 1-3" (13) (17) (18)

Mean parameters	*Study 1: Is within-normal range liver enzymes associated with metabolic syndrome in adults?*	*Study 2: Association between liver function and metabolic syndrome in Chinese men and women*	*Study 3: A Study of AST/ALT Ratio in Metabolic Syndrome*
Type of study	Cross-sectional	Cross-sectional	Case control studies
Date of publication	Available online 03/08/2017	20/03/2017	01/2017
Place of publication	Clinics and Research in Hepatology and Gastroenterology (2018)	SCIENTIFIC RepoRts	International Journal of Contemporary Medical Research
Diagnostic criteria	NCEP ATP III criteria	the International Diabetes Federation Task Force	NCEP ATP III criteria
Number of participants	700	32 768	100
Gender ratio (male : female)	1 : 1.4	1.7 : 1	1 : 1
Diagnosed with MetS	259 (37%)	10 654 (32.51%)	50 (50%)
Age (years)	42.40 ± 12.38	48.65 ±11.05	40.96 ± 8.84
BMI (kg/m2)	27.69 ± 4.94	25.48 ±3.46	23.49 ± 2.78
Ethnicity	Iran	China	India
WC (cm)	92.96 ± 13.34	86.93 ±10.69	90.5 ± 5.65
SBP (mmHg)	120.67 ± 17.66	124.51 ±17.90	115.48 ± 14.6
DBP (mmHg)	78.18 ± 11.58	79.28 ±11.69	78.22 ± 6.35
FBG (mg/dl)	88.86 ± 23.88	95 ± 22	95 ± 8,63
TC (mg/dl)	-/-	202.49 ± 38.55	176.14 ± 38.28
TGL (mg/dl)	159.60 ± 98.66	154.87 ± 123	161.49 ± 21.75
HDL (mg/dl)	43.81 ± 10.24	53.349 ± 14.02	39.9 ± 3,7

Firstly presented and analyzed is the long term community-based project for prevention and control of non-communicable diseases in East Azerbaijan (Iran) including 3000 randomly enrolled participants between 15-65 years of age. The authors of "Is within-normal range liver enzymes associated with metabolic syndrome in adults?" ("*Study 1*") (13) worked with data collected in 2015 as a part of the major lifestyle promotion project. Focus was drawn to participants having normal range of ALT and AST, while those with self-reported chronic liver diseases were excluded. After the selection, a group comprising 700 Iranian adults resulted. Further, standard protocols were used to measure weight, height, and waist circumferences. MetS was diagnosed based on one of the most widely used diagnostic procedures, which requires the presence of three out of five criteria, as they are according to the NCEP ATP III. The liver enzymes AST and ALT were assessed using the ultraviolet method considering

normal range ALT in men < 40, ALT in women < 34 U/l and AST in both < 34 U/l. As this study worked with the Pearson correlation and Logistic regression it is useful as comparative study for statistical analysis. (13) The cross-sectional design of study is its main limitation. Consequently, this implies limitations in causal inferences.

Secondly, a large cohort comprising 32 768 seemingly healthy Chinese citizens (further referred to as "*Study 2*") investigated the association between MetS and liver function. Sen Wang and his co-workers published the article "Association between liver function and metabolic syndrome in Chinese men and women" in 2017, analyzing the results of the research project, focusing on characteristics of liver function in different gender. The comparative study was conducted in Tianjin Medical University General Hospital from 2011 till 2016, as cross-sectional and community-based health-check investigation, using the following criteria to diagnose MetS from "the International Diabetes Federation Task Force" (19)

Yet another comparative study was carried out at Thanjavur Medical College, Thanjavur, Tamilnadu *("Study 3")*. In January 2017, P. Deepa and N. Sasivathanam published the article "A Study of AST/ALT Ratio in Metabolic Syndrome" in the International Journal of Contemporary Medical Research, analyzing the results of 100 subjects (3). The researchers compared AST/ALT ratio in individuals with and without MetS to correlate it with the current diagnostic components of metabolic syndrome as waist circumference, fasting blood glucose and triglycerides levels. To establish the diagnosis of MetS participants were classified according to the already expounded NCEP ATP III criteria (see *"Study 1*"). Exclusion criteria correspond to those established for this literature review by excluding participants with evidence of hepatitis, alcohol use or hepatotoxic drug use. Two major research limitations are claimed by the authors: lack of dietary habit evaluation in the study group and absence of liver biopsy and imaging studies like ultrasound to establish the diagnosis of NAFLD.

As already remarked, there is a significant difference in size of the projects. "*Study 1*" includes in total 700 participants (287 male : 413 female) with 259 cases of MetS (88 / 32,61% male : 171 / 24,15% female). Explicitly larger is "*Study 2*" with 32 768 individuals (20 643 male : 12 125 female) with 37.72% males MetS positive and 23.65% of females. The average age varies within a span of ten years (~ 41 years till ~ 49 years of age) and the percentage of gender distribution of MetS patients is similar. In contrast, in "*Study 3*" from altogether 100 participants, 50 were diagnosed with MetS while the control group was composed of the other half, as healthy individuals, matched in age and gender. All three studies were carried out in

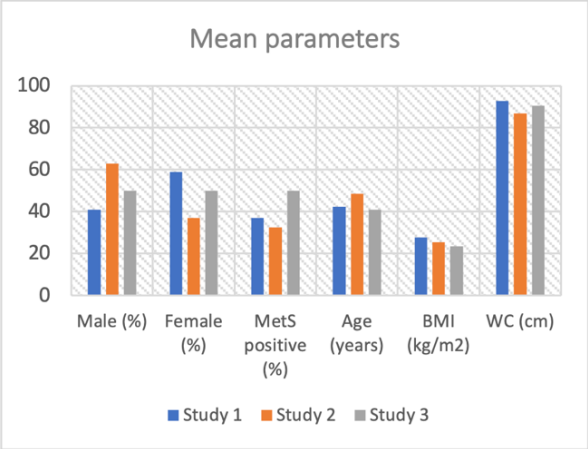

Diagram 2 – Comparison of descriptive statistics (13) (17) (18)

Asia, which facilitates the comparison from an ethnical perspective. Attention must be drawn to the fact that the average body mass index in "*Study 3*" with values of 23.49 ± 2.78 is within the range of normal weight (18.5 – 24.9), whereas "*Study 2*" average BMI is already classified as overweight (range: 25.0 – 29.9 (20)) and "*Study 1*" mean values are even manifest overweight 27.69 ± 4.94.

