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Plasma Fetuin-A Changes in the Gluco-lipotoxicity Interplay Between Non-alcoholic Fatty Liver Disease and Coronary Heart Disease

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Abstract

Mammals have evolved mechanisms to store energy during periods of plenty, which helps to guarantee survival during periods of drought and famine. The fat-thriftiness accomplishes our ability to effectively detect, metabolize, and store fats. For so, variable amounts of extra fat are safely stored subcutaneously, either locally hip and thigh (in adults) or spread over total body subcutaneous (in children), where it confers a metabolically protective reserve in both genders and supports pregnancy and lactation in females. For so, dedicated fat-storing cells were necessary, because the tissues of the lean body mass lack the storage capacity to meet the fuel demands. Consequently, hypertrophy and hyperplasia of adipocytes can occur in energy surplus, with adipocyte hypertrophy controlling both, the differentiation of neo-adipocytes(increasing fat storage) and the local macrophage density for fat surplus scavenging. Therefore, macrophage-scavenger (pro-inflammatory) actions are part of the cell defense against fat-overstored (hypertrophied) adipocytes. The fat balance in non-adipocyte cell is maintained between uptake (and produced) and oxidation of fatty acids. The process has hormonal control of insulin and adipokines, leptin and adiponectin. The excess of fat is primarily stored in the form of neutral fat, as triglycerides, which are the least toxic form of neutral fat to be stored, whereas its alternative, *de novo* ceramide formation, is probably the most damaging lipid. Increased ceramide synthesis leads to both leptin and insulin resistance. Insulin resistance may be one example of cell-lipotoxicity by lipid accumulation in skeletal muscle(myoesteatosis) and liver(esteatosis). Insulin resistance can be consequent to factors such as inflammation, oxidative stress or Free-Fatty Acids (FFAs) accumulation in hepatocytes. The initial step of Non-Alcoholic Fatty Liver Disease (NAFLD) involves fat accumulation in the liver as a result of the excessive delivery of FFAs from the adipose tissue(lipolysis) and an imbalance between lipid synthesis and export in hepatocyte. NAFLD is the most common cause of chronic liver disease, constituting a major risk factor for progression to liver failure, cirrhosis, and also, hepatocellular carcinoma. Approximately 10–30% NAFLD have the potentially progressive form of Non-Alcoholic Steato-Hepatitis (NASH) and, approximately 25–40% of patients with NASH will develop progressive liver fibrosis, which is associated with hepatocellular injury and inflammation, with a poor long-term prognosis. Our sample showed 59.6% NAFLD and 0.6% liver fibrosis with the latter increased 6X by the presence of NAFLD. The NAFLD progression to NASH occurs through the first hit of hepatocytes by saturated fatty acids coming from peripheral lipolysis, complemented by a second inflammatory hit consequent to gut dysbiosis. In our data plasma Lipopolysaccharide-Binding Protein (LBP) correlates significantly with Fatty-Liver Index(r= 0.90). Additionally, our data showed that inflammation and insulin resistance had similar impact on the prevalence of NAFLD and hepatic fibrosis, both far below the oxidative stress impact. The expected consequences of inflammation and oxidative stress are insulin resistance with fasting hyperglycemia and atherogenic dyslipidemia pointing out toward cardiovascular mortality as the leading cause of death in patients with NAFLD. For though, it is suggested that liver esteatosis predates clinical CVDs, and may trigger or accelerate its occurrence. In our data, the atherosclerotic course of NAFLD to Coronary-Heart Disease (CHD) was accentuated by the presence of T2D. Our proposed mechanism for this NAFLD-CHD interplay is based on the underlying background of gluco-lipotoxicity(of T2D) involving inflammation(of NAFLD) and oxidative stress(of T2D) leading to insulin resistance. Besides highly related with metabolic stress markers of gluco-lipotoxicity, NAFLD and related CVDs showed a wide pattern of liver-born proteins, some positively related to acute-phase inflammation (LBP, CRProtein and Fetuin-A) and, other negatively related such as albumin. Similarly, some peptide of antioxidant defense such as GSH was reduced by the presence of NAFLD and gluco-lipotoxicity, in opposition of increased oxidized products GSSG and MDA. From those markers, Fetuin-A presented a potential diagnostic power as a biomarker for grading insulin resistance and cardiovascular diseases, both related to liver dysfunction. Moreover, we showed that plasma Fetuin-A level responded positively to Lifestyle-change protocols.

