Closing report of "Investigation of adipokine profile and lipid metabolism in obesity" (PD124126)

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1. Background and aim

Healthcare data indicate that the prevalence of obesity and associated comorbidities, i.e. insulin resistance, type 2 diabetes mellitus (T2DM), accelerated atherosclerosis are extensively increasing. The investigation of carbohydrate and lipid metabolism processes, that favor the development of obesity-related cardiovascular complications, is excessively important. Obesity-related dyslipidemia is one of the well-known complications in obese patients. Study of triglyceride rich lipoprotein particles, such as very low-density lipoprotein (VLDL) and low-density lipoprotein (LDL) are still standing in the focus of the researchers' interest for several decades. However, it must be noted that beside high-density lipoprotein (HDL) quantity, the investigation of HDL function have also been frequently studied. White adipose tissue termed adipokines and liver-expressed hepatokines play a harmful but beneficial role in the crosstalk between different cells and organs and may act as a "fine-tuner" of endocrine, autocrine and paracrine actions.

Since data are not fully explored, we planned to characterize the associations between the adipokine profile and various parameters of lipid and carbohydrate metabolisms in morbid obese patients with and without T2DM. Based upon the expected results, we aimed to extend our understanding of the modulating role of adipokines in lipoprotein homeostasis.

2. Major results and discussion

2.1. Plasminogen activator inhibitor-1 (PAI-1) level correlates with lipoprotein subfractions in obese non-diabetic subjects

PAI-1 is the primary regulator of fibrinolysis and it may influence the development of atherosclerotic plaque formation. The elevated levels of PAI-1 in obese subjects with metabolic syndrome and in patients with type 2 diabetes mellitus are well established; however, association of plasma PAI-1 with lipid metabolism is still unclear. The aim of the present study was to determine the relationship between plasma PAI-1 levels and the distribution of lipoprotein subfractions in non-diabetic obese (NDO; n=50; 43F/7M; 44.2±13.5 yrs) and aged and gender matched healthy lean individuals (n=32; 27F/5M; 41.8±6 yrs).

Plasma PAI-1 were found to be significantly higher (obese: 6.58 [5–8.58] vs. control: 2.93 [1.8–5.23] ng/ml; $p<0.001$) in NDO patients. Also, tumor necrosis factor-α, interleukin-6, oxidized LDL (oxLDL) and myeloperoxidase levels were significantly higher, while HDL-linked antioxidant PON1 paraoxonase and arylesterase activities were non-significantly lower in the obese patients. In line with previous literature data, the distribution of lipoprotein subfractions showed an atherogenic profile in NDO subjects. Small-dense LDL concentration was significantly higher ($p<0.001$), while mean LDL size was significantly lower ($p<0.001$)

in the obese group. HDL subfractions showed a shift towards small-sized HDL subfractions ($p<0.01$) in NDO compared to lean subjects. Strong significant negative correlations were found between plasma PAI-1 concentration and mean LDL-size ($p<0.001$); as well as between PAI-1 concentrations and the levels of the large and intermediate HDL subfractions ($p<0.001$ and $p<0.001$, respectively). In multiple regression analysis, PAI-1 was best predicted by waist circumference (WC) ($\beta=0.342$; $p<0.001$) and intermediate HDL subfraction ($\beta=-0,52$; $p<0.001$).

To the best of our knowledge, this is the first report about the correlations between plasma PAI-1 levels and lipoprotein subfractions in vivo, indicating the potential role of disturbed lipid metabolism in PAI-1 overproduction in obesity. The observed differences in the distribution of the lipoprotein subfractions may highlight the importance of the early screening of obese individuals to predict the development of subsequent cardiovascular complications. We detected correlations between LDL size/small-dense LDL levels and plasma PAI-1 concentrations in NDO subjects. These data support the suggested link between LDL and PAI-1 in accelerated atherogenesis and increased cardiovascular risk of obese patients.

The link between HDL subfractions and plasma PAI-1 level is not well understood. Using sequential ultracentrifugation, HDL3-, but not HDL2-bound sphingosine-1-phosphate (S1P) stimulates PAI-1 secretion in a concentration-dependent manner in 3T3 adipocytes. In our study, we could not find a significant correlation between PAI-1 and small HDL subfraction concentration (which mostly corresponds to HDL3) using non-gradient gel electrophoresis for lipoprotein subfraction analysis. Still, based on the in vitro results, S1P may have a potential role in elevated plasma PAI-1 level in NDO patients.