Overall, most of the participants mean parameters do not show significant differences (Diagram 2). Blood pressure is within the normal range in all groups and the mean results from the blood parameters listing FBG, TC (not taken in "*Study 3*"), TGL and HDL don´t show notable differences. Fasting blood glucose physiological range of 70 mg/dL till 100 mg/dL is with mean values of minimum 88.86 ± 23.88 mg/dL till maximum 95 ± 22 mg/dL not namable elevated (21). Likewise, mean total cholesterol and high-density lipids are within normal range in both studies, whereas mean levels of triglycerides with a maximum of 161.49 ± 21.75 mg/dL in "*Study 3*" exceeds the physiological limit of 150 mg/dl in all groups (22).

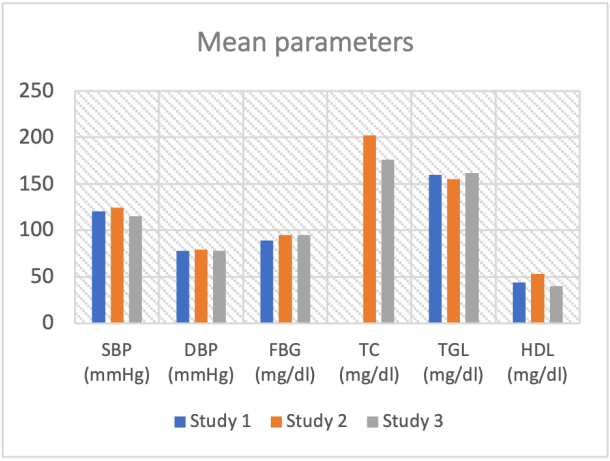

Diagram 3 - Comparison of descriptive statistics (13) (17) (18

Aside of those described studies the following research projects are used for comparison purposes. "Aminotransferase Levels and 20-year Risk of Metabolic Syndrome, Diabetes, and Cardiovascular Disease", by Goessling W and his team investigated the relationship between aminotransferase levels and the long-term risk of developing metabolic syndrome, diabetes, and cardiovascular disease. Specifically, the researchers aimed to determine whether higher levels of aminotransferases, namely ALT and AST, were associated with an increased risk of these conditions over a period of 20 years.

5. CORRELATION OF METABOLIC SYNDROME WITH LIVER ENZYMES

As an exocrine organ, the liver secretes enzymes as alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP) and gamma-glutamyl transferase (GGT). These substances produced by hepatocytes help with various functions such as breaking down food, detoxifying the body, and producing proteins. When the liver is damaged, these enzymes can leak into the bloodstream, causing elevated levels. (23)

Transaminases physiologically participate in gluconeogenesis but are simultaneously markers of hepatocellular injury. The parameter AST at itself is present as cytosolic and mitochondrial isoenzyme not exclusively found in the liver. Additional locations are tissues like skeletal and cardiac muscles, inner organs like kidneys, brain, pancreas and lungs, and in blood components as in leucocytes and erythrocytes. Therefore, AST elevation can also be seen due to secondary non-hepatic causes and is consequently neither as sensitive nor as specific for liver injury as ALT. In contrast, ALT is solely a cytosolic enzyme, found in high concentrations in hepatocytes. In case of liver tissue damage, the concentration of ALT released into the bloodstream is usually higher than AST. (23)
When analyzing these values, it must be noted that the liver is a highly sexually dimorphic organ and accounts for more than 72% of sexually differentiated genes. It is widely recognized that normal levels of liver enzymes, such as aspartate aminotransferase and alanine aminotransferase, tend to be higher in males compared to females. This sexual dimorphism may contribute to differences in the pathophysiological mechanisms involved in various acute and chronic liver diseases. (24) In addition, aminotransferases correlate with obesity, meaning a higher normal reference range in those with higher body mass index (25). (23)

Hepatic fat accumulation may cause damage to hepatocytes resulting in the release of AST and ALT enzymes from damaged liver cells into the blood. More specific, elevated level of ALT is directly associated with liver fat accumulation (26). Nevertheless, the accumulation of triglycerides in the liver can not be seen in direct correlation with liver damage. (27) The AST/ALT ratio is commonly used as a diagnostic tool to differentiate between alcoholic liver disease and NAFLD, as in alcoholic liver disease the ratio is often greater than 1, while in NAFLD the ratio is usually less than 1. However, it is important to note that this ratio is not specific to these two conditions and can also be influenced by other factors such as age, sex, body mass index, and the presence of other underlying liver diseases. Therefore, other

diagnostic tests such as imaging studies and liver biopsies may be needed for accurate diagnosis and treatment of liver diseases. (28) (29)

To visualize the pathophysiology of MetS, in Figure 2 the developmental stages are marked by numbers. The process begins with an excessive caloric and dietary fat intake, that provides a nutritional oversupply. Resulting is the production and consequently accumulation of adipose tissue, marked by number “1”. Expanded adipose tissue mass releases excessive free fatty acids (FFA), which is further transported to organs as liver, skeletal muscles, and blood vessels (number ”2”).

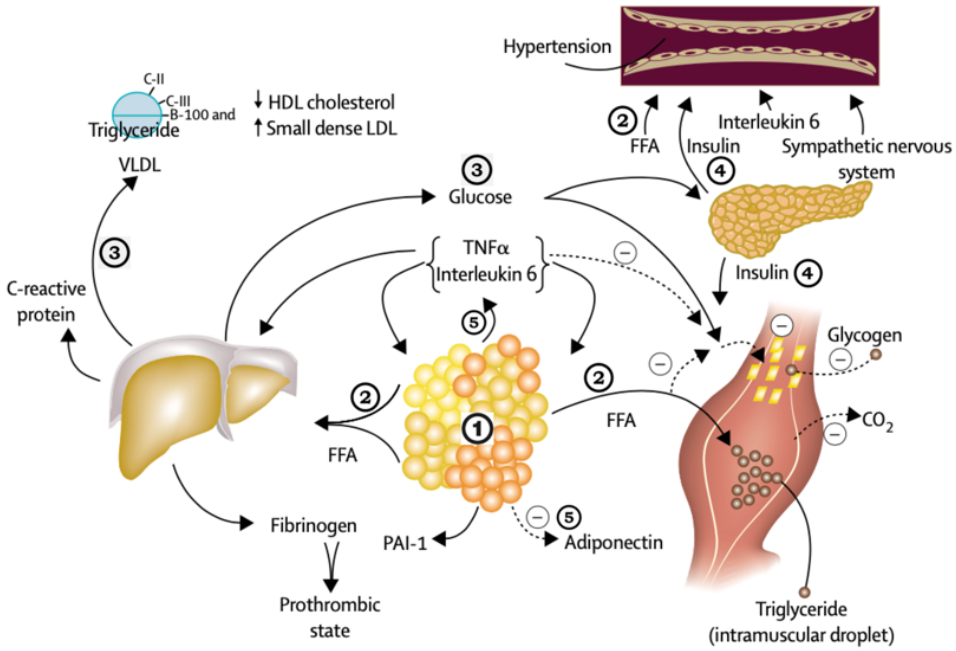

Figure 2 – Pathophysiology of MetS (4)

