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B. CHOROMANSKA[1], P. MYSLIWIEC[1], H.R. HADY[1], J. DADAN[1],
H. MYSLIWIEC[2], T. BONDA[3], A. CHABOWSKI[4], A. MIKLOSZ[4]

THE IMPLICATION OF ADIPOCYTE ATP-BINDING CASSETTE A1 AND G1 TRANSPORTERS IN METABOLIC COMPLICATIONS OF OBESITY

[1]1st Department of General and Endocrine Surgery, Medical University of Bialystok, Bialystok, Poland;
[2]Department of Dermatology and Venereology, Medical University of Bialystok, Bialystok, Poland;
[3]Department of General and Experimental Pathology, Medical University of Bialystok, Bialystok, Poland;
[4]Department of Physiology, Medical University of Bialystok, Bialystok, Poland

Obesity is characterised by imbalance in lipid metabolism manifested by high concentrations of circulating triacylglycerols and total cholesterol as well as low high-density lipoprotein (HDL) levels. Abnormalities related to these lipids lead to metabolic complications such as type 2 diabetes, arterial hypertension and cardiovascular disease. Despite extensive research, it is still unclear why a subset of obese subjects develop metabolic syndrome, while others do not. The aim of our work was to assess total and plasma membrane expressions of cholesterol transport proteins: adipocyte ATP-binding cassette A1 (ABCA1), adipocyte ATP-binding cassette G1 (ABCG1), class B scavenger receptor (SR-BI) in visceral and subcutaneous adipose tissue of obese subjects with and without metabolic syndrome. To keep our preliminary study group uniform, we focused on women, who constitute the majority of bariatric patients. The study was performed on 34 patients: 24 morbidly obese women subjected to bariatric surgery, half of whom had metabolic syndrome; and 10 lean subjects undergoing elective laparoscopic cholecystectomy. Total and plasma membrane expressions of cholesterol transport proteins (SR-BI, ABCA1 and ABCG1) were assessed in samples of both visceral and subcutaneous adipose and analysed in relation to other clinical and laboratory parameters. We demonstrated lower plasma membrane expressions of ABCG1 in visceral adipose tissue of obese patients with metabolic syndrome as compared to lean ones. In addition, total ABCG1 expressions in both types of adipose tissue were lower in morbidly obese patients with metabolic syndrome compared to those without metabolic syndrome. Plasma membrane ABCA1 expressions in visceral adipose tissue were lower in the group of morbidly obese patients without metabolic syndrome, compared to lean patients. We did not find any significant differences in SR-BI expressions. Because of ABCG1 is responsible for cholesterol efflux to HDL, reduced plasma membrane expression of ABCG1 in VAT of morbidly obese women with metabolic syndrome may leads to a significantly decreased concentration of HDL in serum. This may be also confirmed by high positive correlation between both parameters.

Key words: *adipose tissue, cholesterol transport proteins, metabolic syndrome, obesity, high-density lipoprotein, triacylglycerols*

INTRODUCTION

A sedentary lifestyle and the increased consumption of high-calorie Western diet contribute to the elevation of body mass index (BMI) and directly increase occurrence of overweight (BMI 25 – 29.9), obesity (BMI ≥ 30) and morbid obesity (BMI ≥ 40) (1). Obesity is a serious health condition leads to insulin resistance, type 2 diabetes, arterial hypertension, cardiovascular diseases and hormonal disorders as well as primary and metastatic cancer (2-7). Because of exploding prevalence of obesity worldwide the molecular pathways that link obesity with metabolic dysfunctions, collectively referred as to metabolic syndrome (MetS), become a subject of intensive scientific investigation. It has been shown that the development of obesity and its metabolic complications are affected by adipokine secretion such as leptin, adipokine or resistin (8, 9). Nevertheless, recent studies emphasise also the role of cholesterol transport proteins in the development of metabolic complications of obesity (10-12). Adipose tissue contains approximately 25% of total cholesterol in human body (13). In obese individuals, a considerable increase in cholesterol content in adipocytes can be observed, which may result in a dysfunction of adipocytes and development of metabolic syndrome, associated with low HDL concentration in the plasma (13-15). Approximately 95% of adipocytes cholesterol is present in a free form in cell membranes or in the membrane-cytosol interface (16, 17). Efflux of cholesterol is accompanied by cholesterol transport proteins including adipocyte ATP-binding cassette A1 (ABCA1), adipocyte ATP-binding cassette G1 (ABCG1) and class B scavenger receptor (SR-BI). ABCA1 and ABCG1 belong to the family of ATP binding cassette proteins and are tightly regulated by transcription control of LXR alpha receptor (liver X-a) (18). ABCA1 is a mediator in a cholesterol efflux to lipid free

apolipoprotein A-I (apoAI), which then become nascent HDL (19). It has been shown that decreased expression of ABCA1 or ABCG1 is related to reduced HDL levels (20). Moreover, ABCG1 deficiency leads to a decreased lipid influx to HDL fraction (21) and activates proinflammatory cytokines (22). Furthermore, cholesterol esters uptake from HDL and LDL (low-density lipoprotein) is also regulated by SR-BI. SR-BI is an integral membrane protein in numerous cells/tissues and contrary to ABCA1 and ABCG1, it is beyond the control of LXR alpha receptor. It facilitates bidirectional cholesterol flow depending on its intracellular content (18). SR-BI is most known for its role in facilitating absorption of cholesterol esters from high density lipoproteins in the liver. Indeed, this process triggers cholesterol movement from peripheral tissues to the liver and is called reverse cholesterol transport. It is a potentially protective mechanism against development of atherosclerosis and cardiac disorders (23).

So far, there are no data linking expression of cholesterol transport proteins in the adipose tissue with the serum concentration of HDL in morbidly obese subject with metabolic syndrome. Additionally, we would like to explore depot specific differences in cholesterol transporters expression. Therefore, the aim of the study was to evaluate total and plasma membrane expression of cholesterol transport proteins (SR-BI, ABCA1 and ABCG1) in both visceral and subcutaneous adipose tissue of the patients with morbid obesity without and with metabolic syndrome and correlate it with serum HDL concentration.

MATERIALS AND METHODS

Patients

The study group consisted of 24 patients with class 3 obesity (BMI ≥ 45), women aged from 19 to 65 years), who underwent elective bariatric surgery. The patients were divided into two groups: individuals without metabolic syndrome (designation of the group: MetSx-) (n = 12) and obese individuals with metabolic syndrome (designation of the group: MetSx+) (n = 12). The control material was collected from the group of 10 lean patients (BMI ≤ 25) (women aged from 21 to 60 years) who underwent elective laparoscopic cholecystectomy. The characteristics of both groups are shown in *Table 1*. MetS was diagnosed in accordance with the International Diabetes Federation. Additionally, seven patients were treated for type 2 diabetes mellitus (T2DM), and five patients were treated for hypertension. All patients gave their informed consent to participate in the study. Patients with acute inflammatory changes or history of malignancy were excluded from the study.