These results were published in a paper entitled **Somodi S, Seres I, Lőrincz H, Harangi M, Fülöp P, Paragh G. Plasminogen activator inhibitor-1 level correlates with lipoprotein subfractions in obese non-diabetic subjects. Int J Endocrinol. 2018 May 30;2018:9596054. https://doi.org/10.1155/2018/9596054 eCollection (Q2, IF: 2.287)**.

2.2. PAI-1 level correlates with the components of metabolic syndrome in a PAI-1 -675 4G/5G promoter polymorphism dependent manner

A common -675 4G/5G single guanine insertion/deletion polymorphism in the promoter region of the PAI-1 gene is of functional importance in regulating PAI-1 expression. The presence of the 4G allele does not influence plasma PAI-1 levels under normal conditions; however, it may exacerbate the effect of several factors on PAI-1 level during certain pathological conditions. Also, previous studies suggested that VLDL was capable of increasing the PAI-1 level via a VLDL response element localized in the promoter region of the PAI-1 gene, mediating VLDL-induced PAI-1 transcription in endothelial cells. We aimed to study the potential different correlations of carbohydrate and lipid parameters and plasma PAI-1 levels in the 4G and 5G carriers in ninety-three NDO (age: 44.7±12.5 yrs; BMI: 42.5±8.1 kg/m^2) and thirty-two healthy, normal weight subjects (age: 41.8±6 yrs; BMI: 24.5±2.5 kg/m^2).

The genotype distribution of PAI-1 4G/5G polymorphism was not significantly differed in NDO patients (4G/4G 27.9%; 4G/5G 45.2% and 5G/5G 26.9%) compared to controls (4G/4G

31.3%; 4G/5G 46.9% and 5G/5G 21.9%, p=0.8). Slightly higher PAI-1 levels were found in 4G carriers (4G/4G+4G/5G) in the obese and control group (p=0.07 and p=0.02, respectively). In all subjects with 4G/5G genotype, plasma PAI-1 correlated negatively HDL-cholesterol (HDL-C) (p=0.02) and apolipoprotein AI (apoAI) levels (p<0.001). In 5G/5G participants, PAI-1 also correlated negatively with HDL-C (p=0.025) and apoAI (p=0.007) concentrations, moreover positively with triglyceride (p=0.02), fasting glucose (p=0.002) and hemoglobin A1c (HbA1c) (p=0.027). These correlations are lacking in 4G/4G participants.

In this pilot study, the observed correlations between PAI-1 levels and the components of metabolic syndrome suggest a closer link between PAI-1 and lipid and carbohydrate metabolism in subjects with 5G/5G genotype. Based on the preliminary data we plan to enroll patients with T2DM. Measurement of HDL and LDL subfraction distribution may add further valuable information about the role of PAI-1 in lipid metabolism.

The results were presented on the European Atherosclerosis Society 90th Congress in Milan, Italy and published as a congress abstract in Atherosclerosis journal: **Lőrincz H, Galgóczi E, Katkó M, Ratku B, Ötvös T, Harangi M, Paragh G, Szbó Z, Somodi S. Plasminogen activator inhibitor-1 level associated with the components of metabolic syndrome in a 4G/5G polymorphism dependent manner. Atherosclerosis, 2022, 355:66.** **https://doi.org/10.1016/j.atherosclerosis.2022.06.705**

2.3. Low levels of circulating fetuin-A and retinol-binding protein 4 (RBP4) in morbid obese non-diabetic subjects

Fetuin-A is primarily a hepatokine and partially an adipokine, which is originally described as an essential developmental factor in the fetal circulation. Elevated fetuin-A level is also considered as a biomarker for obesity, obesity-related dyslipidemia, metabolic syndrome and atherogenesis. RBP4 is a hepatokine wich transfers retinol (vitamin A) from the liver to the target tissues within the circulation in combination with transthyretin, which prevents renal filtration and catabolism of RBP4. To date, results are highly controversial about the direct association of fetuin-A and RBP4 to HDL particle.

