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Afr. J. Biomed. Res. Vol. 23 (September, 2020);385 - 390

Research Article

Systemic Hypoxia Biomarkers in Asymptomatic Simple Non-Alcoholic Hepatosteatosis

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ABSTRACT

The high prevalence of the risk factors for Non-Alcoholic Fatty Liver Disease (NAFLD) made it one of the most common non-communicable diseases with high morbidity, nationally and worldwide. Early pathogenic implication of cellular hypoxia in Asymptomatic Simple Non-Alcoholic Isolated Hepatosteatosis (ANIHS) was investigated in this cross-sectional study and correlated changes in systemic hypoxia biomarkers with body mass index (BMI) in apparently healthy ANIHS participants with normal liver enzymes and size. We enrolled 180 adult consented volunteering Saudi participants in the period from January 1 to June 1, 2019. They comprised of normal lean healthy controls (n = 40; BMI = 18.5-25) and ANIHS participants (n 140) that were subdivided as overweight (n = 64; BMI = 25.1 – 30) and obese (n = 76; BMI >30 - <40). Male/female ratio was 1:1 and age range was 24 – 50 years (37.0 ± 7.85) without significant differences among groups. The cellular hypoxia biomarkers; lactate, pyruvate, lactate: pyruvate (L/P) ratio & hypoxia inducible factor (HIF)-1α were estimated in fasting plasma. Lactate was significantly higher in obese ANIHS compared to each of overweight ANSHS participants and controls. Pyruvate was significantly lowest in overweight ANIHS followed by obese ANIHS participants - compared to highest level in controls. L/P ratio was highest in obese ANIHS followed by overweight ANIHS participants, compared to controls. HIF-1α was more than 3-folds higher in obese ANIHS participants compared to healthy controls, with a mild increase in overweight ANIHS participants. Pyruvate and the ratio were significantly connected to steatosis, whereas lactate and HIF-1α were significantly connected to both BMI and hepatosteatosis. Cellular hypoxic changes may be implicated in pathogenesis of ANIHS. Plasma HIF-1α level reflects the correlation between BMI and occurrence/progression of ANIHS. Cellular hypoxia with responsive increases in HIF-1α could be prognostically good provided that it correlates a cytoprotective functional transcriptional response.

Keywords: *Non-alcoholic fatty liver, isolated hepatic steatosis, obesity, cellular hypoxia biomarkers, lactate, pyruvate, lactate-pyruvate ratio, hypoxia-inducible factor-1 alpha.*

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Received: March, 2019; Accepted: August, 2020

Abstracted by:

Bioline International, African Journals online (AJOL), Index Copernicus, African Index Medicus (WHO), Excerpta medica (EMBASE), CAB Abstracts, SCOPUS, Global Health Abstracts, Asian Science Index, Index Veterinarius

INTRODUCTION

Overweight and obesity reached a pandemic level worldwide, with a consequent significant increase in the incidence of metabolic syndrome, insulin resistance, diabetes, non-alcoholic fatty liver disease (NAFLD) and coronary heart disease. Obesity is a chronic low grade inflammatory status. Adipocytic hypertrophy accompanied with cellular hypoxia, lipids toxicity and endoplasmic reticulum stress lead to adipokine dysfunction. Hypoxia-induced increase in vascular permeability with enhanced immune cell infiltration and release of inflammatory effectors establish a proinflammatory milieu (Santareno *et al*. 2008; Cheng *et al*., 2018; Engin, 2017; Zhou *et al*., 2017). In such a state, the already low vascularized adipose tissue became more hypoxic (Santareno *et al*. 2008). To a great extent, this hypoxia is due to the expanded size of adipocytes, diminished adipose tissue vascularization and elevated fatty acid metabolism that exhaust oxygen (Pasarica *et al*., 2009).

Abnormal accumulation of lipids, mainly triglycerides, in hepatocytes accompanies onset of obesity. Lipid accumulation badly affects hepatocytes in a progressive course spanning simple reversible/irreversible steatosis, steatohepatitis, cirrhosis, to hepatocellular carcinoma and death (Guerrero *et al*., 2009; Younossi, 2019). The non-alcoholic form of the fatty liver disease - NAFLD - is one of the most common liver diseases that affect around 25-35% of the population worldwide. It has a high rate of morbidity and mortality as it

is a main cause of chronic liver disease and transplantation. The industrialized world with high rate of obesity, diabetes mellitus, and metabolic syndrome observed the surge first. However, improvement in living standards and obesogenic changes in lifestyle and dietary habits induced a serious rapid rise in prevalence of NAFLD in developing countries, as well (Chalasani *et al*., 2018; Mantovani *et al*., 2018; Li *et al*., 2019). The prevalence of NAFLD in Saudi Arabia is strongly associated with known risk factors that include the obesity epidemic, diabetes mellitus, metabolic syndrome, hyperlipidemia and drug misuse (Akbar and Kawther, 2003; Al-Nozha *et al*., 2005).