All the patients were treated at the 1st Department of General and Endocrinology Surgery of the University Hospital in Bialystok. All procedures were designed, conducted, and reported in compliance with the Declaration of Helsinki and were approved by the Ethics Committee of the Medical University of Bialystok (permission R-I-002/187/2017), functioning according to the Guidelines for Good Clinical Practice.

Table 1. Clinical characteristics of lean subjects, patients with morbid obesity without metabolic syndrome (MetSx-) and patients with morbid obesity and metabolic syndrome (MetSx+) (mean ± SEM). Significant differences are shown as: different from lean subjects: * P < 0.05, ** P < 0.01, *** P < 0.001; different from MetSx-: # P < 0.05. *Abbreviations*: ALT, alanine transaminase; AST, aspartate transaminase; BMI, body mass index; CRP, C reactive protein; HCT, hematocrit; HDL, high-density lipoprotein; HGB, hemoglobin: HOMA-IR, insulin resistance index; INR, international normalized ratio; LDL, low-density lipoprotein; PLT, platelet count; RBC, red blood cells; TAG, triacylglycerol; WBC, white blood cells; WHR, waist hip ratio.

	Lean subjects (n = 10)	**Obese MetSx-** (n = 12)	**Obese MetSx+** (n = 12)
Age	42.6 ± 13.21	39.8 ± 14.65	46.7 ± 8.32
BMI (kg/m^2)	23.2 ± 1.61	44.3 ± 1.59***	45.7 ± 1,92***
WHR	0.7 ± 0.04	0.91 ± 0.04**	0.93 ± 0.05***
Waist (cm)	79 ± 2.14	125.1 ± 4.23 **	129.3 ± 4.27 ***
Blood pressure (mmHg)	120/83 ± 2.1/2.4	127/81 ± 3.4/2.1	148/97 ± 2.8/2.7*#
CRP (mg/l)	5.5 ± 0.43	9.7 ± 1.72*	11.6 ± 1.88**#
Glucose (mmol/l)	4.2 ± 0.11	5.7 ± 0.27 **	7.7 ± 0.51 ***#
Insulin (pmol/l)	35.41 ± 3.38	112.4 ± 15.89 ***	145.84 ± 19.13 *** #
HOMA-IR	1.2 ± 0.1	5.7 ± 0.61 ***	8.1 ± 1.19 *** #
ALT (UI/l)	20.1 ± 6.2	29.3 ± 6.7	34.4 ± 4.7
AST (UI/l)	21.0 ± 7.4	23.0 ± 6.4	24.0 ± 8.2
Cholesterol (mmol/l)	4.5 ± 0.08	5.2 ± 0.41	5.6 ± 0.28 *
LDL (mmol/l)	3.2 ± 0.11	3.3 ± 0.31	3.9 ± 0.17 *
TAG (mmol/l)	1.3 ± 0.09	1.5 ± 0.16	1.9 ± 0.17 *#
HDL (mmol/l)	1.6 ± 0.06	1.4 ± 0.09 *	1.2 ± 0.07 ** #
WBC ($10^3/\mu l$)	6.2 ± 0.9	8.5 ± 1.2*	9.6 ± 3.3**#
RBC ($10^6/\mu l$)	4.7 ± 0.2	4.6 ± 0.3	4.6 ± 0.7
HGB (g/dl)	13.6 ± 1.0	13.0 ± 1.0	13.1 ± 1.7
HCT (%)	41.4 ± 3.1	39.5 ± 2.8	40.1 ± 5.5
PLT ($10^3/\mu l$)	296.6 ± 47.2	344.6 ± 48.3	275.4 ± 55.4
Fibrynogen (mg/dl)	377.2 ± 15.0	457.0 ± 25.0*	480.3 ± 25.3**
INR	0.8 ± 0.1	0.9 ± 0.1	0.9 ± 0.1

Samples

Blood samples were taken from obese and lean patients in the overnight fasting state to EDTA coated tubes, prior to the surgery. The blood samples were centrifuged for 10 min 4000 r.p.m.

At the end of the surgical intervention, the samples of visceral adipose tissue (VAT) and subcutaneous adipose tissue (SAT) were excised from the upper abdomen. The tissues were promptly frozen in liquid nitrogen and stored at –80°C until final analysis.

The studies were carried out in the Department of Physiology, Medical University of Bialystok.

Laboratory measurements

Plasma triglycerides, total cholesterol, HDL and LDL cholesterol, CRP, ALT, AST, full blood count, fibrinogen, INR, glucose and insulin were quantified using Abbott analyzer (Wiesbaden, Germany).

Subcellular fractionation

Subcellular fractionation was made using the sucrose technique according to protocol of Gargiulo *et al*. (24) with some modifications. Adipose tissue (250 mg) was resuspended in 2.5 ml sucrose homogenization buffer (SHB) containing a cocktail of protease and phosphatase inhibitors (Roche Diagnostics GmbH, Mannheim, Germany) as well as Triton-X100 (Sigma-Aldrich, St. Louis, MO, USA). The samples were homogenized and centrifuged 10 min at 500 g. The pellet was resuspended in 0.5 mL SHB buffer, layered onto 1 ml sucrose cushion and centrifuged for 25 min at 180,000 g. The membrane fractions was yielded from the supernatant. Protein concentration was determined by bicinchoninic acid (BCA) assay.

Western blot analysis

The protein expressions of ABCA1, ABCG1 and SR-BI were determined in total and plasma membrane adipose tissue homogenates (30 µg). Routine Western blot procedure was used to detect proteins, as described previously (25-27). Briefly, the samples were homogenized in an ice-cold RIPA buffer containing a cocktail of protease and phosphatase inhibitors (Roche Diagnostics GmbH, Mannheim, Germany). Protein content in each sample was determined by bicinchoninic acid method with BSA assay bovine serum albumin as a standard. Then, the proteins in each sample were separated using electrophoresis method. Next, the membranes were immunoblotted with primary antibodies: anti-ABCA1, anti-ABCG1, anti-SR-BI and β-tubulin (Novus Biologicals, US), anti-Na^+/K^+ (Santa Cruz Biotechnology, USA). The primary antibodies were detected with a horseradish peroxidase-conjugated secondary antibody (Santa Cruz Biotechnology, CA). The proteins were visualised using an enhanced chemiluminescence substrate (Thermo Scientific, USA) and quantified by densitometry (Bio-Rad, Poland). Equal protein concentrations were loaded in each lane as confirmed by Ponceau staining the blot membrane. Protein expression (Optical Density Arbitrary Units) was normalized to β-tubulin expression. Finally, the control was set to 100 and the experimental groups were expressed relatively to the control.