Keywords: Lipodystrophy • Non-alcoholic fatty liver disease • Lipotoxicity • Gluco-lipotoxicity • Cardiovascular diseases • Metabolic-stress markers • Plasma liver-proteins • Pleiotropic Fetuin-A

Introduction

Mammals have evolved mechanisms to store energy during periods of plenty, which helps to guarantee survival during periods of drought and famine. Normally, variable amounts of extra fat are safely stored subcutaneously, either locally hip and thigh (in adults) or spread over total body subcutaneous (in children), where it confers a metabolically protective reserve in both genders and supports pregnancy and lactation in females [1]. In addition to storing surplus calories, adipocytes appear to protect against the lipotoxic damage to lean tissues that occurs in the lipoatrophic states such as famine and fasting [2]. From our energy-yelding nutrients, fatty acids provide doubled kcal/g than CHO or protein and are stored anhydrously either occupying intracellular or inter organs spaces, insulating parenchymal organs and working as subcutaneous cushion [3]. However, storage of even a modest caloric surplus in lean tissue maybe ultimately be manifested clinically by hepatic-steatosis (fatty liver), myo-steatosis (fatty muscle), cardiomyo-lipodistrophy, renal-lipodistrophy and, consequently non-insulin- dependent diabetes mellitus and lipid-renal or cardiomyopathy [2].

Controlling fat storage

Several environmental, nutritional, and hormonal factors appear to influence body weight and therefore also body adiposity [4-6]. The fat-storage capacity of the cell (hypertrophy) is limited, once adipocytes reach a critical size. The action of growth factors secreted by the hypertrophied adipocytes

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is observed and neo-adipocyte differentiates leading to hyperplasia. So, both, hypertrophy and hyperplasia of adipocytes can occur in energy surplus [7]. On the other hand, following adipocyte hypertrophy, when expanded fat cells leak or break open, resident macrophages mobilize to clean up and embed into the adipose tissue. Macrophages are attracted by the chemoattractant protein 1 (MCP-1), secreted by the enlarging adipocytes. An additional source could be the trans-differentiation of pre-adipocytes to residual macrophages. Thus, adipocyte hypertrophy controls both, the differentiation of neo-adipocytes (increased fat storage) and the local macrophage density for fat surplus scavenging. Macrophage-scavenger actions are part of the cell defense against fat-overstored (hypertrofied) adipocytes [8].

Lipodystrophy and lipotoxicity

The lipid-induced dysfunction in the lean tissues is referred to as lipotoxicity [9],and lipid-induced programmed cell death is called lipoapoptosis [10]. In lipotoxic cells, there is an imbalance between the amount of lipids produced and the amount used. Hence, lipotoxicity is a syndrome that results from the accumulation of lipid intermediates in non-adipose tissue, leading to cellular dysfunction and death [11]. Lipotoxicity affects tissues such as liver, skeletal muscles, heart and kidneys, with believed roles in obesity, diabetes and heart failure. The lipid-induced dysfunction in the lean tissues is referred to as lipotoxicity [9], and lipid-induced programmed cell death is called lipoapoptosis [10].

Fat storage in non-adipose tissue

Liver is a fat-metabolizing organ but not a fat-storage organ. In the non-adipocyte cell, there is a balance between uptake (and produced) and oxidation of fatty acids. The process has hormonal control of insulin and adipokines, leptin and adiponectin. The excess of fat is primarily stored in the form of neutral fat, as triglyceride, in the adipose tissue but in other tissues as well. Triglycerides are probably the least toxic form in which the lipid surplus can be sequestered and may, at least in the short term, actually protect against severe metabolic trauma therefore, the harmful lipids are quite probably not in the form of neutral fat [12].

The contribution of the *De Novo* Lipogenesis (DNL), in the fasting state accounts for less than 5% of hepatic fatty acids in healthy subjects. DNL is induced by the activation of SREBP-1c (triacylglycerol genesis) and SREB-2 (cholesterol genesis). Sterol-Regulatory Binding Protein 1c (SREBP-1c), is a transcription factor with stimulatory effects on the expression of genes that regulates the expression of enzymes involved in the synthesis of fatty acids (DNL) and in triglyceride synthesis, in the liver. The activation of SREBP-1c stimulates pyruvate kinase, thus increasing the glycolysis of glucose into pyruvate, which forms acetyl-CoA and then malonyl-CoA, which is required for FFA synthesis. The higher activation of SREBP-1c might be consequent to both, the increased insulin (by insulin resistance) and decreased adiponectin (by liver adipocytes).