We enrolled one-hundred NDO patients (83F/17M; 44.7±12.5 yrs; BMI: 42.5±8.1 kg/m^2) and thirty-two aged and gender matched healthy, lean volunteers (27F/5M; 41.8±6 yrs; BMI: 24.5±2.5 kg/m^2). Serum fetuin-A were found to be unexpectedly lower in NDO patients compared to lean controls (fetuin-A: 944.6±201.9 vs. 1068.8±253.3 µg/ml, p<0.01). Fetuin-A correlated negatively with BMI (p=0.01) and WC (p<0.01) in all participants. Additionally, fetuin-A showed a positive correlation with HDL-C (p=0.02), apoAI (p<0.001), VLDL subfraction (p=0.05), as well as large HDL subfraction levels (p=0.001) in overall subjects. Backward stepwise multiple regression analysis showed that fetuin-A is best predicted by large HDL subfraction (ß=0.364, p<0.001).

RBP4 was significantly lower in NDO patients compared to controls (32.3±15.0 vs. 41.4±14.4 µg/ml, p<0.01). RBP4 negatively correlated with BMI (p=0.02), WC (p=0.02) and fasting glucose (p<0.01) in all the enrolled subjects; while there was a positive correlation between RBP4 and C-peptide levels (p<0.01) in NDO patients. We found positive correlations between RBP4 level and HDL-C (p=0.025), apoAI (p=0.01), VLDL subfraction (r=0.37;

p<0.001), intermediate HDL subfraction (r=0.01) and small HDL subfraction levels (p=0.02) in overall participants. RBP4 level was predicted by VLDL subfraction (ß=0.477, p<0.001).

This is the first complex clinical examination of the correlations of fetuin-A and RBP4 in relation to lipoprotein subfractions in NDO patients. Circulating fetuin-A inhibits the activity of insulin receptors mediating phosphatildylinositide 3-kinase (PI3K) and Akt signaling pathways; it also inhibits GLUT4 translocation, which could lead to lower HDL-C level. Meanwhile, HDL can promote glucose uptake in the adipocytes and glycogen synthesis through enhancing GLUT4 involving PI3K/Akt via AMPK signaling pathways. Previously, fetuin-A was described as one of the HDL-associated proteins in proteomic analyses, but its affinity to various HDL subclasses is not clarified. A positive correlation between the levels of fetuin-A and large HDL subfraction may indicate that fetuin-A is associated to large HDL particles, and its glycosylation, reported previously, may promote its enhanced clearance from the circulation, leading to a lower level of large HDL subfraction. Further studies on HDL function are needed to evaluate this hypothesis.

A previous study showed a U-shaped association between RBP4 concentration and risk of incident of T2DM. Contradictory results are published about the role of RBP4; also, both atherogenic and cardioprotective effects of RBP4 have been described. These results can support our recent data. Proteomic analyses on HDL described an extended list of HDL-associated proteins. Beside structural and enzymatic proteins other small amounts of proteins could link to HDL particles including fetuin-A and RBP4. In patients with chronic kidney disease, almost a two-fold abundance of RBP4 was associated with lower estimated glomerular filtration rate (eGFR) in HDL particles. However, some authors indicated that the data of HDL particle proteome composition are highly variable and strongly depend on the HDL isolation and purification method. These precise results of proteomic analyses could verify our results; however, further examinations are necessary to clarify these findings in obesity.

We published our results in a paper entitled **Low levels of serum fetuin-A and retinol-binding protein 4 correlate with lipoprotein subfractions in morbid obese and lean non-diabetic subjects. Lőrincz H, Csige I, Harangi M, Szentpéteri A, Seres I, Szabó Z, Paragh G, Somodi S. Life (Basel). 2021 Aug 27;11(9):881. doi: 10.3390/life11090881. (Q2, IF: 3.251).**

2.4. Role of afamin in oxidative stress and lipid metabolism in obese non-diabetic and obese type 2 diabetic patients

Afamin is a liver-produced bioactive glycoprotein, the fourth member of the albumin gene family and features vitamin D and E binding sites. Previous studies showed that circulating afamin is elevated in obesity, metabolic syndrome, polycystic ovary syndrome and gestational diabetes and is correlated well with components of metabolic syndrome. Additionally, a recent study suggested afamin as a potential novel biomarker of increased hepatic lipid content in prediabetes and T2DM. To date, afamin concentrations, correlations between afamin and α- and γ- tocopherols, afamin and lipoprotein subfractions in NDO and obese T2DM patients have not been studied.

Afamin concentrations were significantly higher in NDO patients (n=50; 43F/7M; 44.2±13.5 yrs) compared to gender and aged matched healthy controls (n=32; 27F/5M; 41.8±6 yrs): 70.4±12.8 vs. 47.6±8.5 μg/ml, $p<0.001$. α-, γ- tocopherol and oxLDL levels were higher in NDO patients ($p<0.05$, $p<0.001$ and $p<0.005$, respectively).