The gluconeogenic function of liver clears up to 70% of blood lactate (Phypers and Pierce, 2006). Under hypoxic conditions, this burden increases proportion to lactate production, and, become worse in case hepatocytes themselves become hypoxic. Any degree of hepatic dysfunction, as in obesity and NAFLD, instigates lactate accumulation (Van Hall, 2010). Proportionation with pyruvate depends on the source of lactate; hypoxic vs. non-hypoxic (Suistomaa *et al*., 2000). Organisms are adapted to the changeable oxygen level via developing multiple oxygen-sensitive systems that maintains oxygen homeostasis. Major orchestrator of the hypoxic cellular response is the heterodimeric hypoxia-inducible transcription factors (HIFs)-1 and -2 (Roth and Copple, 2015). Glucose transporters, glycolytic enzymes, pyruvate dehydrogenase kinase-1 and insulin receptor substrate-2 are HIF-1-responsive genes. Oppositely, HIF-1α lowers the expression of key regulatory enzymes of fatty acid synthesis and de novo lipid synthesis, reduces serum cholesterol levels, and, protected against hepatic steatosis in mice (Rahtu-Korpela *et al*., 2014). Upregulation of HIF-2α protects hepatocytes from hypoxia-induced oxidative stress and apoptosis, and doweregulates the expression of pro-fibrogenic mediators (Liu J *et al*., 2017). Experimental hypoxia and lead toxicity-induced liver damage is correlated with massive increases in serum HIF-1α levels (Das *et al*., 2015). Enhancement of HIF-1α activity in mice by knockdown or chemical inhibition of its hydroxylase prevents high-fat diet-induced adiposity and adipose tissue inflammation, and, improved glucose tolerance and insulin sensitivity. In chronic hypoxia, increases in HIF-1α are due to its salvage from ubiquitin-dependent degradation and translocation into the nucleus, where it activates the hypoxia response element (HRE). The latter decreases T-regulatory cells and release of anti-inflammatory cytokines (IL-10, IL-35, TGF-β), while increasing T-helper cells and proinflammatory cytokines (NF-κB, IL-6, and IL-17) (Liu Z *et al*., 2018). Increased expression of HIF-1α induced by chronic hypoxia activates the fibrogenic enzyme lysyl oxidase. The anti-steatotic metformin induced lactic acidosis could reflect an effect of HIF-1α orchestrated processes (Han *et al*., 2019).

We were the 1st worldwide to characterize the changes in circulating levels in HIF-1α as a disease biomarker (Hamed *et al*., 2012; Elsayh *et al*., 2014). The target of this study was to investigate the changes in circulating levels of cellular hypoxia biomarkers (lactate, pyruvate, lactate/pyruvate ratio and HIF-1α) in apparently healthy, overweight and obese Saudi participants with simple/isolated hepatosteatosis compared to lean healthy controls. Correlations among these biomarkers stratified to the body mass index (BMI) were assessed. This illustrates the early implication of cellular hypoxia in the pathogenesis of fatty liver disease.

MATERIAL AND METHODS

Participants and Setting: The present cross-sectional study anonymously enrolled 180 adult consented Saudi volunteering participants. Participants were randomly selected by simple consequent inclusion from Sakaka community personnel, referred to undergo routine health checkup at a private Poly-Clinic Center, Sakaka, Aljouf, Saudi Arabia, in the period from January 1 to June 1, 2019. They comprised lean healthy Controls [body mass index ((BMI) = 18.5 – 25 kg/m^2; n = 40), and, apparently healthy participants with asymptomatic non-alcoholic isolated hepatosteatosis (ANIHS). The latter group was subdivided into overweight ANIHS (n = 64; BMI = 25.1 – 30 kg/m^2) and obese ANIHS (n = 76; BMI >30 - <40 kg/m^2) participants. Their male/female ratio was 1:1. Their age ranged from 24 – 50 years (37.0 ± 7.85) without statistically significant differences among groups or within each group (male/female) considering the age. Fatty liver participants with increased liver size or increased hepatocyte integrity biomarkers were excluded. None of our participants is on specific medication and none of them qualify to have metabolic syndrome as defined by National Cholesterol Education Program (NCEP) Adult Treatment Panel III (ATP III) and later updates. Alcoholics and those with fatty liver disease due to drug exposure and abuse, pregnancy, viral hepatitis, autoimmune hepatitis, primary biliary cirrhosis, diabetes, thyroid disease and hemochromatosis, hypertensive and those on dieting regimens were excluded. Participants were free of Helicobacter pylori (serology and fecal antigen negative) and other chronic metabolic, autoimmune, endocrine, and renal diseases. Following the tenets of the Declaration of Helsinki, each participant signed a written informed consent and the study was approved by Jouf University Bioethical Committee (Approval # 9-15-9/40).