In the fasting state the main origin of the FFAs in the systemic plasma pool is considered to be sub cutaneous fat. In fact, after lipolysis and extracellular release, these endogenous free-fatty acids enter the liver bound to albumin. In fed state, mostly of the free-fatty acids enter the liver from exogenous (dietary) sources. FFAs are taken up by the hepatocytes and are bound to Coenzyme A (CoA). FA-CoA can be oxidized, involving the transcription factors PPAR-alpha and PPAR-delta and involvement of AMPK. The activation of these factors is through leptin and adiponectin adipokines. AMPK activation (by adiponectin) is also involved in the suppression of lipogenesis. By promoting fatty acid oxidation and deterring lipogenesis, the hyperleptinemia maintains the lean tissue content of lipids at a near-normal level. Physiologically, both hepatic and plasma TG levels are substantially reduced by a direct antisteatotic effect of endogenous hyperleptinemia. For though it presents two major effects, the hypothalamic effects of leptin in the regulation of food intake and, it most probably direct antisteatotic effects, by enhancing lipid oxidation and inhibiting lipogenesis in tissues [2]. Deficiency of and/or unresponsiveness to leptin prevents these protective events and results in ectopic accumulation of lipids. The leptin insufficiency can be attributed to the visceral adiposity once the leptin produced by visceral fat is less effective than that from subcutaneous fat. Then, visceral adiposity decreases the activities of AMPK, PPAR-alpha and PGC-1alpha, consequently altering the appetite (reducing satiety) and reducing FFA beta-oxidation. The consequences of lower leptin activity would be appetite overruling satiety (hypothalamic effect) and decreased FFA oxidation (perypheric action). It is accepted that liver-expanded adipocytes are biologically active and secretes inflammatory adipocytokines leading to both insulin and leptin resistance. Complementary to leptin, mechanisms of adiponectin action include increase in lipid oxidation in liver and skeletal muscle. Mechanisms of adiponectin action in lipid oxidation in liver and skeletal muscle occur via activation of AMP-Activated Protein Kinase (AMPK) and induction of Peroxisome Proliferator Activated Receptor (PPAR) [13-15]. Furthermore, adiponectin decreases the activity of enzymes involved in fatty acid synthesis [16]. By summing up, leptin and adiponectin are hormones that enhance oxidation of surplus lipids in nonadipose tissues and reducing the activity of lipogenic enzymes. Commonly, NAFLD is associated with insulin and leptin resistance and lower production of adiponectin. Hence, by its decreased sensitivity of insulin and leptin and decreased levels of adiponectin, NAFLD might have cell-FFA accumulation (by lowering beta-oxidation) aggravated by the increased De Novo Lipogenesis (DNL). Thus, the initial step of NAFLD involves fat accumulation in the liver as a result of the excessive delivery of Free Fatty Acids (FFAs) from the adipose tissue (lipolysis) and an imbalance between lipid synthesis and export in hepatocyte. Regarding the liver-lipid export, the remained (non-oxidized) FA-CoAs are assembled to Triglycerides (TG) that are secreted in the form of Very-Low-Density Lipoprotein (VLDL). The VLDL secretion is regulated by the two enzymes Stearoyl-Coa-Desaturase (SCD) and acyl:CoA:DGAT2 (diacylglycerol acyltransferase 2), as well as the MTP (Microsomal Transfer Protein) and the availability of apolipoprotein . RE membrane formation, plays a main role in the synthesis of apolipoprotein and therefore in the assembled VLDL, in the liver. RE membrane formation is dependent of hepatocyte supplying with lipotropic agents (methionine, choline, folate and B12 vitamins).