Positive correlations were found between afamin and BMI ($p<0.001$), WC ($p<0.001$), fasting glucose ($p<0.001$), HbA1c ($p<0.001$), hsCRP ($p<0.001$), triglyceride ($p<0.01$), and oxLDL levels ($p=0.02$), as well as the amount and ratio of small HDL subfractions ($p<0.001$). Negative correlations were observed between afamin and mean LDL size ($p<0.001$), as well as the amount and ratio of large HDL subfractions ($p<0.001$). However, the α- and γ-tocopherol levels did not correlate with the afamin concentrations in obese patients ($r = 0.20$; $p = 0.2$ and $r = 0.22$; $p = 0.1$, respectively). After multiple regression analysis, WC ($\beta=0.685$; $p<0.001$), HbA1c ($\beta=0.291$; $p<0.01$) and small HDL ($\beta=0.282$; $p<0.05$) turned out to be independent predictors of afamin.

After this examination we added another fifty NDO and 38 morbid obese patients with T2DM (28F/10M, age: 49±7 yrs, BMI: 42±9 kg/m^2) to the study. Afamin was significantly elevated in T2DM group compared to NDO and controls (102.4±19.7 vs. 81.1±18.8 vs. 47.6±8.5 μg/ml, $p<0.001$). We observed strong significant negative correlations between large, intermediate HDL and afamin ($p<0.001$ and $p<0.001$, respectively) and positive correlations between small HDL and afamin ($p<0.001$) in NDO+T2DM (n=138) group.

Our results suggest that afamin - despite the fact that it features specific α- and γ-tocopherol binding sites - does not play a crucial role in the regulation of vitamin E levels. However, increased α- and γ-tocopherol concentrations could be beneficial in reducing endogen oxidative stress in NDO patients. The strong correlation observed between HDL subfractions and afamin levels is a remarkable finding and may help us to better understand the function of HDL subfractions. However, the increased amount and percentage of small, dense HDL subfractions alone - as small HDL levels were only 18.1% higher NDO patients - does not explain the significant increase in afamin levels.

Afamin showed markedly close associations with carbohydrate parameters and lipoprotein subfractions, therefore determination of afamin level might be an excellent biomarker in assessing the condition of morbid obese patients with or without manifest insulin resistance. The strong correlation of afamin with HDL and LDL subfractions highlights the link between afamin and lipid metabolism in obesity and T2DM.

These results were published in a paper entitled **Juhász I, Ujfalusi S, Seres I, Lőrincz H, Varga VE, Paragh G Jr, Somodi S, Harangi M, Paragh G. Afamin Levels and Their Correlation with Oxidative and Lipid Parameters in Non-diabetic, Obese Patients. Biomolecules. 2022 Jan 12;12(1):116. doi: 10.3390/biom12010116. (Q2, IF:6.064)** Furthermore, additional data on T2DM subjects are presented at the Hungarian Diabetes Association XXX. Congress: **Lőrincz H, Ratku B, Ötvös T, Szentpéteri A, Seres I, Katona É, Páll D, Paragh G, Harangi M, Somodi S: A szérum afamin korrelál a szénhidrát paraméterekkel és a lipoprotein szubfrakciókkal elhízott 2-es típusú diabeteses és nem diabeteses betegekben. Diabetologia Hungarica, 30 évf. 2. szuppl. 2022. szeptember. doi: 10.24121/dh.2022.S2.**

2.5. Other ongoing projects

Adipokine profile can show significant alterations in obesity during a follow-up and these changes may contribute to increased cardiovascular risk and atherogenesis. Based upon these observations, a 5 or 10-years follow-up study may reveal some further, presumably unfavorable, changes in the LDL and HDL subfraction profile compared to the baseline. Because of the COVID-19 pandemic we cannot involve the planned number of participants to this follow-up; however, anthropometric, anamnestic and routine laboratory data collection of enrolled subjects is in progress; and we plan to enroll 50-60 patients until the end of the year.

3. Summary

Our results on morbid obese non-diabetic and T2DM patients may help to define efficient therapeutic strategies and to find novel treatment options to alter lipoprotein levels and to reduce the risk of atherogenesis. Investigation of structural and qualitative properties of HDL particles may provide further detailed information about impaired HDL function.

The results were published in three papers (Q2, IF: 11.612), in five citable abstracts and in three lectures and posters at national conferences. The project was paused two years (2018 and 2021) because of the PI’s maternal leave.