First, we evaluated the purity of the tissue homogenate plasma membrane. Using the Western blot method, we confirmed the presence of subunit Na^+/K^+ pump (Santa Cruz Biotechnology, Inc., Dallas, Texas) in the plasma membrane fraction and the lack of mitochondrial cytochrome C (Abcam, Cambridge, Massachusetts). Additionally, we not found the presence of sarcoendoplasmic reticulum calcium ATPase and P-nuc (Abcam), which are nuclear proteins.

Triacylglycerides content

Triacylglycerides (TAGs) tissue were analyzed by gas liquid chromatography (GLC) as described previously (28-30). Briefly, the frozen muscle samples were pulverized in an aluminum mortar precooled in liquid nitrogen. The adipose tissue samples (10 mg) were extracted with chloroform-methanol (2:1 vol/vol) with antioxidant (0.01% butylated hydroxytoluene). Next, an internal standard (100 µl) containing heptadecanoic acid (C17:0 FFA), 1,2-diheptadecanoin (C17:0 DG) and triheptadecanoin (C17:0 TG) (Sigma-Aldrich, St. Louis, MO, USA) was added. After overnight extraction, the samples were separated using TLC on silica gel plates (Silica Plate 60, 0.25 mm; Merck, Darmstadt, Germany) with a heptane: isopropyl: acetic acid (60:40:3, vol/vol/vol) resolving solution. After visualization the lipid bands containing TAG were scraped off and methylated. The fatty acid methyl esters (FAMEs) were extracted using pentane. Thereafter samples were dissolved in hexane and analyzed using a Hewlett-Packard 5890 Series II gas chromatograph with Varian CP-SIL88 capillary column (50 mm × 0.25 mm internal diameter) and a flame-ionization detector (FID) (Agilent Technologies, CA, USA). The individual fatty acids were quantified according to the standard retention times. The adipose tissue TAG concentration was estimated as the sum of particular fatty acid species content. The value was expressed as nanomoles per gram of adipose tissue wet weight.

Statistical analysis

The results were statistically analysed using Statistica 13 (StatSoft, Cracow, Poland). The data were analysed using one-way analysis of variance (ANOVA) followed by *post hoc* Tukey's test for groups with normal distribution test and non-parametric Kruskal-Wallis for groups without normal distribution. Spearman's test was used to evaluate correlations between studied parameters. For consistency of data in the graphs, the results are presented as mean ± standard error. Values $P < 0.05$ were considered statistically significant.

RESULTS

Clinical and laboratory parameters

The clinical data are summarized in the *Table 1*. In obese MetSx- ($P < 0.01$) and MetSx+ ($P < 0.001$) patients we observed increased waist, waist/hip ratio and blood as compared to lean patients (*Table 1*). Insulin and HOMA-IR index were increased in both MetSx- and MetSx+ patients as compared to the control group ($P < 0.001$). Blood pressure, total cholesterol, LDL, TAG were increased only in MetSx+ patients ($P < 0.05$). HDL was decreased, while inflammatory parameters: CRP, WBC and fibrinogen were increased in MetSx- ($P < 0.05$) and MetSx+ ($P < 0.01$) as compared to lean patients. As regard the comparison MetSx+ versus MetSx- patients, we noticed that MetSx+ patients had increased: blood pressure, glucose, insulin, HOMA-IR, CRP, WBC and TAG and decreased HDL ($P < 0.05$ for all mentioned) (*Table 1*).

Adipocyte ATP-binding cassette A1 expressions

There were no significant differences in the total expression of ABCA1 in VAT and SAT in both morbidly obese study groups as compared to lean patients (*Fig. 1A*).

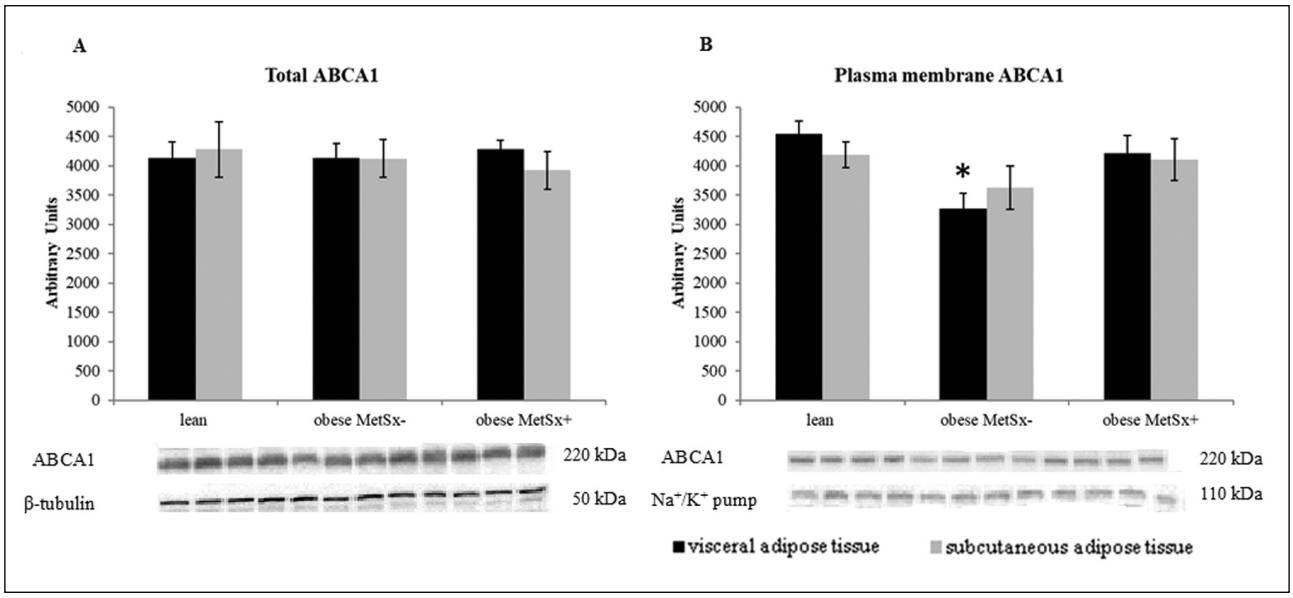

Fig. 1. Total (A) and plasma membrane (B) expression of ABCA1 protein in visceral (VAT) and subcutaneous adipose tissue (SAT) of lean, with obesity without metabolic syndrome (MetSx-) and with obesity with metabolic syndrome (MetSx+) subjects. Results are shown in arbitrary units and presented as mean ± SEM. Significant differences are shown as: * different from the visceral adipose tissue of lean patients, * $P < 0.05$.