Choline is a lipotrophic factor such as methionine, folate and B12 vitamins, whose deficiencies result in lower VLDL output leading to fatty liver (NAFLD). Choline is a vital component of cell membranes as a constituent of phosphatidyl-choline (lecithin) formed in methionine-transmethylation pathway. It is central to liver-lipid metabolism (RE membrane formation), and plays a role in the synthesis of Very-Low-Density Lipoprotein (VLDL) in the liver. Insufficient levels of choline in the diet are associated with altered gut microbial ecology and liver steatosis Deficiencies in choline have been implicated in the development of NAFLD and enhanced progression to NASH. Insufficient levels of choline in the diet are associated with altered gut microbial ecology and liver steatosis.

Dietary choline is first processed by the enteric microbiota into metabolite-intermediate trimethylamine (TMAs). Hepatic enzyme FMO3 further converts TMAs to Trimethylamine-N-Oxide (TMAO)s. Therefore, three metabolites of phosphatidylcholine were found in humans: choline, Trimethyl Amine (TMA), TMA-N-Oxide (TMAO), and betaine. The methylamines are produced exclusively by the gut microbiota as a downstream metabolite of choline. High levels of urine TMA indicates increased bacterial- choline metabolism and decreased choline bioavailability (to the liver). Hence, TMA is an example of a harmful bacterial metabolite and an increase in the urine concentration of trimethylamine (TMA) suggests that the decreased plasma levels of (phosphatidyl) choline and may be the result of increased microbial metabolism of dietary choline. These transformations may decrease the levels of bioavailable choline and are suggested to trigger Non-Alcoholic Fatty Liver Disease (NAFLD).

Lipotoxicity and insulin resistance

Triglycerides are probably the least toxic form of neutral fat to be stored whereas its alternative, de novo ceramide formation, is probably the most damaging lipid [2]. Ceramide formation occurs via condensation of unoxidized palmitoyl CoA and serine catalyzed by the enzyme serine palmitoyl transferase [17]. Increased ceramide synthesis leads to both leptin and insulin resistance by increasing SOCS-3 expression. Insulin resistance may be one example of cell-lipotoxicity by lipid accumulation in skeletal muscle and liver [18].

Ceramides induce insulin resistance by increasing c-Jun NH2-terminal

Kinase (JNK) activity and therefore, inhibiting Akt/PKB signaling. The effects of JNK are through the serine phosphorylation of the Insulin Receptor Substrate (IRS)-1, which inhibits normal tyrosine phosphorylation of IRS-1 and downstream insulin signal transduction [19-21]. In mitochondria, ceramide suppresses the electron transport chain and induces production of reactive oxygen species [22]. All have been shown to activate both JNK and NF-kB [23,24].

Thus, overall, insulin signaling transduction can be inhibited through serine-kinase activation induced by the transcription factors NF-kB and JNK, activated in pro-inflammatory and pro-oxidant states, found in situations of intracellular ceramide formation [25]. Therefore, insulin resistance can be consequent to factors such as inflammation, oxidative stress or FFA accumulation in hepatocytes. Consequently, expansion of visceral fat mass, as well as ectopic fat accumulation in liver and skeletal muscle is driven by insulin resistance of sub-cutaneous adipose tissue. Insulin resistance may be one example of cell-lipotoxicity by lipid accumulation in skeletal muscle (myoesteatosis) and liver (hepatic-esteatosis). Lipotoxicity is believed to cause insulin resistance and ultimately failure of pancreatic beta-cells [26].

Overall, increased de novo ceramide formation is probably the most damaging lipid abetted by a decline in tissue Bcl-2. Therefore, de novo ceramide pathway, has been implicated in both, insulin resistance (by lipotoxicity) and the lipoapoptosis of beta-cells and myocardiocytes of obeses.

In the presence of obesity, the liver uptake of palmitic acid originated from peripheral lipolysis, induces inflammasome-pathway activation involving mitochondrial reactive oxygen species. Additionally to inflammasome activation (by saturated fatty acids), liver immune cells sensitived by gutborn LPS and macrophages recruitment would secrete the pro-inflammatory cytokines (TNF-a, IL-1, IL-6 and IL-8) leading to insulin and leptin resistance. Though, it has been hypothesized that hepatic steatosis might induce a subacute inflammatory response in liver that is similar to the adipose tissue inflammation, after adipocyte lipid accumulation [27]. In fact, inflamed adipose tissue secretes high amounts of proinflammatory cytokines as TNF-a and ILs, particularly IL-6, which suppress the production of the insulin-sensitizing adipokine adiponectin. Adiponectin insufficiency leads to an increased lipogenesis and decreased cell-FFA oxidation, accumulating intracellular FFA and therefore ceramide formation [28].