ANIHS Assessment and BMI measurement: According to the international scanning guidance and protocol, simple hepatosteatosis was transabdominal ultrasonographically characterized by a single independent consultant radiologist. Only patients with normal liver enzymes and normal liver size were included in this study. Normal hepatocyte function/integrity (measured as serum AST, ALT, AST/ALT ratio, LDH, alkaline phosphatase, γ-glutamyl transpeptidase, indirect, direct, and total bilirubin, glucose, prothrombin time, and, albumin and globulin and their ratio along with lipid profile) and increased parenchymal real-time ultrasonographic echogenicity were used for diagnosing simple fatty liver in our ≥10-hour overnight fasting participants as previously described for the same cohort of patients (Elasbali *et al.*, 2020). Hepatic steatosis index (HSI) calculated as 8 × ALT/AST + BMI (+2, if female; +2, if diabetes mellitus; that does not apply here) was used to substantiate the ultrasonography; giving a score between 30 and 36 (Lee *et al.*, 2010).

Biochemical markers analysis: Fasting peripheral blood samples of 2.5 mL were collected on EDTA from each participant, and, plasma was recovered, and aliquot stored at -70 ºC until used. Specific ELISA kit from Cloud-Clone Corp. (Wuhan, Hubei, PRC; cat# SEA798Hu) was used to measure human HIF-1α level. Lactate and pyruvate were measured using quantitative enzymatic colorimetric assay kits in plasma aliquots immediately deproteinized by perchloric acid (cat# MAK064 and MAK071; Sigma-Aldrich Co., St. Louis, MO, USA) and lactate: pyruvate (L/P) ratio was calculated.

Statistical analysis: Statistical analysis was conducted using SPSS version 23.0 (IBM Corp. IBM SPSS Statistics for Windows. Armonk, NY). Continuous variables were expressed as mean ± standard deviation (SD) and median ± Interquartile range (IQR) in case of non-normal distribution. Normal distribution of variables was checked by Shapirowilk W-test, where, a significant W-test means non-normal distribution. Variables with normal distribution were compared with analysis of variance (ANOVA) test and independent samples t-test. Intragroup correlation among variables was assessed by Pearson or Spearman correlation coefficient test. Variables with asymmetric distribution were analyzed by Kruskal-Wallis and Mann-Whitney U tests. ROC analysis was carried out assuming nonparametric distribution. P value <0.05 was considered significant.

RESULTS

In this study, we observed that there is significant difference in plasma lactate levels comparing healthy controls (1.5 ± 0.7 mg/dL) and otherwise healthy asymptomatic ANIHS participants (1.8 ± 0.9 mg/dL; P = 0.001). Lactate was highest in obese ANIHS participants (2.1 ± 1 mg/dL), followed by overweight ANIHS participants (1.7 ± 0.9 mg/dL) vs. the lowest level in controls. This revealed significantly higher plasma lactate level in obese vs. each of controls and overweight ANIHS participants (p <0.003 and <0.027, respectively), while the level was non-significantly different comparing controls and overweight heptosteatotic group. Similar significant changes were observed for other plasma hypoxia biomarkers; pyruvate (3.91 ± 2.73 vs. 2.938 ± 2.504 mg/dL), L/P ratio (0.86 ± 1.29 vs. 1.946 ± 2.51) and HIF-1α (221.05 ± 223.95 vs. 522.81 ± 1235.67 pg/dL) comparing controls and heptosteatotic participants (P = 0.01, = 0.001 and <0.001, respectively). This revealed a significant difference in pyruvate level only comparing controls vs. overweight ANIHS persons (2.49 ± 2.54 mg/dL; P = 0.013), but nonsignificant difference for obese ANIHS (2.804 ± 2.22 mg/dL) vs. each of controls and overweight ANIHS persons (P = 0.058 and = 0.732). L/P ratio was nonsignificantly different comparing overweight ANIHS (2.01 ± 2.03) vs. each of controls (P = 0.055) and obese steatotic participants (2.465 ± 3.15; P = 0.521), but significant difference comparing controls and obese steatotics (P = 0.003). Similar pattern was evident for HIF-1α, with nonsignificant differences comparing overweight ANIHS (350.96 ± 381.28 pg/dL) vs. each of controls (P = 0.856) and obese steatotics (826.34 ± 1824.50 pg/dL; P = 0.057), but significant difference comparing controls and obese steatotics (p = 0.031). These differences in plasma levels of hypoxia biomarkers implicate an early involvement of cellular hypoxia in ANIHS with overweight/obesity, and, may explain the higher comorbid cardiometabolic events among them.

Correlation among parameters within healthy lean controls showed significant positive correlations between plasma lactate vs. L/P ratio (P/r =; 0.001/0.516), pyruvate vs. HIF-1α (0.011/0.363), while, L/P ratio negatively significantly correlated with each of plasma pyruvate (0.001/-0.830) and HIF-1α (0.05/-0.281). Among overweight steatotics, lactate significantly positively correlated with each of L/P ratio (0.001/0.411) and HIF-1α (0.041/0.219), and, plasma pyruvate level significantly negatively correlated with plasma L/P ratio (0.001/-0.934). Among obese steatotic participants, significant positive correlations were evident between plasma lactate vs. L/P ratio (0.001/0.517), and pyruvate vs. HIF-1α (0.05/0.258), while L/P ratio significantly negatively correlated vs. each of pyruvate (0.001/-0.871) and HIF-1α (0.05/ -0.249).