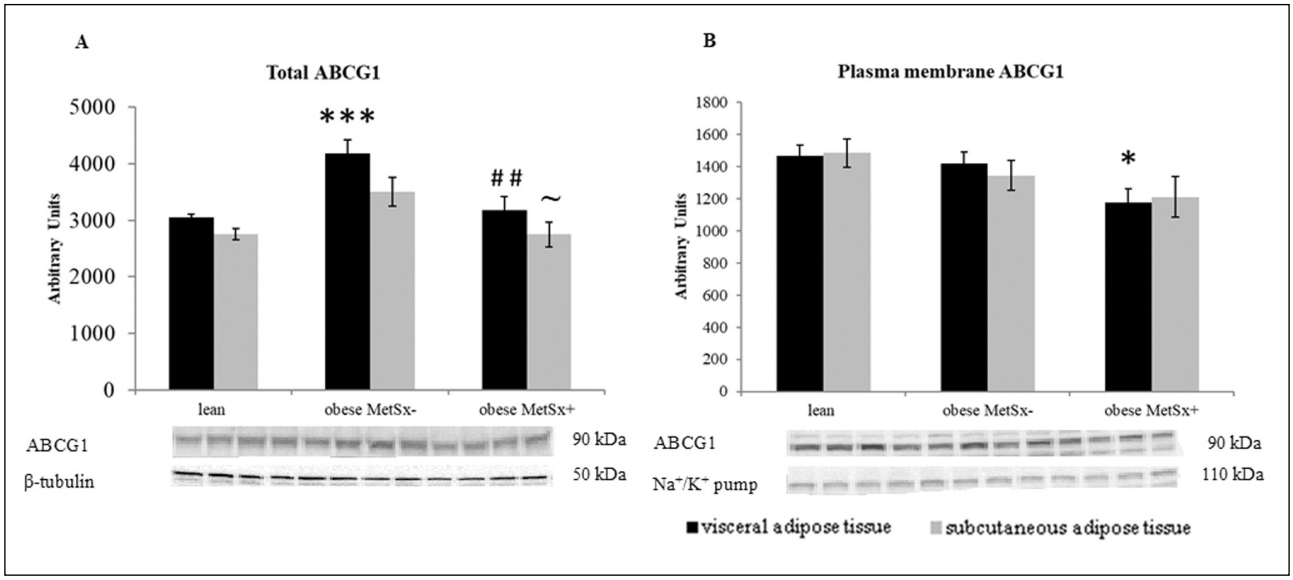

Fig. 2. Total (A) and plasma membrane (B) expression of ABCG1 protein in visceral (VAT) and subcutaneous adipose tissue (SAT) of lean, with obesity without metabolic syndrome (MetSx-) and with obesity with metabolic syndrome (MetSx+) subjects. Results are shown in arbitrary units and presented as mean ± SEM. Significant differences are shown as: * different from the visceral adipose tissue of lean patients, * $P < 0.05$, *** $P < 0.001$; # different from VAT MetSx-: ## $P < 0.01$; ~ different from subcutaneous adipose tissue (SAT) of patients with obesity without metabolic syndrome (MetSx-) ~ $P < 0.05$.

The plasma membrane ABCA1 expression in VAT was significantly decreased in the group of morbidly obese patients without metabolic syndrome (–28%, MetSx- versus lean, $P < 0.05$; *Fig. 1B*), compared to lean patients. The expression of plasma membrane ABCA1 in SAT showed no changes in morbidly obese patients without metabolic syndrome compared to lean patients. Similarly, the plasma membrane ABCA1 expression in both types of adipose tissue did not change in morbidly obese patients with metabolic syndrome compared to lean patients (*Fig. 1B*).

Adipocyte ATP-binding cassette G1 expression

Total ABCG1 protein expression in VAT was significantly higher in morbidly obese patients without metabolic syndrome (+37%, MetSx- versus lean, $P < 0.001$; *Fig. 2A*), compared to lean individuals. Total ABCG1 expression in SAT showed a trend towards higher values in morbidly obese patients without metabolic syndrome (+27%, MetSx- versus lean, $P = 0.67$) compared to lean patients. In addition, total ABCG1 expression in VAT and SAT significantly decreased in morbidly obese

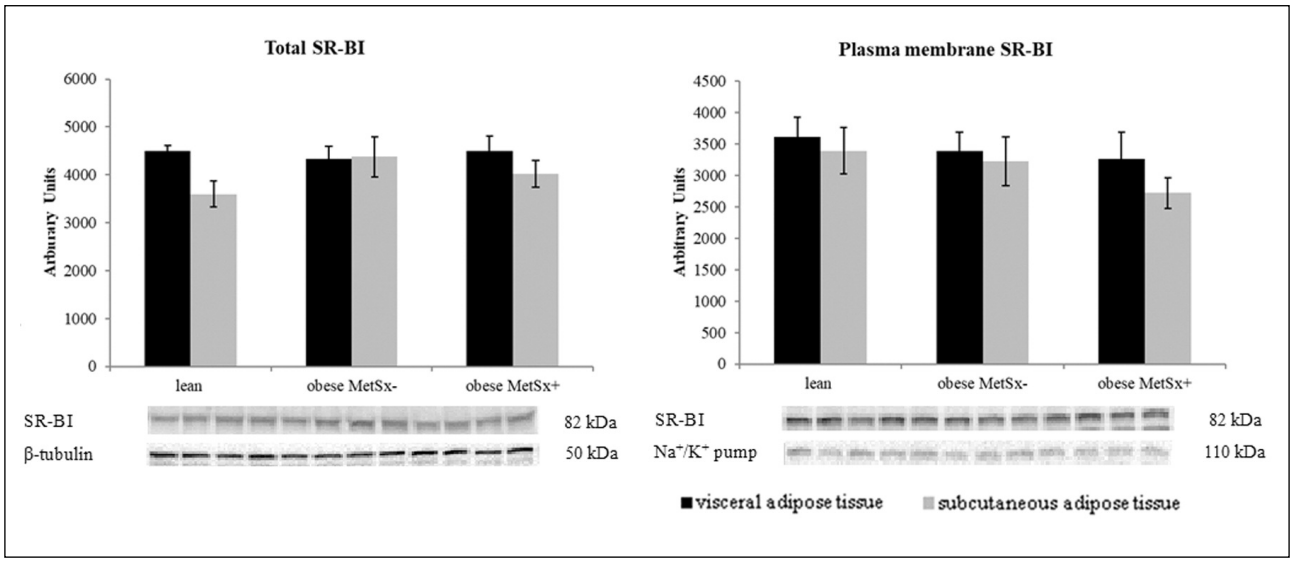

Fig. 3. Total (S) and plasma membrane (B) expression of SR-BI protein in visceral (VAT) and subcutaneous adipose tissue (SAT) of lean, with obesity without metabolic syndrome (MetSx-) and with obesity with metabolic syndrome (MetSx+) subjects. Results are shown in arbitrary units and presented as mean ± SEM.