Non-alcoholic fatty liver disease

Non-alcoholic fatty liver disease (NAFLD) is an increasingly common chronic-condition seen in patients with obesity, type 2 diabetes, atherogenic dyslipidemia and arterial hypertension [29].

NAFLD is present when >5% of hepatocytes are steatotic in patients who do not consume excessive alcohol consumption (<20 g/day for women and <30 g/day for men) and ranges in severity from simple steatosis (fat without significant inflammation or hepatocellular injury), to steatohepatitis (fat with hepatocellular injury and hepatic inflammation), through to advanced fibrosis and cirrhosis [29]. Previously, we had shown data from a dynamic cohort with free-demand of urban-adult population as presenting 39% obesity, 17.2% T2D, 32.1% highrisk PAI from abnormal TG(51.2%) and HDL-chol.(16.7%) and 28.7% blood hypertension [30]. Non-Alcoholic Fatty Liver Disease (NAFLD) was found in 59.6% of the sample [30]. The accumulation of lipid in the liver often accompanies and parallels weight gain and obesity [31]. In general population the prevalence of NAFLD varied from 20% to 30% in different countries [32]. In our free-demand sampling, NAFLD was found 20.6% higher in the presence of obesity [33].

Pathogenicity of NAFLD

NAFLD is the most common cause of chronic liver disease, constituting a major risk factor for progression to liver failure, cirrhosis, and also, hepatocellular carcinoma [34-36]. Our diagnosis showed 59.6% NAFLD and 0.6% liver fibrosis [30]. with the latter increasing 6X in the presence of NAFLD. Similarly, both NAFLD and Hepatic Fibrosis (HFI) increased (1.69X) by either, inflammation or insulin resistance. However, the presence of oxidative stress (higher MDA) led to higher increase in both, NAFLD (2.03X) and HFI (3.33X). Taken together our data show that inflammation and insulin resistance have similar impact on prevalence of NAFLD and hepatic fibrosis, both far lower than the oxidative stress impact (MDA). Consequences of insulin resistance would be fasting-plasma hyperglycemia and dyslipidemia. In our data, abnormal levels of triglycerides and HDL-chol. were considered as algorithm for atherogenic index, either systemic (PAI) or Coronary-heart diseases (CHD-Framingham score). From that, PAI high-risk increased more NAFLD (2.27X) than the abnormal HFI (1.77X). Similarly, Framingham's higher risk of CHD was higher influenced by NAFLD (2.29X) than by abnormal HFI (1.86X). By summing up, atherogenic dyslipidemia was more associated to NAFLD than to Hepatic Fibrosis. Dyslipidemia such as used for PAI or Framingham's score of CHD are examples of a inflamed-lipotoxicity, whereas T2D is considered an example of glucotoxicity and liver fibrosis as gluco-lipotoxicity. As mentioned above, NAFLD and hepatic fibrosis were more associated with the oxidative stress (MDA) than to inflammation hs-CRP). Furthermore, the atherosclerotic course of NAFLD to CHD was accentuated when passing through T2D, once the presence of T2D led to higher-risk of CHD through NAFLD (4.19X) than through the liver fibrosis (2.73X). In our proposed mechanism, the NAFLD course to CHD has underlied background of gluco-lipotoxicity (of T2D) involving metabolic stress accomplishing inflammation (of NAFLD)/oxidative stress (of T2D) and insulin resistance (of both). Further on pancreatic beta-cells and myocardiocytes are cellular victims of the lipoapoptosis process, leading to non-insulin-dependent diabetes and lipotoxic cardiomyopathy. Part of this process is considered to be attributed to Endoplasmic Reticulum (ER) oxidative stress largely mediated by activation of JNK resulting in impairment of insulin signaling [24].

As stated before, our data have shown that inflammation and insulin resistance have similar impact on prevalence of both NAFLD and hepatic fibrosis, far lower than the oxidative stress impact (MDA). We can go forward saying similar to both, Atherosclerotic Index (PAI) and CHD higher risk that increased 1.69X in the presence of abnormal hs-CRP against more than 3X in the presence of higher MDA. Taken together, the studies raise the possibility that the link between NAFLD and cardiovascular mortality might not simply be mediated by shared underlying common risk factors but rather that NAFLD independently contributes to increasing this risk [37].