Each organ has a different response to an excess of FFA. The liver increases the secretion of glucose and triglycerides. In addition, the production of very low-density lipoproteins (VLDL) is enhanced (number “3”). This abnormally high amount of circulating glucose and to some extent FFA cause hyperinsulinemia due to increased pancreatic insulin secretion (number “4”). Eventually that state results in insulin resistance, which is defined as "an impaired response of insulin-sensitive tissues (e.g., liver, skeletal muscle, adipose tissue) to insulin that results in decreased glucose uptake and utilization, increased hepatic glucose production, and elevated plasma insulin concentrations." (30) This can eventually lead to hepatic insulin resistance and diabetes mellitus type II. Insulin resistance not only in the muscle and adipose tissue but also

in the liver is associated with an abundance of proinflammatory cytokines and relative deficiency of the anti-inflammatory cytokine adiponectin that is produced exclusively by adipocytes (number “5”). (31). Excessive hepatic fat accumulation and insulin resistance have a causal relationship that is strongly associated. This relationship forms a vicious circle, as hepatic fat accumulation can enhance glucose production in the liver, increase hepatic production of very-low-density lipoprotein (VLDL), and induce muscular insulin resistance. This, in turn, exacerbates insulin resistance and further promotes hepatic fat accumulation.

Non-alcoholic fatty liver disease (NAFLD) is a common hepatic manifestation of MetS, that can progress to non-alcoholic steatohepatitis (NASH) and ultimately to hepatic fibrosis and cirrhosis if left untreated. A widely used definition of NAFLD in the medical community is formulated by The European Association for the Study of the Liver (EASL) and the European Association for the Study of Diabetes (EASD). They define NAFLD as "a condition characterized by hepatic fat accumulation exceeding 5% of liver weight in the absence of significant alcohol consumption, viral hepatitis or other specific causes of liver disease". (32) Liver enzymes have commonly been used to indicate liver damage in individuals with NAFLD. Recent research has provided insight into the function of different enzymes in diagnosing and predicting the progression of the disease. (33) ALT is considered to be a more specific marker of liver injury in NAFLD than AST. A study published in the Journal of Gastroenterology and Hepatology in 2021 found that ALT levels were significantly higher in individuals with NAFLD compared to healthy controls, while AST levels were only slightly elevated. These recent studies support the notion that ALT is a more specific marker of liver injury in NAFLD than AST. (34) Even more reliable are serum ALT levels as predictors of liver histology in patients with NASH. (33) Other relevant enzymes to mention are serum gamma-glutamyl transferase (GGT) and alkaline phosphatase (ALP) levels. Elevated serum levels of ALP are linked to an increased risk of developing NAFLD, while patients with already diagnosed NAFLD who have elevated serum GGT levels are at an increased risk of mortality. (35) (36)

6. OUTCOME

STUDY 1 - "IS WITHIN-NORMAL RANGE LIVER ENZYMES ASSOCIATED WITH METABOLIC SYNDROME IN ADULTS?"

To analyze the data, participants were grouped into quartiles according to their number of metabolic abnormalities from zero till more than 4. First quartile corresponds with one metabolic abnormality (MA) and includes n=89 men and n=91 women. In the second quartile there are n=75 men and n=121 women, while n=53 men and n=93 women fall into the category of the third quartile. Finally, n=28 men and n=59 present more than 4 metabolic abnormalities. A clear trend is discernible in both genders, visualized in Diagram 4. With increasing the number of metabolic abnormalities, the mean concentrations of AST and ALT rose. It is noteworthy to mention that transaminase levels are typically higher in males compared to females as explained in "C".

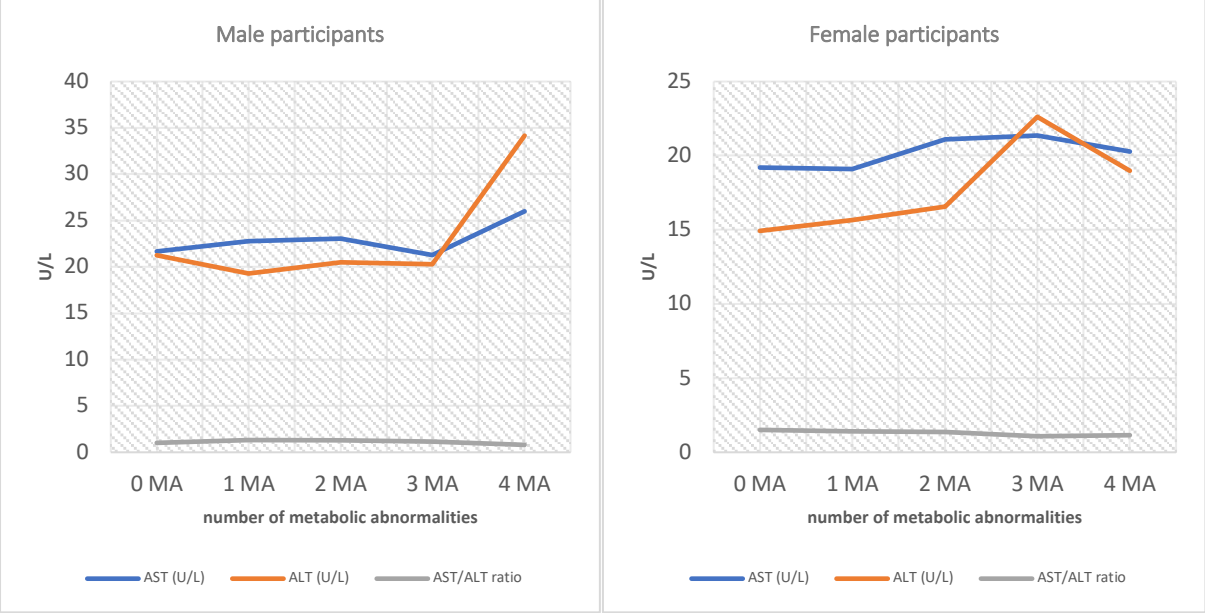

Diagram 4 - Mean concentration of liver markers in relation to number of metabolic abnormalities (MA) in men and women (13)