To determine whether the differences in plasma hypoxia biomarkers among patients with and without simple non-alcoholic hepatosteatosis can be attributed to obesity alone or not, we compared the plasma levels of lactate, pyruvate, their ratio and HIF-1α in overweight and obese ANIHS groups (Table 2). It was found that lactate and HIF-1α are significantly different comparing overweight and obese steatotic participants (P = 0.01 and = 0.004, respectively), while pyruvate and L/P ratio were statistically non-different. Given the fact that they all are higher than healthy lean controls, these results implicate that among the four plasma hypoxia biomarkers utilized, pyruvate and L/P ratio are more specific for hypoxic injury related to ANSHS. Distinctively, lactate and HIF-1α are more sensitive to the combined effect of obesity and ANIHS.

Table 1:

Comparison of the levels of the investigated plasma hypoxia biomarkers among adult lean healthy controls (Controls; n = 40), and, overweight (OW-SHS; n = 64) and obese (O-SHS; n = 76) participants with simple non-alcoholic hepatosteatosis (SHS).

Parameter	Controls	OW-SHS	O-SHS	All SHS (n = 140)	X^2 (P)
Lactate, mg/dL	1.5 ± 0 .7 (1.3 ± 1)	1.7 ± 0.9 (1.4 ± 1)	2.1 ± 1 (2.0 ± 1.2)	1.8 ± 0.9 (1.7 ± 1.2)	13.52 (0.001)
Pyruvate, mg/dL	3.91 ± 2.73 (3.45 ± 3.6)	2.49 ± 2.54 (1.27 ± 3.5)	2.804 ± 2.22 (3.091 ± 4.4)	2.938 ± 2.504 (2.546 ± 3.6)	9.01(0.01)
Lactate to Pyruvate Ratio	0.86 ± 1.29 (0.35 ± 0.7)	2.01 ± 2.03 (1.278 ± 2.7)	2.465 ± 3.15 (0.864 ± 4)	1.946 ± 2.51 (0.784 ± 2.7)	14.39 (0.001)
Hypoxia-inducible Factor 1α, pg/mL	221.05 ± 223.95 (154.88 ± 105.8)	350.96 ± 381.28 (218.97 ± 132.2)	826.34 ± 1824.50 (256.382 ± 210.2)	522.81 ± 1235.67 (215.669 ± 146.9)	35.96 (<0.001)

Data shown are mean ± SD (Median ± IQR), and X^2 (P) Kruskal Wallis test. Shapiro Wilk Test (W-test) was significant for all 4 parameters (asymmetrical distribution).

In this study, we measured area under curve to dig deep into the differentiation power of four plasma hypoxia biomarkers (Table 3 and Figure 1). All four markers, i.e., lactate, pyruvate, L/P ratio and HIF-1α can differentiate significantly between cases and controls, but the best one was HIF-1α. Higher levels of HIF-1α and lactate were significantly associated with ANIHS and higher BMI. However, pyruvate and L/P ratio failed to differentiate between overweight and obese participants with ANIHS. This confirms our other finding that pyruvate and L/P ratio are more specific for hypoxic injury related to ANIHS.

Table 2:
Distribution of the plasma hypoxia parameters among overweight (OW; n = 64) vs. obese (O; n = 76) individuals with simple non-alcoholic hepatosteatosis (SHS).

Parameter	OW-SHS	O-SHS	P
Lactate, mg/dL	60.91 (3898.0)	78.58 (5972.0)	0.010
Pyruvate, mg/dL	67.16 (4298.0)	73.32 (5572.0)	0.365
Lactate to Pyruvate Ratio	70.19 (4492.0)	70.76 (5378.0)	0.933
Hypoxia-inducible Factor 1α, pg/dL	59.72 (3822.0)	79.58 (6048.0)	0.004

Data shown are mean rank (sum of ranks) and Mann Whitney U-test p value.

DISCUSSION

Although simple/isolated hepatosteatosis is viewed as a mild reversible issue, it has morbid ramifications to the liver as it becomes liable to inflammation, fibrosis, cirrhosis and cancer. This mandates its prevention, early diagnosis and treatment. NAFLD is a major cause of liver-related and cardiometabolic morbidity and mortality worldwide not only in obese, diabetic, and dyslipidemic but also increasingly in normal-weight individuals. The multifaceted initiation and progression of the disease encompass modulation of the metabolism of glucose, lipid, and bile acids, inflammation, apoptosis, and fibrosis (Paik *et al*., 2019). This necessitates a complex interaction among environmental factors (i.e., Western diet and fast foods), obesity, insulin resistance, and changes in microbiota, with predisposing genetic variants. They all disturb lipid homeostasis with a toxic accumulation of triglycerides and other lipid species in hepatocyte. The latter exhibits endoplasmic reticulum stress, disturbed autophagy, and, ultimately, hepatocyte injury and death. This triggers hepatic inflammation, hepatic stellate cell activation, and progressive fibrogenesis that entails liver transplant (Arab *et al*., 2018).