The leading cause of death in patients with NAFLD is cardiovascular mortality [38]. It is suggested that liver steatosis predates clinical cardiovascular disease, and may trigger or accelerate its occurrence [39-41]. The relationship between steatosis, associated cardiometabolic risk factors and cardiovascular events have been detailed by some studies [42,43]. The results suggested that while fibrosis is ultimately associated with increased CV mortality. Steatosis and low-grade inflammation play a key role in occurrence and progression of early atherosclerotic lesions [37].

Non-alcoholic steatohepatitis

Up to 90% of patients with NAFLD have simple steatosis, which carries a relatively benign prognosis. However, approximately 10–30% have the potentially progressive form of NAFLD, Non-Alcoholic Steatohepatitis (NASH) and, approximately 25–40% of patients with NASH will develop progressive liver fibrosis, which is associated with hepatocellular injury and inflammation, with a poor long-term prognosis [44]. According to the two-hit hypothesis, the NAFLD progression to NASH occurs through the first hit of saturated fatty acids coming from peripheral lipolysis complemented by a second inflammatory hit consequent to gut dysbiosis. Changes in the gut microbiome stimulate microbial translocation, i.e., the transfer of microbial organisms or some of their products across intestinal mucosal barriers without definite low-grade bacteremia [45].

The presence of Lipopolysaccharides (LPS) lysed from the cell membrane of Gram-negative bacteria in the gut lumen is thought to promote the development of a balanced gut immune response whilst the entry of the same LPS into systemic circulation may lead to a deleterious pro-inflammatory systemic immune response [46]. One possible explanation is that upstream changes in the gut microbiota promote alterations in intestinal permeability or cause microbial translocation that would result in low levels of systemic LPS [45].

The immune cells cannot by themselves mediate a response to LPS as it must be presented in the appropriate molecular context. Once in the circulation, LPS forms a complex with LBP [47] that would bind to CD14 [48] resulting in innate immunity events and development of a low-grade systemic inflammatory response with production of Tumor Necrosis Factor (TNF)-α and interleukin (IL)-6. Within the vascular system LPS forms aggregates (micelles) and the micelles of LPS are sequestered by LPS-Binding Protein (LBP) and, transferred from LBP to HDL and other serum lipoproteins e.g., Very Low-Density Lipoprotein (VLDL) and chylomicrons [49,50]. LPS-loaded lipoproteins are subsequently transported to the liver [51] where LPS molecules undergo modification. On arrival in the liver, LPS is deacylated by the lipase Acyloxyacyl Hydrolase (AOAH) in hepatic Kupffer cells.

Lipopolysaccharide-binding protein

The LBP-mediated transport of LPS is important in the induction of the LPS-dependent immune response [52]. Therefore, Lipopolysaccharide-Binding Protein (LBP), LPS-Binding Protein (LBP) is one of the endogenous reactive biomarkers produced in response to microbial translocation that recognizes and binds the lipid A moiety of LPS, enhancing host immune response to endotoxin. Increasing evidence shows that LBP plays an important role in the pathophysiology of diseases associated with insulin resistance [53-55].

Lipopolysaccharide-Binding Protein (LBP) is a soluble 60-kDa glycoprotein, which is present in high concentrations in plasma. LBP is produced primarily in the liver as an acute-phase protein, and its plasma concentration increases exponentially during acute inflammatory responses [56] LBP production is up-regulated in response to mediators (TNF-α, IL-1β, IL-6) that similarly induce other hepatic acute-phase production. It is noteworthy that LBP production by the liver is increased by elevated circulating levels of IL-1β, IL-6 and TNF-α [57] after LPS challenge. There is evidence that LBP levels significantly correlate with levels of proinflammatory cytokines (IL-6 and IL-8) and also, with liver enzymes (ALT and γ–glutamyltransferase) [58].

Thus, LBP may be a surrogate marker of underlying low-grade endotoxemia caused by LPS from the gut, which ultimately leads to the development of metabolic derangements [58]. Taken together, these observations suggest that measurement of plasma LBP levels might provide a potential biochemical indicator for subjects at risk for metabolic abnormalities. The found significant relationship of LBP with FLI was r=0.90 [33].