Analyzing the graph representing the outcome of male participants a clear positive trend is evident. Serum ALT levels showed an impressive progression with a peak of 34.16 ± 13.85 U/L in the fourth quartile. The serum AST graph also shows a positive trend, but lower peak heights in the fourth quartile with a maximum of 26.00 ± 5.67 U/L. AST/ALT ratio, however,

showed a different course. The trend is not constant, and the highest value was measured in patients suffering from one metabolic abnormality (1.32 ± 0.42). After that it declines till the lowest value of 0.80 ± 0.40. In women significant increase of transaminases should be mentioned with a p-value of 0.01 in AST and <0.001 in ALT. Comparing the trend of serum AST and ALT, the changes seen in serum AST are much smaller. Highest values are seen in the third quartile with 21.36 ± 11.92 U/L AST and 22.62 ± 11.92 ALT. Interestingly, a slight decline is then noticeable in female participants with four or more metabolic abnormalities. Similar as in men, the trend of serum ALT concentration raises faster than in serum AST, with an amplitude of 7,69 U/L.

Diagram 5 presents the percentage of participants with MetS based on the quartiles of serum ALT and AST levels. In the 4th quartile with serum ALT of 35,7% in men and 37% in women the maximum is reached. In contrast, in serum AST only women peak in the 4th quartile with 35,7%. The highest percentage in serum AST in men can be observed in the 3rd quartile with 33,9%.

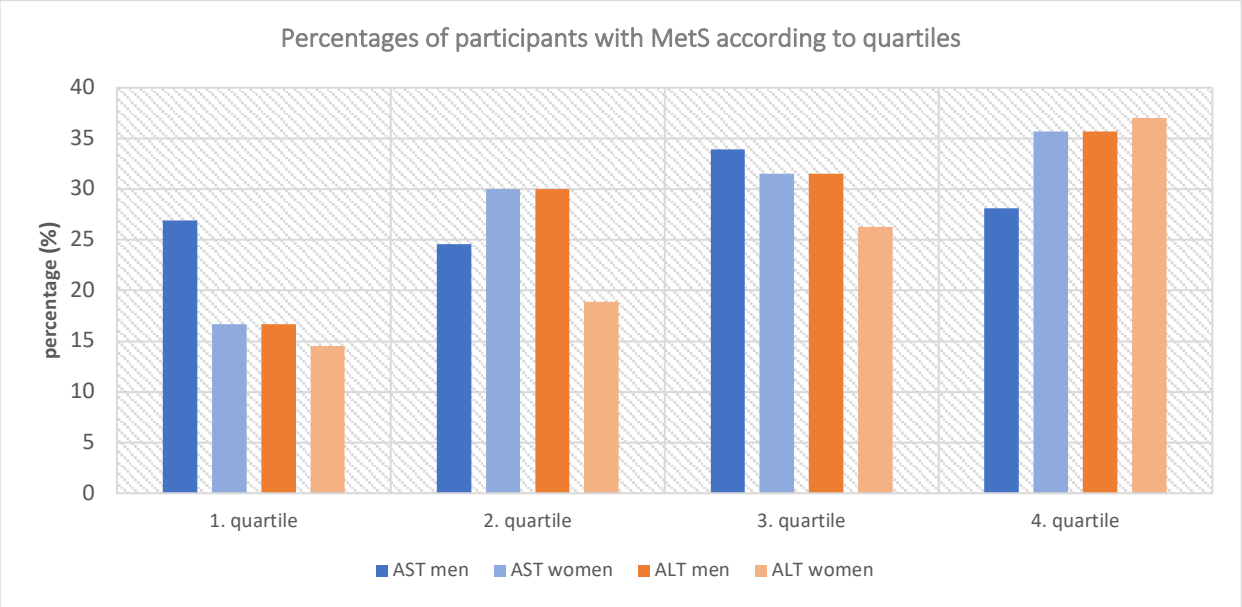

Diagram 5 - Percentages of participants with the metabolic syndrome according to quartiles of each of the within normal limits AST and ALT. P-value of trend for ALT in men: 0.08; P-value of trend for ALT in women: 0.04; P-value for AST in men: 0.36; P-value for AST in women: 0.26. (13)

Directly comparing the values from different gender, it may be noticed, that the trend of serum ALT is similar, whereas in female the serum AST trend is much steeper. In addition, nearly 10% more men are MetS positive already in the first quartile, then women. The data indicates

that the percentage of male participants with metabolic syndrome rose, as the quartiles of serum ALT and AST within normal range increased. Especially it is pointed out that the difference in percentage of MetS positive men is much more significant in serum ALT then in AST. Here the values more than double from 16,7% till 35,7%. However, this association was only found to be statistically significant for serum ALT in women (with a P-trend value of 0.04).

Additionally, the study presented the Pearson correlation between liver markers and specific metabolic abnormalities as waist circumference, triglyceride and cholesterol levels, blood pressure and fasting blood glucose (see Diagram 6).

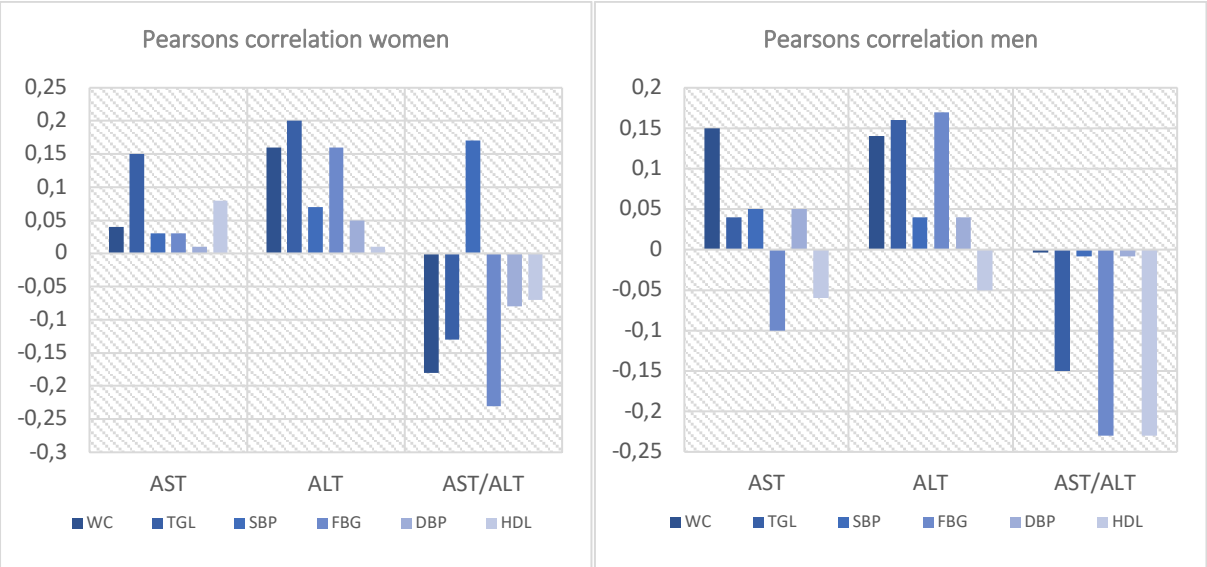

Diagram 6 - Pearson correlation between liver markers and specific metabolic abnormalities (13)