HIF-1α is a highly conserved transcription factor that plays a key role in tissue response to hypoxia. Obese adipose tissue is glucose intolerant, inflammatory and hypoxic. This hypoxic milieu, due to imbalance between the metabolic demand and oxygen supply, have a crucial role in initiating many obesity-related disorders by abnormal expression of multiple genes in adipocytes (Mazzatti *et al*., 2012) - in particular a high expression of HIF-1α.

Adipocyte-specific knockout of HIF-1α, in mice, reverses all of these disorders, prevent liver fibrosis, and enhanced postprandial insulin secretion and increased serum level of each of glucagon-like peptide (GLP)-1 and adiponectin (Mesarwi *et al*., 2016; Kihira *et al*., 2014).These consequences highlight the crucial role of HIF-1α in the development of liver fibrosis in NAFLD patients. In our participants, a reciprocal relationship was evident between the induced HIF-1α and our previous reported reduction in circulating levels of GLP-1 for the same cohort (Elasbali *et al*., 2020).

Table 3:
Area under receiver operating characteristic (ROC) curve (AUC) for differentiation between overweight vs. obese simple non-alcoholic hepatosteatosis cases (SHS; n = 140), and them vs. lean healthy controls (n = 40).

Parameter	SHS Cases vs. Controls		Overweight vs. Obese SHS	
	AUC (95% CI)	P	AUC (95% CI)	P
Lactate, mg/dL	0.642 (0.542 to 0.742)	0.006	0.626 (0.532 to 0.721)	0.010
Pyruvate, mg/dL	0.354 (0.264 to 0.444)	0.005	0.544 (0.447 to 0.641)	0.371
Lactate to Pyruvate Ratio	0.697 (0.609 to 0.785)	<0.001	0.504 (0.406 to 0.602)	0.933
Hypoxia-inducible Factor 1α, pg/dL	0.781 (0.684 to 0.878)	<0.001	0.642 (0.548 to 0.735)	0.004

Data shown are AUC (95% CI) and p values.

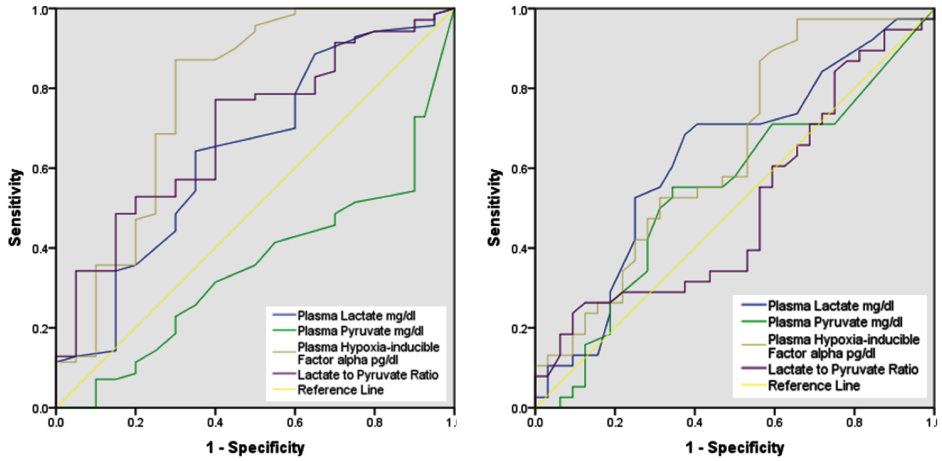

Figure 1.
Left: Area under receiver operating characteristic (ROC) curve (AUC) for differentiation between overweight/obese simple non-alcoholic hepatosteatosis cases (n = 140) and lean healthy controls (n = 40). Right: AUC for variance in the measured hypoxia biomarkers vs. obesity in obese cases with simple non-alcoholic hepatosteatosis cases (n = 76). For detailed data and p value, see Table 3. Diagonal segments are produced by ties.