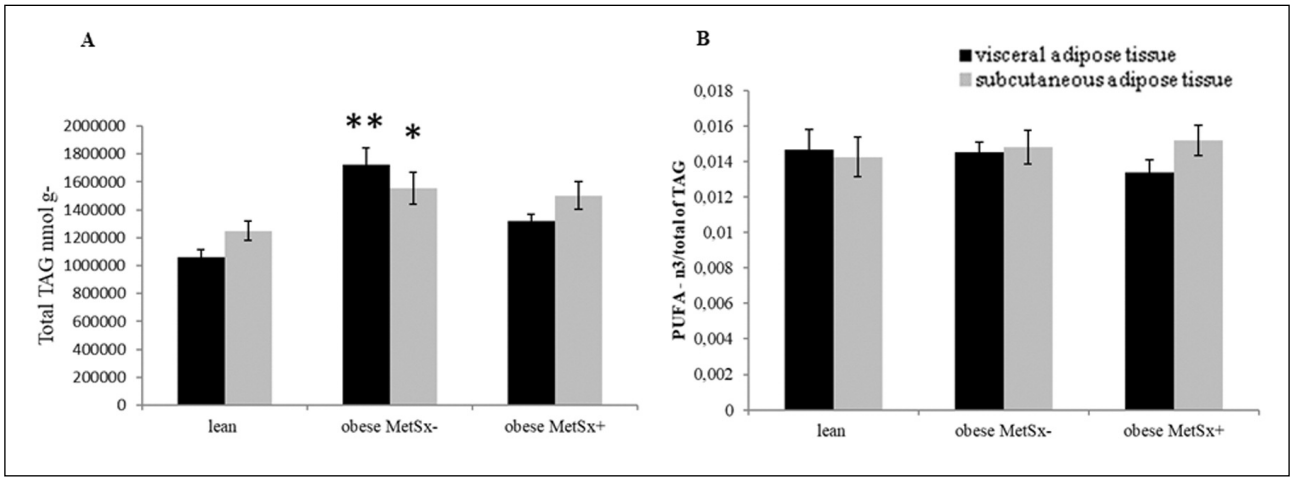

Fig. 4. Total content (A) and the ratio of polyunsaturated (PUFA) n3 / total triacylglycerols (B) fatty acids of TAG in visceral (VAT) and subcutaneous adipose tissue (SAT) of lean subjects, patients with obesity without metabolic syndrome (MetSx-) and with metabolic syndrome (MetSx+) subjects. Results are presented as mean ± SEM. Significant differences are shown as: * different from visceral adipose tissue of lean patients * $P < 0.05$; ** $P < 0.01$.

patients with metabolic syndrome (–24%, $P < 0.01$, –22%, $P < 0.05$, respectively, MetSx+ versus MetSx-; *Fig. 2A*) compared to those without metabolic syndrome. The plasma membrane ABCG1 expression in VAT significantly decreased in morbidly obese patients with metabolic syndrome (–20%, MetSx+ versus lean, $P < 0.05$; *Fig. 2B*) as compared to the lean ones. There were no significant differences in the plasma membrane expression of ABCG1 in VAT and SAT in morbidly obese patients without metabolic syndrome as compared to lean patients (*Fig. 2B*).

Class B scavenger receptor expression

There were no statistically significant differences in the total and plasma expression of SR-BI in VAT and SAT in both morbidly obese study groups as compared to lean patients (*Fig. 3A*, *3B*).

Content of triacylglycerides

The total fatty acid content of TAG in VAT was significantly greater in morbidly obese patients without metabolic syndrome (+62%, MetSx- versus lean, $P < 0.01$) compared to lean patients (*Fig. 4A*, *Table 2*). Similarly, we observed a greater concentration of TAG in subcutaneous adipose tissue of MetSx- subjects (+24%, MetSx- versus lean, $P < 0.05$; *Fig. 4A*, *Table 2*).

The content of saturated fatty acids of TAG in VAT and SAT increased significantly in morbidly obese patients without metabolic syndrome (+48% and +16%, MetSx- versus Ctrl, respectively, $P < 0.05$; *Table 2*) in comparison with lean subjects. Additionally, the content of saturated fatty acids of TAG in VAT was significantly diminished in morbidly obese patients with metabolic syndrome compared to morbidly obese patients without metabolic syndrome (–26 %, MetSx+ versus MetSx-; *Table 2*). As regard, the content of unsaturated fatty acids of TAG,

Table 2. Fatty acid - triacylglycerols (nmol/g) composition in lean subjects, patients with morbid obesity without metabolic syndrome (MetSx-) and patients with morbid obesity and metabolic syndrome (MetSx+) (mean ± SEM). Significant differences are shown as: different from VAT lean subjects: * P < 0.05, ** P< 0.01; # different from VAT MetSx-: # P < 0.05; ^ different from VAT of MetSx+: ^ P < 0.05. VAT, visceral adipose tissue; SAT, subcutaneous adipose tissue.

	Lean subjects (n = 10)		**Obese MetSx-** (n = 12)		**Obese MetSx+** (n = 12)	
	VAT	**SAT**	**VAT**	**SAT**	**VAT**	**SAT**
14:0	34244 ± 1602	38660 ± 2011	48934.3 ± 4317.4	41519.7 ± 2727.2	30798 ± 3038#	33219 ± 3575
16:0	273579 ± 12972	335043 ± 14889	434338 ± 25696*	415279.1 ± 27302.8*	327606 ± 12012	376032 ± 24616
16:1	53128 ± 2718	60516 ± 8230	139654 ± 15834*	103183.9 ± 12417.2*	90752 ± 8677	101074 ± 12961*
18:0	53097 ± 3733	55876 ± 5664	52823 ± 3405	44034.1 ± 2704.7	39938 ± 2295	46859 ± 3414
18:1n9c	509827 ± 31708	594901 ± 40283	820038 ± 62414*	742273.1 ± 61729.3	658216 ± 34094	745578 ± 49753*
18:2n6c	115898 ± 7003	139317 ± 9706	195113 ± 16833*	177033.8 ± 11653.4*	151055 ± 8960	169037 ± 10824
20:0	1943 ± 144	1412 ± 146	1616 ± 210	891.0 ± 63.6*	1252 ± 93*	769 ± 69*^
18n3	12869 ± 1232	15285 ± 1565	21640 ± 1801*	19824.1 ± 2038.9	15031 ± 1183	18538 ± 1579
22:0	477 ± 55	322 ± 58	387 ± 53	220.9 ± 41.1*	272 ± 19	134 ± 14*^
20:4n6	2071 ± 181	3375 ± 453	3591 ± 388	5578.5 ± 567.1*	3054 ± 210	5434 ± 389*^
20:5n3	327 ± 31	237 ± 48	53 ± 29*	7.6 ± 0.6*	49 ± 49	9 ± 0.2*
24:1	331 ± 29	255 ± 31	450 ± 59	224.4 ± 41.8	242 ± 51	388 ± 145
22:6n3	2881 ± 661	2904 ± 748	3669 ± 642	3581.3 ± 727.2	2782 ± 434	4434 ± 735
24:0	535 ± 120	802 ± 212	1135 ± 183	1316.6 ± 215.8	648 ± 99	1609.8 ± 230
saturated	363875 ± 16614	432115 ± 19597	539232 ± 30676*	503261.6 ± 31373*	400515 ± 15730 #	458624 ± 30522
unsaturated	697006 ± 41533	816552 ± 58428	1184155 ± 90686*	1051699.3 ± 86351*	921133 ± 41786	1044483 ± 71244*
MUFA	563286 ± 33241	655672 ± 47261	960142 ± 73799*	845681.4 ± 73420	749210 ± 36309	847041 ± 59919*
PUFA - n3	15750 ± 1719	18188 ± 2123	25310 ± 2230	23405.5 ± 2651	17813 ± 1355	22972 ± 2254
PUFA – n6	117970 ± 7066	142692 ± 10014	198703 ± 17114*	182612.3 ± 12093*	154109 ± 8903	174470 ± 11042
Total	1061208 ± 52774	1248904 ± 68128	1723259 ± 117933**	1554968.6 ± 117006*	1321697 ± 44931	1503117 ± 97719