It was observed that in men, there was a noteworthy positive correlation between ALT and waist circumference ($r = 0.14$, $P < 0.05$), serum triglycerides ($r = 0.16$, $P < 0.05$), and fasting plasma glucose ($r = 0.17$, $P < 0.01$). On the other hand, in women, a significant correlation was found between serum AST level and serum triglycerides ($r = 0.15$, $P < 0.05$). The study also revealed that serum ALT had a significant positive correlation, with waist circumference, serum triglycerides, and fasting blood glucose in women. Notably, serum ALT showed the strongest positive correlation with serum triglycerides and fasting plasma glucose in women, while the correlation of AST:ALT ratio with these parameters was negative. Furthermore, there was no significant correlation between serum AST level and any of the metabolic abnormalities in men, except for waist circumference ($r = 0.15$, $P < 0.05$).

These findings suggest that even in the normal range, elevations in serum AST and ALT levels are associated with a greater likelihood of metabolic abnormalities and a higher risk of developing metabolic syndrome. Notably, the study found statistically significant positive linear trends between serum ALT concentration and the number of metabolic abnormalities, both in men and women. After adjusting for covariates such as age, BMI, smoking status, and physical activity level, the study also found that the highest quartile of the normal range of ALT and AST:ALT ratio was markedly associated with an increased risk of metabolic syndrome in women only.

STUDY 2 - ASSOCIATION BETWEEN LIVER FUNCTION AND METABOLIC SYNDROME IN CHINESE MEN AND WOMEN BY WANG, S. ET AL.

The cross-sectional study focused not exclusively on the association of liver enzymes and MetS, but also on the correlation between MetS and other parameters. It additionally presented the percentage distribution of serum ALT, GGT, TBIL in different genders and ages. The prevalence of MetS increased with higher serum GGT and ALT values in both genders, whereas it decreased with higher levels of TBIL. “In other words, serum GGT and ALT were identified as risk factors for MS, while TBIL as a protective factor against MS.” (17)

This statement is visualized in Diagram 7, where the prevalence of MetS in different serum ALT subgroups is shown. It outlines an ascending trend from an odds ratio (OR) of 1.341 with confidence interval (CI) 1.217–1.477, to OR=2.068 with CI=1.858–2.302 in men. The graph presenting female participants develops likewise.

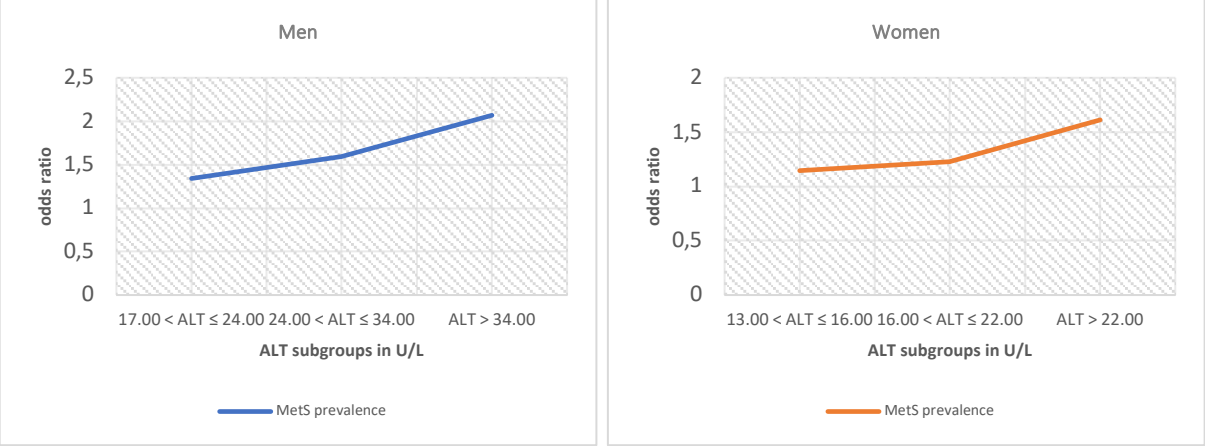

Diagram 7 - Prevalence of metabolic syndrome in different alanine aminotransferase (ALT) subgroups (17)

Aside from serum ALT, the prevalence of metabolic syndrome in different gamma glutamyl transferase (GTT) subgroups was calculated. In Diagram 8 both graphs for men as for women show a definite rising trend. In men reference range is serum GGT ≤20.00 U/L, so the first classified subgroup contains participants with serum GGT levels between 20.00 U/L and 30.00U/L. Odds ratio of 1.710 with an CI of 1.546–1.892 in this group is more than two times lower as in subgroup 3 (GGT >48.00U/L) with an odds ratio of 3.715 (CI=3.335–4.138).

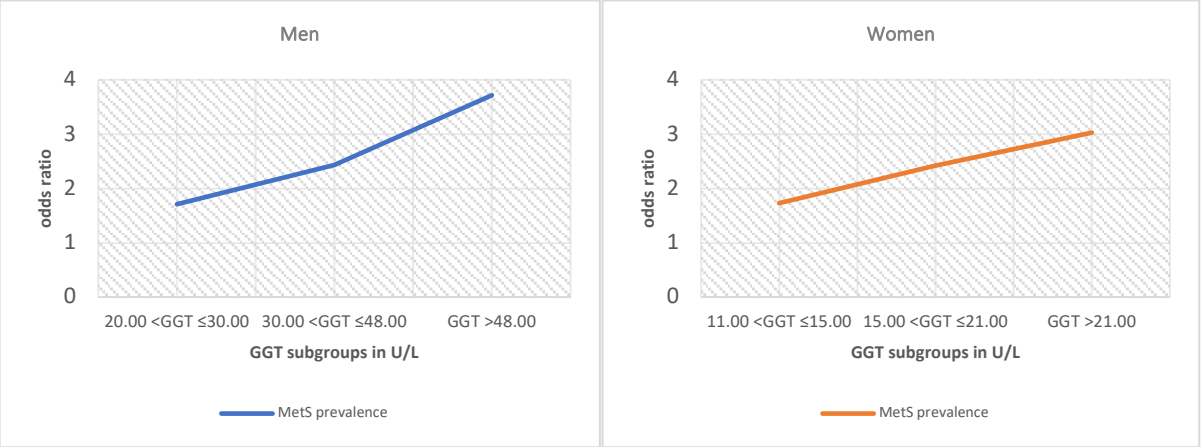

Diagram 8 - Prevalence of metabolic syndrome in different gamma glutamyl transferase (GTT) subgroups (17)

The graph in the female diagram describes a nearly linear trend. From the first to the second subgroup the prevalence of metabolic syndrome doubles from an odds ratio of 1.730 (CI=1.458–2.052) to an odds ratio of 2.425 (CI=2.048–2.871). The difference to the third subgroup with serum GGT >21.00 is not as prominent as in men.