Our observation of a progressive increase in level of plasma HIF-1α and L/P ratio with the rise in body weight is in agreement with the results reported by Golzar *et al*. (2019) showing increased expression of HIF-1α in adipose tissue of mice given high fat diet for 9 weeks. This may be explained by expansion of adipose tissue with inadequate compensatory blood supply to hypertrophic adipocytes resulting in local hypoxia (Ludzki *et al*., 2018). Increased HIF-1α expression in obese adipose tissue induces a potent profibrotic transcriptional program leading to fibrosis and induces the obesity-associated inflammation - through recruiting M1-macrophages that secrete proinflammatory cytokines and connective tissue growth factor (Warbrick and Rabkin, 2019). Similar to our findings, at the onset of obesity, increases in blood free fatty acids prompt increases in concentrations of lactate and HIF-1α and replace the beneficial gut microbiota with harmful proinflammatory lipopolysacchide-secreting microbiota (Dai and Wang, 2015). The free fatty acids-induced HIF-1α is due to blockage of succinate dehydrogenase. Accumulation of succinate induces the synthesis of HIF-1α. The latter increases activities of hexokinase, phosphofructokinase, and lactate dehydrogenase, and, inhibits pyruvate dehydrogenase. This leads to slower oxidative phosphorylation and increased lactate production. Also, the state of hypoxia associated with obesity augments glucose fermentation with higher lactate production than normal cells (Ray *et al*., 2018). Moreover, The hepatoprotective anti-steatotic oroxylin A flavonoid prevents nuclear translocation of HIF-1α, while activation of HIF-1α attenuates the effect of oroxylin A on lipid droplets accumulation and genes related to lipid metabolism in vitro and in vivo (Shen *et al*., 2016). Specific inhibition of HIF-1α by 3-(5'-hydroxymethyl-2'-furyl)-1-benzylindazole alleviates hepatosteatosis and inflammation with reduced NF-κB expression and reduced liver fibrosis degree in a rat model of non-alcoholic fat liver diseases (Jin *et al*., 2018).

Other than quality control factors, high blood lactate could be due to primary and secondary mitochondrial dysfunction, tissue hypoxia/ischemia, or specific vitamin deficiency. L:P molar ratio reflects lactase dehydrogenase product and substrate equilibrium and is indirectly mirrored as cytoplasmic NADH:NAD+ ratio that is controlled by cellular respiration. The extent of dysfunction of the latter proportionally correlates elevation of L:P molar ratio (Lane *et al*., 2016). The progressive increase in plasma lactate, L/P ratio & HIF-1α found in the present study in asymptomatic otherwise healthy overweight and obese ANIHS participants was confirmed by significant positive correlations among lactate & L/P ratio in the 3 studied groups & between lactate and HIF-1α in overweight steatotic participants. The elevated plasma lactate level associated with high L/P ratio are similar to results of a study done by Moon *et al*. (2017) on rats fed high fat diet for 6 weeks - indicating enhancement of anaerobic glycolysis. This may be the result of impaired hepatic glucose production & impaired gluconeogenesis, as lactate is the major substrate for this process - at the expense of elevated hepatic lipid synthesis. Besides, the increased adipose tissue mass, associated with obesity, augments plasma lactate level - as adipose tissue is a major source of lactate reflecting up-regulated anaerobic glycolysis (Xie *et al*., 2012). It is worth noting that, utilizing several molecular mechanisms, accumulation of lactate is immunosuppressive – a phenomenon ascribed to chronic liver illness (Comito *et al*., 2019; Heymann and Tacke, 2016). With NAFLD as the instigator, HIF-1α is implicated in hepatocellular carcinogenesis, aggression and progression (Wang *et al*., 2017; Liu Z *et al*., 2017; Liu Z *et al*., 2018; Dou *et al*., 2019). In this concern, we observed a connection between changes in plasma lactate and HIF-1α with both of ANSHS and changes in BMI, whereas, changes in pyruvate and L/P ratio was connected to ANIHS only.

Although we reviewed about 900 individuals, due to our stringent exclusion criteria, we enroll except the presented number of participants. We know that liver biopsy histopathological analysis is the standard diagnostic approach for grading hepatosteatosis, fibrosis and/or inflammation. However, nobody would resort to that it would be indicated or accepted by participants in a setting like ours; needless to mention its inherent complications. The other alternatives, magnetic resonance spectroscopy and 2D-SWE ultrasound elastography, were not available for our use. Although we excluded morbid obese participants, with criteria used for characterizing simple/isolated hepatosteatosis, still we could have missed participants with low degree of steatosis. Since we excluded participants with diabetes or prediabetes, we did not correlate our finding with insulin resistance.

In conclusions, there is an early induction of cellular hypoxia in apparently healthy overweight and obese Saudi participant with ANIHS – measured as plasma lactate and HIF-1α. In this context, HIF-1α is an attractive druggable target that could ameliorate the early course of the disease when controlled. Such pathogenetic link among cellular hypoxia, BMI and hepatosteatosis requires further dissection utilizing multicenteric longitudinal studies with specific anti-hepatosteatotic treatment and/or HIF-1α-blocking intervention – that would confirm a causal relationship. Moreover, circulating levels of HIF-1α could be characterized as a prognostic/diagnostic biomarker for ANIHS in conjunction with obesity.

Acknowledgement

This study was partially funded by a grant from Deanship for Scientific Research, Jouf University, Sakaka, Saudi Arabia (Grant #309/35).

Competing Interests:

The authors have declared no competing interest exists.

REFERENCES

Akbar DH, Kawther AH (2003): Nonalcoholic fatty liver disease in Saudi type 2 diabetic subjects attending a medical outpatient clinic. Diabetes Care 26(12):3351-3352.

Al-Nozha MM, Al-Mazrou YY, Al-Maatouq MA, *et al* (2005): Obesity in Saudi Arabia. Saudi Med J. 26(5):824-829.

Arab JP, Arrese M, Trauner M (2018): Recent insights into the pathogenesis of nonalcoholic fatty liver disease. Annu Rev Pathol. 13:321-350.