we noticed significantly higher UNSAT content in both adipose tissue of morbidly obese patients without metabolic syndrome (VAT: +70%, SAT: +29%, MetSx- versus lean, P < 0.05; *Table 2*). Similarly, the content of UNSAT of TAG fraction was enlarged in SAT of obese patients with metabolic syndrome (+28%, MetSx+ versus lean, P < 0.05; *Table 2*). The level of MUFA among UNSAT fatty acids of TAG fraction was increased in VAT of MetSx- (+70%, MetSx- versus lean, P < 0.05) and in SAT of MetSx+ subjects (+29%, MetSx+ versus lean, P < 0.05) as compared to lean. Despite visible tendency there were no differences in saturated/unsaturated fatty acids of TAG between study groups nor PUFA-n3/total fatty acids (*Fig. 4B*). Moreover, in VAT and SAT of morbidly obese patients without metabolic syndrome the content of PUFA-n6 of TAG was significantly greater than in lean patients (+68% and +30%, respectively, MetSx- versus lean, P < 0.05; *Table 2*).

Correlations

In patients with MeSx+ there was a high positive correlation between plasma HDL and plasma membrane ABCG1 expressions in both VAT (P < 0.05, R = 0.82; *Fig. 5A*) and SAT

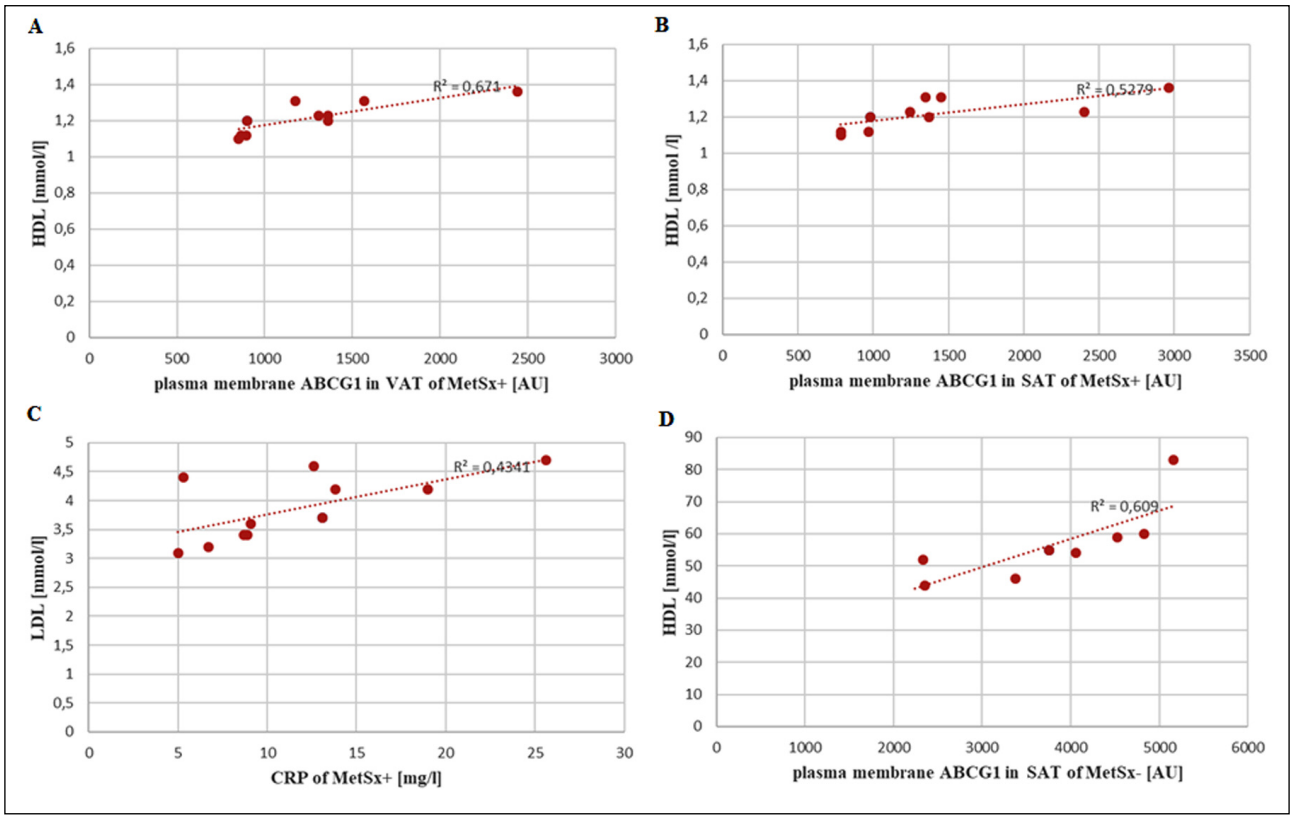

Fig. 5. Correlations of plasma high -density lipoprotein (HDL) and plasma membrane ABCG1 in visceral (VAT) (A) and subcutaneous (SAT) (B) adipose tissue of patients with obesity with metabolic syndrome (MetSx+); plasma low-density lipoprotein (LDL) with plasma C reactive protein (CRP) levels in MetSx+ (C); plasma HDL with plasma membrane expressions of ABCA1 in SAT of patients without obesity with metabolic syndrome (MetSx-) (D).