Diagram 9 provides information about correlations of serum ALT among key variables which are partially already included in the definitions of MetS. In both gender, a positive correlation with WC, TGL, SBP, FBG, DBP, LDL and TC can be noted. Highest positive correlation is seen in waist circumference with 0,251 in women and 0,238 in men. In both genders, the lowest positive correlation exists between low-density lipoproteins and serum ALT. Negative correlations exists between serum ALT and HDL only with a value of -0,071 in women and -0,092 in men.

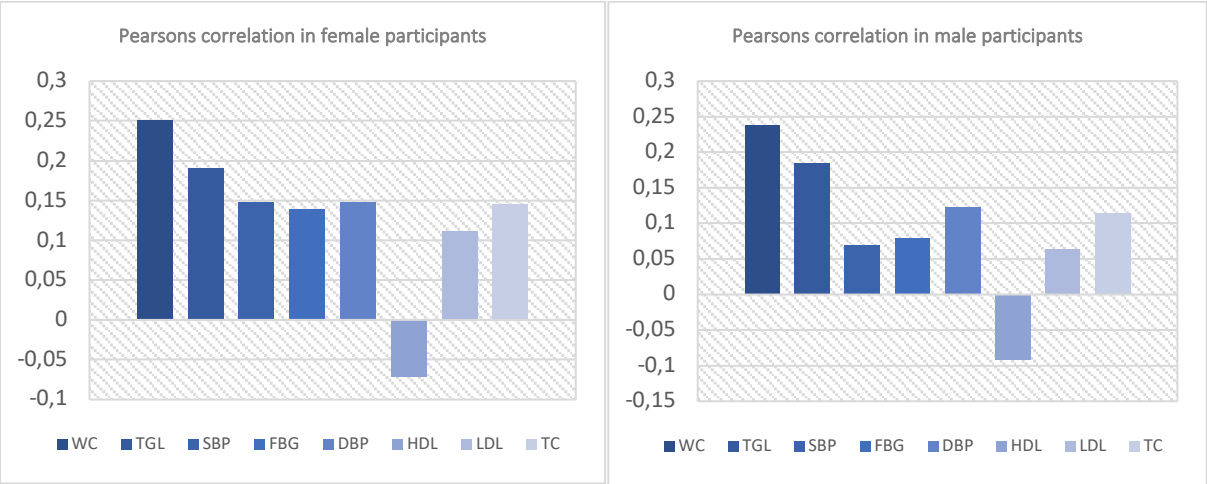

Diagram 9 - Pearson bivariate correlations of ALT among key variables (17)

STUDY 3 - A STUDY OF AST/ALT RATIO IN METABOLIC SYNDROME

With data from the study "A Study of AST/ALT Ratio in Metabolic Syndrome" (18) in Diagram 10 the mean levels of serum ALT, AST and its ratio in MetS diagnosed are compared to a control group. AST/ALT ratio is one of several laboratory tests that can be used to evaluate liver injury. While a ratio higher than one can suggest alcoholic liver disease, it is not specific or sensitive enough to be used as a sole diagnostic criterion. D. Dufour notes in this article that "the AST/ALT ratio has an accuracy rate for the diagnosis of alcoholic liver disease of only about 65%.". (37) Meaning that in differential diagnostics score lower than one is suggestive of NAFLD or NASH. Concluding, a high AST/ALT ratio does not lead to the suspicion of high alcohol consumption, but reflects the severity of hepatitis or underlying liver diseases (38).

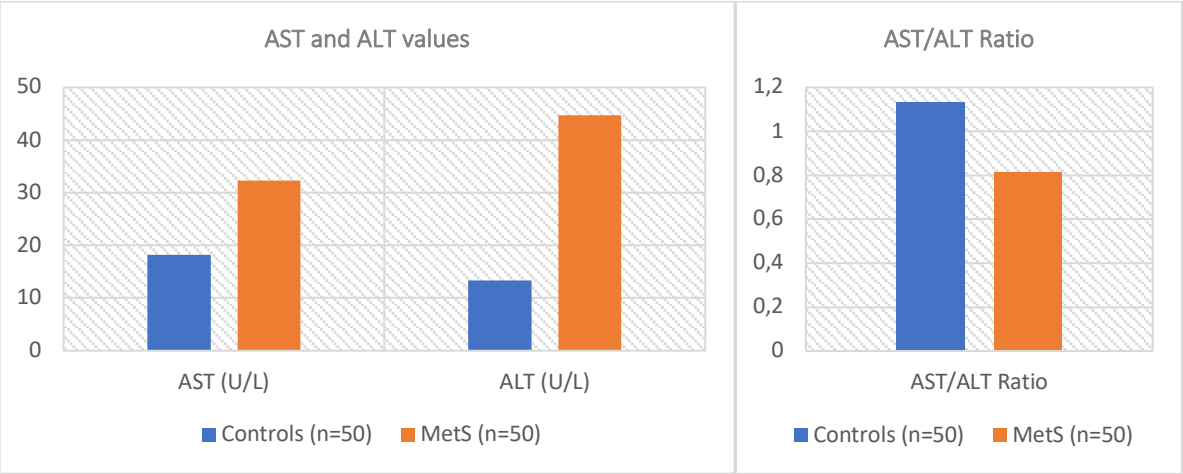

Diagram 10 – Mean AST, ALT and AST/ALT ratio values (18)