Plasma liver-born acute-phase proteins

As an acute-inflammatory response, the liver-born protein pattern in plasma is characterized by increased positive responsiveness (CRP, serum amyloid-A, alpha 1 acid glycoprotein, alpha-2-anti tripsin etc) and decreased negative responsiveness (albumin, transferrin, transtyretin, retinol-binding protein etc). This pattern of responsiveness is associated with the predominantly pro-inflammatory pattern of cytokines [59].

C-reactive protein

Intracellular ceramide formation and JNK activation leads also to activation of multiple stress responses associated with ER stress. Among the responses is the specific activation of CREBP, a hepatocyte-specific transcription factor that may have an important role in hepatic acute-phase response such as the induction of transcription of the serum amyloid P-component and C-Reactive Protein (CRP) genes [60]. Plasma CRP is produced predominantly by hepatocytes and is under transcriptional control by IL-6 and other proinflammatory cytokines [61].

Systemic subclinical inflammation can be estimated by measurement of circulating CRP. The plasma levels of this acute-phase protein are very low under healthy conditions but increase in response to a pathological inflammatory process. Because of its relatively low half-life of 18 h, CRP represents a useful, early nonspecific marker of inflammation [62]. Circulating CRP is positively correlated with liver fat [63-66]. CRP levels are higher in patients with histologically proven NASH compared with simple steatosis [66]. Circulating CRP correlates with insulin resistance [67-70] and atherosclerosis [62] being considered a marker for cardio-vascular risk.

Fetuin-A

A candidate for NAFLD-related cardiovascular risk would be the Fetuin-A, the former Alpha-2 Heremans-Schmid Glycoprotein (AHSG), a multifunctional 60 kDa protein, whose plasma level is 95% secreted by the liver [71].

Fetuin-A plays a role in insulin resistance (as inhibitor of tyrosine kinase and reducing the expression of adiponectin), stimulates inflammation and dyslipidemia [71]. However, regarding CVDs, it has been suggested a biphasic association of fetuin-A, depending on the stage of atherosclerosis. In early stages of CVD, Fetuin-A exacerbates the disease due to its effects to promote insulin resistance and dyslipidemia. In later stages of CVD, the high concentrations of Fetuin-A would have protective results due to Fetuin-A ability to prevent vascular calcium deposition. It is known that Fetuin-A also regulates bone remodeling and calcium metabolism being an important inhibitor of calcium salt precipitation and vascular calcifications [71].

Objective

NAFLD is an obesity comorbidity leading to possibilities of clustering inflammatory abnormalities that may lead to fatal outcome. The source of inflammatory agents either from peripheral adipocytes, gut dysbiosis or liver by its own has to be dimensioned. Fetuin-A is a liver-born protein responsive to inflammatory stress likely as albumin and C-reactive protein. Differently from the other two, the role of Fetuin-A in the NAFLD-related metabolic stress is yet to be clarified.

Materials and Methods

Cross-sectional and longitudinal studies were conducted with subjects participating in a Lifestyle Modification Program (“Move for Health”), a community-based dynamic cohort study conducted by professionals linked to the Metabolism Exercise and Nutrition Center (CeMENutri) at UNESP Medical School (Sao Paulo, Brazil), since 1991. This lifestyle changing program (LiSM) introduces healthy lifestyle into subject’s daily activities as alternative care for chronic non-communicable diseases. Participants come to the Center spontaneously, looking for preventive health examination with further non-medicated interventions including nutrition reeducation and supervised physical exercises. Subjects were aware of the study and signed a consent form based on the “experiments involving humans” of the Brazilian “National Council of Health, Ministry of Health” and the declaration of Helsinki. Both the design and consent form were submitted and approved by the Research Ethics Committee of the UNESP-Botucatu (SP) Medical School.

Baseline data obtained from 1030 individuals, selecte from 2005 to 2017, had shown 39% obesity, 17.2% T2D, 32.1% highrisk PAI from abnormal TG(51.2%) and HDL-chol.(16.7%) and 28.7% blood hypertension [30].