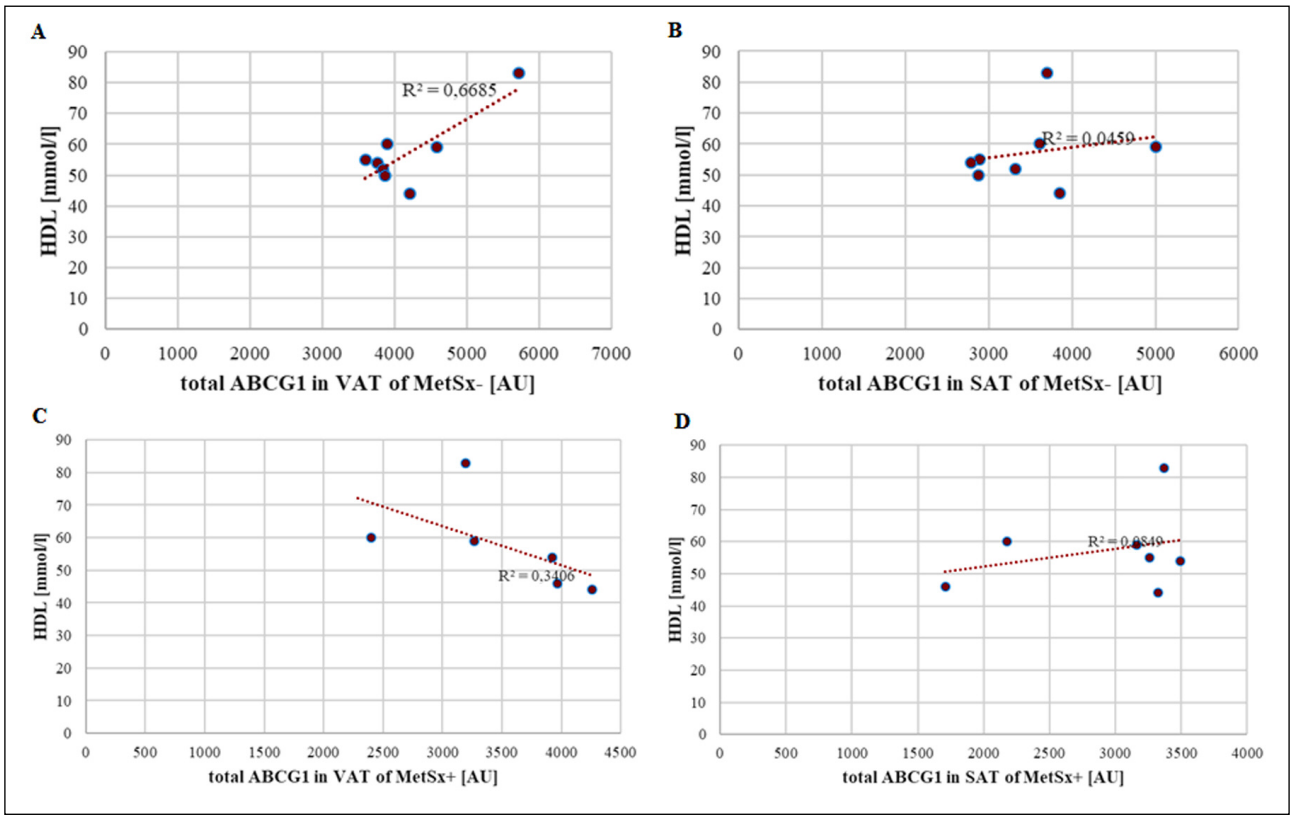

Fig. 6. Correlations of plasma high -density lipoprotein (HDL) and total ABCG1 in visceral (VAT) (A) and subcutaneous (SAT) (B) adipose tissue of patients with obesity without metabolic syndrome (MetSx-) and with metabolic syndrome in visceral (VAT) (C) and subcutaneous (SAT) adipose tissue (D).

($P < 0.05$, $R = 0.73$; *Fig. 5B*). Additionally, in patients MetSx- there was a high positive correlation between plasma HDL and total ABCG1 in VAT ($P < 0.05$, $R = 0.81$; *Fig. 6A*) whereas in MetSx+ in both VAT ($P = 0.224$, $R = 0.58$) and SAT ($P = 0.526$, $R = 0.29$) not were any significant correlation (*Fig. 6C*, *6D*) as well as in SAT of MetSx- ($P = 0.6105$, $R = 0.21$; *Fig. 6B*). Plasma CRP levels in MetSx+ patients were associated with plasma LDL concentrations ($P < 0.05$, $R = 0.66$; *Fig. 5C*). In MetSx- patients, HDL was highly positively correlated with plasma membrane expressions of ABCA1 in SAT ($P < 0.05$, $R = 0.78$; *Fig. 5D*).

DISCUSSION

Adipose tissue is not only storage depot for triacylglycerols, but it also contains one of the largest pools of cholesterol in the human body (31). It is suggested that intracellular cholesterol takes part in adipose tissue dysfunction. According to previous observations, the content of both TAG and cholesterol in adipocytes of obese individuals is increased (14).

According to our knowledge, this is the first study evaluating total and plasma membrane expression of major cholesterol transport proteins: ABCA1, ABCG1 and SR-BI in two types of adipose tissue (VAT and SAT) of patients with class 3 obesity. Additionally, the group of obese patients were divided into the patients with and without metabolic syndrome and compared to lean individuals. We also wanted to explore a possible link between cholesterol transporters expression and metabolic derangements associated with obesity in humans.

ATP-binding cassette transporter A1

So far, there have been no publications available on ATP-binding cassette transporter A1 (ABCA1) expression in human adipose tissue. Vast majority of the studies refer to this protein's expression in monocytes/macrophages or adipocytes of animal models.

Adipose dysfunction and obesity are associated with changes in adipocyte cholesterol homeostasis (14). De Haan *et al*. (32) studied ABCA1-deficient mice on a high fat high cholesterol diet and observed increased intracellular cholesterol content with a concurrent accumulation of TAG in adipose tissue, associated with increased body weight. Additionally, it has been reported that myeloid-specific ABCA1 deficient mice exhibit higher occurrence of adipose tissue inflammation and metabolic abnormalities in mild obesity (33). According to *in vitro* studies, ABCA1(–/–) macrophages showed an increased inflammation caused by saturated fatty acids (34). These studies prove that in the obesity related to ABCA1, inflamed tissue macrophages stimulate the production of increased number of monocytes, leading to the increase in the severity of inflammatory condition and related disorders (33, 34). Whereas, silencing ABCA-1 reversed the anti-inflammatory effect of apoA-I but not on the effect of intact HDL (35). Also our study confirms increased indices of inflammatory markers (CRP, WBC and fibrinogen) in obese patients even more pronounced in MetSx+ group.

Interestingly, our research showed decreased plasma membrane expression of ABCA1 in VAT only in obese MetSx- patients with plasma cholesterol and TAG concentration within the reference range. Moreover, TAG content in adipocytes was considerably higher compared to the individuals of a normal body mass (*Table 1)*. In obese subjects with metabolic syndrome, tissue TAG content remained unchanged (MetSx+ versus lean), while plasma concentration of TAG and cholesterol was higher compared to lean and MetSx- individuals.