As visualized in Diagram 10 the mean level of serum ALT is significantly higher in participants diagnosed with MetS as in the control group. With a mean number of 44.7 U/L including a standard deviation of 9.21U/L in the MetS group, the mean value of the control group was more than 3 times lower with a value of 13.4 U/L (S.D = 6.3 U/L). Whereas the difference of 14.1 U/L between the outcome of serum AST measurement was less prominent. Mean AST/ALT ration in the control group is 1,13, whereas the MetS positive group presents mean values of 0,81. From this it can be deduced that the parameter was significantly higher in participants of the healthy control subjects ($p<0.001$), comparing to MetS positive group. "Metabolic syndrome showed a marked association with decreased AST/ALT ratio." (18)

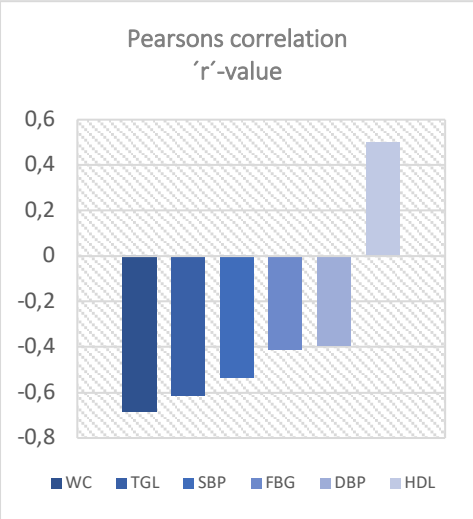

Diagram 11 – Pearson correlation coefficient for AST/ALT ratio with components of Metabolic Syndrome (18)

Focusing on the correlation of AST/ALT ratio with different parameters a clear negative correlation with WC, TGL, SBP, FBG and DBP is seen (Diagram 11). Interesting results were noticed comparing the parameter of waist circumference and AST/ALT ratio using Pearson's correlation coefficient. The value of -0,686 suggests a strong connection of these parameters. Additional negative correlations were observed in triglycerides, fasting blood glucose and blood pressure. In contrast, a positive correlation of 0,499 with HDL was remarked. P. Deepa and N. Sasivathanam conclude that their "study shows that the AST/ALT ratio was strongly associated with all the components of metabolic syndrome." (18)

7. DISCUSSION

The studies presented had different focuses and approaches. Nevertheless, all three coincide on the existing correlation between transaminases and metabolic syndrome. These are now compared with the results of other recent studies.

A significant association of liver function markers alanine transferase and gamma glutamyl transferase with MetS could be demonstrated in "Association between liver function and metabolic syndrome in Chinese men and women". The outcome of the cross-sectional study showed that for a major part of MetS positive participants, the parameters of liver function were within physiological ranges. It is precisely this aspect that "Is within-normal range liver enzymes associated with metabolic syndrome in adults?" focused on more in detail. From the outcome it can be deduced that "increasing the mean level of AST and ALT even in the normal range [is] associated with increasing metabolic abnormalities and also metabolic syndrome risk."(13) In both genders the prevalence of MetS increased with higher quartiles of serum ALT and GGT. Especially, significant association was observed in women between fourth quartile of serum ALT and MetS risk. In men, there was no evident association found (see Diagram 5). The authors expressed the hypothesis of an influence of hormonal contraceptives use, pregnancy, lactation, and menopause with liver enzymes in women. Several studies have been conducted to investigate about a possible association and indeed found interesting results. A study by Madhavan et al. (2019), for example investigated the effect of lactation on liver enzymes in Indian women. The study found that lactating women had significantly higher levels of serum ALT, AST, and GGT compared to non-lactating women. (39) Based on the presented findings, to enhance the sensitivity of MetS diagnostic and earlier identify individuals at risk, the scientists propose to adapt the range of liver function. (17).

Now we will go more into detail regarding the specific role of alanine transferase and its correlation with parameters of MetS. The results the work presented above support Ph.D Villegas statement, that "the prevalence of elevated serum ALT levels was positively associated with each individual component of metabolic syndrome, the number of metabolic syndrome components present, and the prevalence of metabolic syndrome" (40). Alanine transferase showed in all three analyzed studies the highest positive correlation with serum triglycerides, waist circumference and fasting plasma glucose in both genders (see Diagram 6, Diagram 9, Diagram 11). It suggests that, depending on the severity of the diagnosis, the liver damage increases by high levels of TGL, WC and FBG. This correlation is already observed in children.

Overweight adolescents tend to have more elevated alanine transferase. A study conducted by Park Hye Soon, with Korean adolescents claim that abdominal obesity is the most sensitive predictor of elevated serum ALT concentrations in children. (41) Since TGL, WC and FBG rise with the development of MetS, a direct correlation between increase in serum ALT and severity of MetS can be assumed. This observation has been described in previous studies (42) (43) (3) (44), as in a population-based cohort "Liver Enzymes, Type 2 Diabetes, and Metabolic Syndrome in Middle-Aged, Urban Chinese Men", where a strong association between WC and ALT was found (40). Ph. D Villegas and his team discovered that the association between ALT and WC is even higher than between AST and WC. Another interesting result from this study is the inverse association of intrahepatic fat and transaminases with physical activity. (40) Studies have shown mixed results regarding the association between physical activity and serum ALT and AST levels. Some studies have found that regular physical activity is associated with lower ALT and AST levels in both healthy individuals and those with liver disease. (45) (46) While others have found no significant association: "The liver enzymes, alanine aminotransferase, aspartate aminotransferase and γ-glutamyl transpeptidase, were not significantly altered with exercise." (47) One possible explanation for the mixed findings is that the relationship between physical activity and serum ALT and AST levels may depend on the type, intensity, and duration of physical activity, as well as other factors such as age, sex, and body composition. For example, high-intensity exercise may temporarily increase ALT and AST levels due to muscle damage, but regular exercise may ultimately lead to lower levels as the liver becomes healthier. (48)

Negative correlations were found with high density lipoproteins only (see Diagram 6, Diagram 9). This is also confirmed by Zhang et al. (2021), who observed a negative correlation between serum ALT levels and HDL cholesterol levels, and that this relationship is stronger in individuals with MetS. The author also found that including HDL cholesterol as a diagnostic criterion for MetS improved the sensitivity and specificity of the diagnosis. (49) (1)

Aspartate transferase presented an independent outcome from alanine transferase. At this point, it is of importance to remark, that AST, as cytosolic and mitochondrial isoenzyme, that is not exclusively found in the liver, is neither as sensitive nor as specific for liver injury as ALT. (50) AST had no significant correlation with any of the metabolic abnormalities, except for waist circumference in men (see Diagram 6). This correlation between waist circumference and AST levels is suggested to be particularly present in individuals with non-alcoholic fatty liver disease. (51) One study published in the Journal of Gastroenterology and Hepatology in 2012 found that waist circumference was independently associated with elevated AST levels in

patients with NAFLD, even after controlling for other factors such as BMI and insulin resistance. The authors of the study also suggested that waist circumference may be a useful marker for predicting liver injury and disease progression in patients with NAFLD. (52) In addition, Ph.D Villegas and his team found out that "participants with elevated serum AST levels had higher BMI and [WC] and were more likely to drink alcohol than those without the condition." (40)