Chalasani N, Younossi Z, Lavine J.E *et al* (2018): The diagnosis and management of nonalcoholic fatty liver disease: Practice guidance from the American Association for the Study of Liver Diseases. Hepatology. 67:328-357.

Comito G, Iscaro A, Bacci M, *et al (2019)*: Lactate modulates CD4+ T-cell polarization and induces an immunosuppressive environment, which sustains prostate carcinoma progression via TLR8/miR21 axis. Oncogene. 38(19):3681-3695.

Dai X, Wang B (205): Role of gut barrier function in the pathogenesis of non-alcoholic fatty liver disease. Gastroenterol Res Pract. 2015:1-6.
Das KK, Jargar JG, Saha S, Yendigeri SM, Singh SB (2015): α-tocopherol supplementation prevents lead acetate and hypoxia-induced hepatic dysfunction. Indian J Pharmacol. 47 (3): 285-291.
Dou C, Zhou Z, Xu Q, *et al* (2019): Hypoxia-induced TUFT1 promotes the growth and metastasis of hepatocellular carcinoma by activating the Ca2+/PI3K/AKT pathway. Oncogene 2019;38(8):1239-1255.
Elasbali AM, Almaeen AH, Alduraywish AA, *et al* (2020): Changes in Plasma Ghrelin, Leptin, Insulin and Glucagon-like Peptide-1 Levels in Early Asymptomatic Non-alcoholic Fatty Liver. International Medical J. 27(6):xx-xx (ahead of print).
Elsayh KI, Zahran AM, El-Abaseri TB, *et al* (2014): Hypoxia biomarkers, oxidative stress, and circulating microparticles in pediatric patients with thalassemia in Upper Egypt. Clin Appl Thromb Hemost. 2014;20(5):536-45. doi: 10.1177/1076029612472552.
Engin A (2017): The pathogenesis of obesity-associated adipose tissue inflammation. Adv Exp. Med. Biol. 960:221-245.
Golzar FAK, Fathi R, Mahjoub S: High fat diet leads to adiposity and adipose tissue inflammation: The effect of whey protein supplementation and aerobic exercise training. Appl Physiol Nutr Metab. 2019;44(3):255-262.
Guerrero R, Vega GL, Grundy SM, Browning JD. Ethnic differences in hepatic steatosis (2009): an insulin resistance paradox? Hepatology 49:791-801.
Hamed EA, El-Abaseri TB, Mohamed AO, *et al* (2012): Hypoxia and oxidative stress markers in pediatric patients undergoing hemodialysis: cross section study. BMC Nephrol. 13:136. doi: 10.1186/1471-2369-13-136.
Han J, He Y, Zhao H, Xu X (2019): Hypoxia inducible factor-1 promotes liver fibrosis in nonalcoholic fatty liver disease by activating PTEN/p65 signaling pathway. J Cell Biochem. 120(9):14735-14744.
Heymann F, Tacke F (2018): Immunology in the liver--from homeostasis to disease. Nat Rev Gastroenterol Hepatol. 13(2):88-110.
Jin H, Lian N, Bian M, *et al* (2018): Oroxylin A prevents alcohol-induced hepatic steatosis through inhibition of hypoxia inducible factor 1alpha. Chem Biol Interact. 285:14-20.
Kihira Y, Miyake M, Hirata M, *et al*: Deletion of hypoxia-inducible factor-1α in adipocytes enhances glucagon-like peptide-1 secretion and reduces adipose tissue inflammation. PLoS One 2014;9(4):e93856;1-9.
Lane M, Boczonadi V, Bachtari S, *et al* (2016): Mitochondrial dysfunction in liver failure requiring transplantation. J Inherit Metab Dis. 2016; 39(3):427-436.
Lee JH, Kim D, Kim HJ, *et al* (2010): Hepatic steatosis index: A simple screening tool reflecting nonalcoholic fatty liver disease. Dig Liver Dis. 42(7):503-508.
Li J, Zou B, Yeo YH, *et al* (2019): Prevalence, incidence, and outcome of non-alcoholic fatty liver disease in Asia, 1999-2019: A systematic review and meta-analysis. Lancet Gastroenterol Hepatol. 4(5):389-398.
Liang C, He Z, Hong J, *et al* (2019): Exposing the causal effect of body mass index on the risk of type 2 diabetes mellitus: A Mendelian randomization study. Front. Genet. 2019;10(94):1-10.
Liu J, Li Y, Liu L, *et al* (2017): Double knockdown of PHD1 and Keap1 attenuated hypoxia-induced injuries in hepatocytes. Front Physiol. 8:291;1-13.
Liu Z, Tu K, Wang Y, *et al* (2017): Hypoxia Accelerates Aggressiveness of Hepatocellular Carcinoma Cells Involving Oxidative Stress, Epithelial-Mesenchymal Transition and Non-Canonical Hedgehog Signaling. Cell Physiol Biochem. 2017;44(5):1856-1868.