Metabolic syndrome is related to changed HDL concentration. Lucero *et al*. (36) evaluated the relationship between the plasma ability of cholesterol efflux and changes in the HDL content in individuals with untreated metabolic syndrome. They observed increased cholesterol transport aided by ABCA1 which was positively correlated with the increase in pre-β1-HDL (it contains apolipoprotein AI, *apoAI,* and phospholipids, it is an acceptor of cellular cholesterol and it is critical for reverse cholesterol transport. The researchers suggest that android obesity may lead to the impairment of cholesterol efflux capacity (37). Aron-Wisnewsky *et al*. (38) evaluated ABCA1 mediated cholesterol efflux in the plasma of obese women before as well as 3 and 6 months after bariatric surgery Roux-en-Y bypass. It turn out that loss of body mass decreased capacity of whole plasma to remove cellular free cholesterol through the ABCA1 pathway, which mediate the transport of cellular cholesterol to lipid-poor and lipid-free apoAI (39). According to further research carried out on morbidly obese women, also a decrease in plasma concentration of pre-β-HDL after Roux-en-Y bypass was observed (40). Finally, reduced ABCA1 mediated cholesterol efflux with a concurrent decrease in plasma concentration of HDL fraction in obese individuals may also result from the low-fat and low-total energy diet (41).

ATP-binding cassette G1 transporter

ATP-binding cassette G1 transporter (ABCG1), apart from playing a mediating role in free cholesterol efflux to HDL, also participates in lipid accumulation (10). The important role of ABCG1 in TAG metabolism is evident from observations in humans and animals. For instance, silencing of ABCG1 resulted in a decreased activity of lipoprotein lipase (LPL) which controls TAG accumulation through the production of free fatty acids from circulating lipoproteins (11, 12). ABCG1 deficient mice fed with high-fat diet showed decreased TAG accumulation in adipose tissue and liver and did not develop fatty acid induced obesity compared to control animals. Furthermore, silencing of genes for ABCG1 in non-obese mice C57BL/6 and fat-rich diet did not result in the increase in the mass of fatty tissue (21). Also, it has been shown a reduction in body weight gain and adipose tissue mass gain high-fat diet in ABCG1(–/–) mice fed high-fat diet (42). Therefore, Frisdal *et al*. (11) suggest that ABCG1 in adipose tissue may be used for therapeutic aims in the treatment of obesity and its metabolic complications. The research conducted by Kennedy *et al*. (43) seem to be contrary to the generally accepted role of ABCG1 in cellular efflux of free cholesterol since decreased expression of this protein led to the intracellular lipid accumulation. However, it is believed that both intracellular TAG accumulation and an elevated expression of ABCG1 protects cells from excessive accumulation of free cholesterol causing cell toxicity (11). In own studies, we observed increased total ABCG1 expression and total TAG content in obese patients without metabolic syndrome. Additionally, we noticed decreased ABCG1 membrane expression and increased total cholesterol concentration in plasma of obese patients with metabolic syndrome in VAT. Increased expression of ABCG1 in adipose tissue was observed also by other authors in case of morbidly obese patients (44). Interesting outcomes were obtained by Johansson *et al*. (45) in human adipose tissue during weight loss on a low-calorie diet and weight maintenance after weight loss. Increased ABCG1 expression was observed in subcutaneous adipose tissue collected from abdomen in both period of weight loss and body mass maintenance in obese persons. The process of mass loss and maintenance of this effect was accompanied by increased HDL concentration and decreased TAG concentration. Increased ABCG1 expression was also observed in adipose tissue after

losing weight in obese women who underwent bariatric surgery (Roux-en-Y bypass) (38).

When comparing the importance of ABCA1 and ABCG1 changes in obese patients, based on our results it can be concluded that the latter is implicated in metabolic complications to a greater extent. MetSx+ patients had significantly decreased plasma membrane ABCG1 expressions, lower HDL, with the link between the two confirmed by a high positive correlation. Furthermore, MetSx+ patients had more metabolic complications of obesity: high blood pressure, higher glucose and insulin levels, and higher HOMA-IR – insulin-resistance index as compared to MetSx- patients. MetSx+ patients also had higher inflammation indices (CRP, WBC and fibrinogen), that have previously been reported to be associated with metabolic complications.

Scavenger receptor class B type 1

We found no significant differences in the total and plasma membrane expression of scavenger receptor class B type 1 (SR-BI) in VAT and SAT in both obese study groups as compared to lean patients (*Fig. 3A*, *3B*).

Unfortunately, studies on SR-BI expression in both obese animals and people are sparse. For instance, SR-BI deficient mice are immune to obesity caused by diet. After 24 weeks on western-type diet these mice showed no capability of accumulating lipids in the liver or intolerance to glucose. Moreover, based on the analysis of gene expression the authors of the work (46) suggest that SR-BI deficient mice may have reduced synthesis *de novo* of fatty acids.

According to the studies of the plasma of women with android overweight/obesity, slightly decreased cholesterol efflux dependent on SR-BI with adequately lower HDL was observed. Based on the results the authors (37) indicate that android overweight/obesity may lead to the impairment of the cholesterol's efflux ability and next to the development of metabolic complications of obesity. A positive effect on cholesterol balance in obese subjects was observed following bariatric surgery Roux-en-Y bypass. Loss of body mass 6 months after the surgery resulted in overall capacity of total plasma to mediate increased cellular cholesterol efflux with SR-BI compared to the data collected prior to the procedure.

Conclusion

This study identifies for the first time the implication of adipocyte ATP-binding cassette G1 (ABCG1) and A1 (ABCA1) cholesterol transporters in metabolic complications of obesity. We showed a link between lower plasma membrane expressions of ABCG1 in VAT and decreased HDL plasma concentrations in MetSx+ as compared to MetSx- patients. The above-mentioned was also confirmed by a high positive Spearman correlation (R = 0.82). Furthermore, those obese subjects exhibited higher concentration of LDL, which was positively correlated with elevated CRP level. We can speculate that these changes were associated with the metabolic derangements like development of inflammation in obese subjects. Neither obesity nor metabolic syndrome did not affect the SR-BI expression in the adipose tissue. However, the reductions in plasma membrane expression of ABCA 1 in MetSx- occurred in VAT. To sum up, our results show that obesity and metabolic syndrome are associated with lower expression of ABCG1 in visceral adipose tissue in humans.

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Author's address: Dr. Barbara Choromanska, 1st Department of General and Endocrine Surgery, Medical University of Bialystok, 24A M. Sklodowskiej-Curie Street, 15-276 Bialystok, Poland.
E-mail: barbara.choromanska@umb.edu.pl