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Clinical Research Article

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Expression of the Adipocyte Progenitor Markers *MSCA1* and *CD36* is Associated With Adipose Tissue Function in Children

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Abstract

Context: *MSCA1* (mesenchymal stem cell antigen 1) and *CD36* (cluster of differentiation 36) have been described as novel adipocyte progenitor markers in adults with a potential relevance for obesity and adipocyte progenitor function.

Objective: With the early manifestation of obesity in children and formation of adipose tissue (AT) dysfunction, children provide the opportunity to characterize the function of *MSCA1* and *CD36* during physiological AT accumulation and with obesity and related disease.

Methods: We investigated *MSCA1* and *CD36* expression in adipocytes and stroma vascular fraction (SVF) cells from 133 children of the Leipzig AT Childhood cohort with regard to AT accumulation and biology. In a subsample we analyzed how *MSCA1* and *CD36* expression is related to adipose progenitor capacities in vitro (ie, proliferation, differentiation and mitochondrial function).

Results: Both *MSCA1* and *CD36* are differentially expressed in adipocytes and SVF cells of children. *MSCA1* expression is positively correlated to obesity-associated AT dysfunction (ie, adipocyte hypertrophy and serum high-sensitivity C-reactive protein), and high SVF *MSCA1* expression is associated with increased mitochondrial respiration in vitro. *CD36* expression is not associated with AT dysfunction but SVF *CD36* expression is downregulated in children with overweight and obesity and shows a positive association with the differentiation capacity of SVF cells ex vivo and in vitro.

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Conclusion: Both *MSCA1* and *CD36* are associated with obesity-related alterations in AT of children. In particular, *CD36* expression predicts adipogenic potential of SVF cells, indicating a potential role in the regulation of adipocyte hyperplasia and hypertrophy with obesity development in children.

Key Words: adipocyte progenitor cells, adipose tissue, adipose tissue dysfunction, adipocyte differentiation, children, obesity

Obesity can manifest during early childhood (1,2). The characteristic feature of obesity is an increase in adipose tissue (AT) mass caused by an increase in the number of adipocytes (hyperplasia) and/or an increase in adipocyte size (hypertrophy). This AT accumulation is not pathological per se. However, if adipocyte hypertrophy with inflammation and adipokine disbalance is prevalent, this so-called AT dysfunction drives development of obesity-related comorbidities (3). Hence, assessment of AT composition and function at an early obesity stage is of relevance. It is assumed that during obesity-associated AT expansion, adipocyte progenitor cells play an important role, particularly through differentiation into metabolically active adipocytes (4). Adipocyte progenitor cells are contained within the stroma vascular fraction (SVF) of AT (5,6). Several studies show that the SVF contains multiple subpopulations of adipocyte progenitor cells with different abilities to proliferate and differentiate (7,8). These subpopulations are characterized by the expression of specific cell surface markers, in particular mesenchymal stem cell antigen 1 (MSCA1), also known as tissue nonspecific alkaline phosphatase or alkaline phosphatase, liver/bone/kidney (ALPL), and scavenger receptor cluster of differentiation 36 (CD36) (9-11).

MSCA1 is encoded by the *ALPL* gene and is expressed in various tissues, including AT, bone marrow, and heart (12). It has been shown that MSCA1 is involved in cellular differentiation processes (13,14), immunomodulatory functions (15,16), and metabolism (17). Recent results from studies in human adults provided evidence that in subcutaneous AT MSCA1 is a surface marker for cells with higher white and brite adipogenic potential compared to other adipocyte progenitor cell subtypes, and both number of MSCA1-positive progenitor cells and magnitude of *MSCA1* expression are positively correlated to body mass index (BMI) in adults (9). In addition, a recent study by Sun et al has provided evidence that mitochondrial tissue nonspecific alkaline phosphatase controls thermogenesis via regulation of the futile creatine cycle in AT of mice (18).

Gao et al demonstrated that the MSCA1-positive adipocyte progenitor population can be further subdivided by the presence of CD36 (10). *CD36* is expressed in many cell types, including adipocytes, endothelial cells, and hematopoietic cells, such as macrophages and platelets (19). In fact, CD36 is an established marker of adipocyte differentiation and its loss of function in mice is associated with decreased adipocyte differentiation, ectopic lipid accumulation in the liver, and insulin resistance (20). In line with this, it has been demonstrated that CD36-positive cells isolated from human adult subcutaneous AT differentiate more efficiently than CD36-negative cells, suggesting that CD36 is a marker of human adipocyte progenitor cells with high adipogenic potential (10).