"A Study of AST/ALT Ratio in Metabolic Syndrome" places emphasize on the correlation of AST/ALT ratio (see Diagram 11). The results can be compared to those in "Is within-normal range liver enzymes associated with metabolic syndrome in adults?" (see Diagram 6), where on both genders was worked individually. A strong negative correlation was documented in men and women with parameters of serum triglycerides, waist circumference, blood pressure and fasting plasma glucose. Furthermore, the documented correlation between MetS and transaminases is explained as "increased enzymes level and decrease AST:ALT ratio most commonly [is] a sign of liver fat deposition and is representative of the presence of visceral fat." (18) Additionally, a significant negative correlation is present between AST/ALT ratio and blood pressure. This finding was also seen in a research project from 2015 by Hsiao et al. (53) Researchers demonstrated "the significant correlation of severe fatty liver with hypertension, triglyceride metabolism and abnormal glucose levels." (18) Unequal results in Pearson correlation were found concerning high-density lipids. Further literature research supports the results from L. Nikniaz. A cross-sectional study from a Chinese population (2021), found a negative correlation between the AST/ALT ratio and HDL cholesterol levels. The authors also reported that this correlation was stronger in individuals with metabolic syndrome compared to those without.

Given the strong association and similar risk profile between the features of NAFLD and MetS, NAFLD has been proposed as a component of metabolic syndrome in recent research. Therefore, the results of the studies can be inferred to NAFLD and were included into the research project "A Study of AST/ALT Ratio in Metabolic Syndrome". (54) The observed negative correlation between AST/ALT ratio and waist circumference suggests that NAFLD is strongly correlated with visceral adiposity, which is reflected by waist circumference. (18) In "Association between liver function and metabolic syndrome in Chinese men and women" the interaction of NAFLD and MetS is discussed based on literature research. It unmasks the non-homogeneous state of research by comparing the different results of recent studies. On the one hand, NAFLD has been referred to as a precursor to MetS on the other hand as a result of the

manifest disease. Further investigations confirm the positive association between NAFLD and an increased risk of developing MetS. (55) Hye Soon Park concludes in his article based on a cross-sectional health survey performed in South Korea, that their results “suggest that the metabolic syndrome, a clinical manifestation of insulin resistance, might be predictive of NAFLD independent of obesity even in adolescents.”. (41)

Insulin resistance is also be involved in the pathogenesis of type II diabetes (T2D) (40). The objective of “Liver Enzymes, Type 2 Diabetes, and Metabolic Syndrome in Middle-Aged, Urban Chinese Men” was to investigate about the relation between aminotransferases with impaired fasting glucose, newly diagnosed type II diabetes (T2D), and MetS. (40) A positive associations between elevated serum ALT, AST, T2D and MetS were found. (40) Hae Ran Kim confirmed this statement by his results also showing an high association of serum ALT and AST with a higher prevalence of MetS and T2D. (3)

8. CONCLUSION AND SUGGESTIONS

Concluding, this thesis determined the value of including serum alanine transferase, gamma glutamyl transferase and aspartate aminotransferase / alanine aminotransferase ratio as a novel biomarker in the metabolic syndrome risk stratification. The levels of liver function were indeed associated with the risk of developing metabolic syndrome. Therefore, liver function should be seriously evaluated in all individuals to judge the metabolic syndrome risk. Since the onset and progression of non-alcoholic fatty liver disease is associated with multiple cardiovascular risk factors, aspartate aminotransferase / alanine aminotransferase ratio could be used as a marker to predict non-alcoholic fatty liver disease in metabolic syndrome. Based on this extensive research, I would also like to support the proposal, to adjust the range of physiological liver function when talking about metabolic syndrome patients. Which ranges are appropriate needs to be investigated first. However, further research is needed to fully understand the mechanisms underlying this association and to determine the clinical significance of liver function testing in the context of metabolic syndrome. Nonetheless, these findings highlight the importance of considering liver health as part of a comprehensive approach to managing metabolic syndrome and its associated risks.

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APPENDIX

Table 4 - Comparable features of "Study 1", "Study 2" and "Study 3"

	Study 1	*Study 2*	*Study 3*
Anthropometric measurements	height, weight	• height, weight • + overall heath check including ultrasonography abdomen and heart	height, weight
Waist circumference	• at the minimum circumference between the iliac crest and the rib cage • anthropometric tape • wearing light clothing		• measured to the nearest 0.5cm at the end of normal expiration • midway between the superior border of the iliac crest and inferior margin of the ribs • steel tape
Fasting glucose	• 12-hour overnight fast • enzymatic colorimetric methods • with commercially available kit (Pars Azmone, Tehran, Iran) • automatic analyzer (Abbott, model Alcyon 300, USA)	• auto-analyzer • range: 3.60–5.80 mmol/L	estimated by Glucose oxidase-peroxidase method
HDL-C	• enzymatic colorimetric methods • commercially available kit (Pars Azmone, Tehran, Iran) • automatic analyzer (Abbott, model Alcyon 300, USA)	• auto-analyzer • range: 0.80–2.20 mmol/L	direct method
TG	• enzymatic colorimetric methods • commercially available kit (Pars Azmone, Tehran, Iran) • automatic analyzer (Abbott, model Alcyon 300, USA)	• auto-analyzer • range: 0.57–1.71 mmol/L	GPO-PAP method
Total cholesterol		• auto-analyzer • range: 3.59–5.17 mmol/L	Cholesterol oxidase PAP method
Total Bilirubin		• auto-analyzer • range: 3.40–20.00 µ mol/L	Jendrassik-Grof's method in XL 300 auto analyser
LDL		• auto-analyzer • range: 1.33–3.36 mmol/L	Friedwald's formula
Blood pressure	• standard manual • sphygmomanometer in sitting position		• right arm • standard sphygmomanometer in sitting position • after relaxation for about 10 min • repeated after 10 minutes • the average of the two readings were noted
AST and ALT	• ultraviolet method • normal range ALT in men < 40 ALT in women AST in both < 34 U/l	ALT 5.00–40.00U/L	oxidase-peroxidase method
Additional parameters	• risk estimation of metabolic syndrome • questionnaire about socio-demographic characteristics and smoking status • short form of international physical activity questionnaire (IPAQ)		