Liu Z, Wang Y, Dou C, *et al* (2018): Hypoxia-induced up-regulation of VASP promotes invasiveness and metastasis of hepatocellular carcinoma. Theranostics 2018;8(17):4649-4663.
Liu Z, Wang Y, Dou C, *et al*: (2018): Hypoxia-induced up-regulation of VASP promotes invasiveness and metastasis of hepatocellular carcinoma. Theranostics 8(17):4649-4663.
Ludzki, AC, Pataky MW, Cartee GD, Horowitz JF (2018): Acute endurance exercise increases VEGFa mRNA expression in adipose tissue of rats during the early stages of weight gain. Appl Physiol. Nutr. Metab. 43(7):751-754.
Mantovani A, Byrne CD, Bonora E, Targher G (2018): Nonalcoholic fatty liver disease and risk of incident type 2 diabetes: a meta-analysis. Diabetes Care;4(2):372-382.
Mazzatti D, Lim FL, O'Hara A, Wood IS, Trayhurn P. A: microarray analysis of the hypoxia-induced modulation of gene expression in human adipocytes. *Arch Physiol Biochem.* 2012;118(3):112-120.
Mesarwi OA, Shin MK, Bevans-Fonti S, Schlesinger C, Shaw J, Polotsky VY (2016): Hepatocyte hypoxia inducible factor-1 mediates the development of liver fibrosis in a mouse model of nonalcoholic fatty liver disease. PLoS One 11:e0168572.
Moon CM, Oh CH, Ahn KY, *et al*: Metabolic biomarkers for non-alcoholic fatty liver disease induced by high-fat diet: In vivo magnetic resonance spectroscopy of hyperpolarized [1-13C] pyruvate. Biochem Biophys Res Commun. 2017;482(1):112-119.
Paik JM, Henry L, De Avila L, Younossi E, Racila A, Younossi ZM: Mortality Related to Nonalcoholic Fatty Liver Disease Is Increasing in the United States. Hepatol Commun. 2019;3(11):1459-1471.
Pasarica M, Sereda OR, Redman LM, *et al*. (2009): Reduced adipose tissue oxygenation in human obesity: evidence for rarefaction, macrophage chemotaxis, and inflammation without an angiogenic response. Diabetes 58:718-725.
Phypers B, Pierce T (2006): Lactate physiology in health and disease. Continuing Education in Anesthesia Critical Care & Pain 6(3):128-132.
Rahtu-Korpela L, Karsikas S, Hörkkö S, *et al* (2014): HIF prolyl 4-hydroxylase-2 inhibition improves glucose and lipid metabolism and protects against obesity and metabolic dysfunction. Diabetes 2014;63(10):3324-3333.
Ramalho R, Guimaraes C. The role of adipose tissue and macrophages in chronic inflammation associated with obesity: Clinical implications. Acta Med. Port 2008;21(5):489-496.
Ray I, Dasgupta A, De RK (2018): Succinate aggravates NAFLD progression to liver cancer on the onset of obesity: An in silico model. J Bioinform Comput Biol. 16(4):1-16.
Roth KJ, Copple BL (2015): Role of hypoxia-inducible factors in the development of liver fibrosis. Cell Mol Gastroenterol Hepatol. 1(6):589-597.
Shen WD, Zhang W, Xu QH, Liu PF (2016): Effect of targeted inhibition of hypoxia-inducible factor-1α by 3-(5'-hydroxymethyl-2'-furyl)-1-benzylindazole on the progression of non-alcoholic fatty liver disease in rats. Zhonghua Gan Zang Bing Za Zhi. 24(10):749-754.
Suistomaa M, Ruokonen E, Kari A, Takala J (2000): Time-pattern of lactate and lactate to pyruvate ratio in the first 24 hours of intensive care emergency admissions. Shock 14(1):8-12.
Van Hall G (2010): Lactate kinetics in human tissues at rest and during exercise. Acta Physiol. 199 (4):499-508.
Wang M, Zhao X, Zhu D, Liu T, Liang X, Liu F, Zhang Y, Dong X, Sun B (2017): HIF-1α promoted vasculogenic mimicry formation in hepatocellular carcinoma through LOXL2 up-regulation in hypoxic tumor microenvironment. J Exp Clin Cancer Res. 36(1):60;1-14.
Warbrick I, Rabkin SW (2019): Hypoxia-inducible factor 1-alpha (HIF-1α) as a factor mediating the relationship between obesity and heart failure with preserved ejection fraction. Obes Rev. 2019;20(5):701-712.
Xie B, Waters MJ, H.J. Schirra HJ (2012): Investigating potential mechanisms of obesity by metabolomics. J. Biomed. Biotechnol. 805683:1-10.
Younossi ZM. Non-alcoholic fatty liver disease (2019): A global public health perspective. J Hepatol. 70(3):531-544.
Zhou Y, Zhang Y, Shi K, Wang C (2017): Body mass index and risk of diabetic retinopathy: A meta-analysis and systematic review. Medicine 96 (22): e6754;1-7.