Non-Alcoholic Fatty Liver Disease (NAFLD) was found in 59.6% of the sample at baseline. Longitudinal analysis were undertaken in data from 583 subjects submitted to a 10-wk LiSM intervention with daily supervised mixedphysical exercises (5x/wk, 80min/session/60-80% VO2max) and dietary counseling [33].

Fetuin-A was assayed in plasma of both, cross-sectional and longitudinal (intervention), experiments. The plasma Fetuin-A determined by immuneenzymatic assay (RayBio Human Fetuin-A ELISA kit).

Results and Discussion

In our sample (n=560), the plasma Fetuin-A showed a significant positive relationship with BMI (r= 0.45; p=0.014), WC (r= 0.48; p=0.014), FLI= 0.56; p=0.014), PAI (r=0.61; p<0.001), CHD-Framingham score (r= 0.58; p<0.001). Plasma Fetuin-A also correlated positively with markers of inflammation (CRP r=0.77), oxidative stress (MDA r=0.75; Hcy r=0.71; GSSG r=0.65) and insulin resistance (HOMA-IR r=0.64), and negatively with markers of anti-oxidant

defenses (GSH r= -0.65 and Cys r= -0.52) and albumin (r= -0.59) (Figure 1).

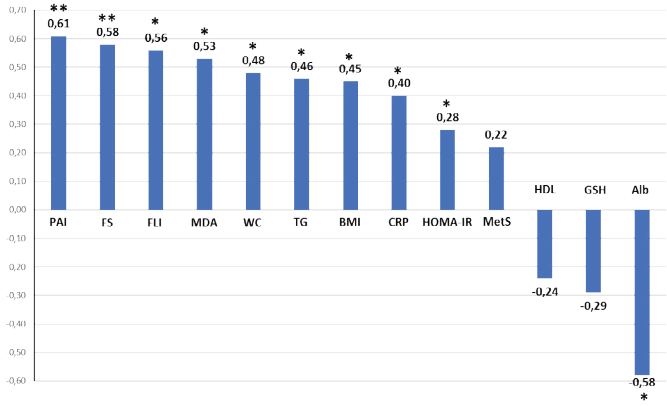

Figure 1. Fetuin-A relationship with co-variables found in the studied sample (n=560). PAI: Plasma Atherogenic Index; FS: Framingham Score; FLI: Fatty Liver Index; MDA: Malondialdehyde; WC: Waist Circumference; TG: Triglyceride; CRP: High sensitivy C-Reactive Protein; HOMA-IR: Homeostasis Model Index - Insulin Resistance; MetS: Metabolic Syndrome components; HDL: HDL-Cholesterol; GSH: Reduced Glutathione; Alb: Albumin.

Hence, high fetuin-A concentrations are found to be associated with NAFLD and atherogenic lipid profile whereas, low fetuin-A levels are connected to vascular calcifications and inflammation.

As described in the preceding paper, FLI was associated with low-quality diet with high-energy manufactured foods along with insulin resistance, pro-inflammatory, and elevated oxidative stress (Burini RC, Burini FHP) [33]. Consequently, the responsiveness to our 10-wk Lifestyle Modification Program (LiSM) was associated with the decreasing of processed-refined foods and the reduced inflammatory-oxidative state. Table 1 shows the effectiveness of our 10-wk LiSM in reducing significantly FLI along with hs-CRP and Fetuin-A. (Table 1).

Table 1. Effects of 10-wk lifestyle modification with supervised physical exercise and counseled dietary adequacy on the liver-born proteins and related hepatic diseases.

	Absence of NAFLD(n=397)	Presence of NAFLD (n=170)	Total(n=327)
FLI	−7.2(0.01)	− 3,7(0,06)	−13,7(<0,001)
HFS	−45(0.77)	−26,5(0,13)	−71,9(0,43)
Albumin	0.1(0.92)	0,0(0,95)	0,1(0,62)
CRP	0,26(0,01)	−0,21(0,02)	−0,26(0,01
Feruin-A	1,92(0,04)	−85,9(0,13)	−549(0,04)

*M1-M0 (p value). FLI: Fatty Liver Index; HFS: Hepatic Fibrosis Score; CRP: High sensitivity C-reactive Protein

Conclusion

Fetuin-A, a liver born protein, is a diagnostic potential biomarker for cardiovascular diseases related to liver dysfunction and, it responds sensitively positive to Lifestyle-change protocols.

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