These preliminary data suggest that *MSCA1* and *CD36* might play a role during obesity-related AT accumulation by affecting processes related to AT function. However, most of the studies have been performed in patients with morbid obesity, and the relevance for processes involved in early obesity progression remains poorly understood. In this regard, studies in children are of special value since the functional relevance of adipocyte progenitor markers can be addressed during AT accumulation with normal development and with early development of obesity and related AT dysfunction (21).

Within this study, we assessed an association of *MSCA1* and *CD36* gene expression with physiological or obesity-related AT accumulation in children. Furthermore, we investigated whether an elevated *MSCA1* and *CD36* expression is related to functional properties of adipocyte progenitor cells, such as proliferation, differentiation, and mitochondrial function.

Materials and Methods

Samples (Leipzig AT Childhood Cohort)

Subcutaneous AT samples were retrieved from the Leipzig AT Childhood Cohort from 133 Caucasian children (0-18 years) undergoing elective surgery (21). All children were free of diseases or medications that potentially affect AT biology. Children with diabetes, generalized inflammation, malignant diseases, genetic syndromes, or permanently immobilized children were excluded. Written informed consent was obtained from all parents. The study was approved by the local ethics committee (Reg. No. 265-08, 265-08-ff, University of Leipzig; NCT02208141).

BMI data were standardized to age- and sex-specific German reference data and are given as BMI SD score (SDS)

(22). A cutoff of 1.28 and 1.88 SDS defined overweight and obesity in children, respectively. Fasting blood samples were obtained prior to surgery. Levels of adiponectin, leptin, high-sensitivity C-reactive protein (hs-CRP), glucose, and insulin were measured by a certified laboratory (Institute of Laboratory Medicine, Clinical Chemistry and Molecular Diagnostics, University of Leipzig, Leipzig, Germany). Homeostasis model assessment-insulin resistance (HOMA IR) was calculated to evaluate insulin resistance (23).

Isolation and Characterization of Adipocytes and Cells of the Stromal Vascular Fraction From Human AT Samples

Immediate isolation of adipocytes and stromal vascular fraction cells from AT after extraction during surgery and subsequent functional analyses was performed as previously described by Landgraf et al (21). The freshly isolated adipocytes were used for in vitro determination of lipolytic capacity or fixed in osmium tetroxide for analysis of adipocyte cell size and number using a Coulter counter (Multisizer III; Beckmann Coulter, Krefeld, Germany) with a 560 µm aperture. Lipolytic activity was normalized to adipocyte number determined by the Coulter counter method and is given as the release of glycerol in ng/mL per 1000 adipocytes.

The in vitro proliferation and differentiation assays of SVF cells were also carried out as previously described (21). To estimate the potential for proliferation of the seeded cells, the cell doubling time was calculated as the fold change from the mean cell numbers on days 8 and 10 relative to day 2. For samples with observed cell proliferation, the growth rate [$gr = (\ln[N(t)/N(t_0)])/t)$] and generation time [=ln (2)/gr] were calculated as the mean from the cell numbers on days 6, 8, and 10 in comparison to day 4. The differentiation efficiency was determined with a fluorescence microscope as the percentage of differentiated cells (Nile Red/Hoechst 33342 double-stained cells) from the total number of cells (Hoechst 33342 stained) and as Oil Red O (Sigma, Darmstadt, Germany) absorbance at 540 nm (CLARIOstar, BMG LABTECH, Offenburg, Germany) per well at day 8.

For immunohistochemical analysis, AT samples were fixed in 4% paraformaldehyde, paraffin-embedded, and sectioned, and macrophages were stained using a monoclonal CD68 antibody (1:100; clone PGM-1, M0876 DAKO).

RNA Isolation and Gene Expression Analyses

Total RNA was extracted using the RNeasy Mini Kit (Qiagen, Hilden, GER), and a total of 250 ng of RNA was reverse transcribed using Moloney Murine Leukemia Virus Reverse Transcriptase (Thermo Fisher, Waltham, MA, USA) and random hexamer primer (Roche, Basel, Switzerland). The amplification of the complimentary DNA sample by quantitative real-time reverse transcription polymerase chain reaction was performed in a 20 µL reaction mix with 10 µL 2 × quantitative polymerase chain reaction MasterMix (Eurogentec, Seraing, Belgium), 900 nM of each primer, and 200 nM probe. Copy numbers of *MSCA1* and *CD36* were determined from a standard curve and normalized to the reference genes *ACTB* (beta actin), *HPRT* (hypoxanthine guanine phosphoribosyl transferase), and *TBP* (TATA box binding protein) (24). The following primers and probes were used: *CD36* (Hs00169627_m1, Thermo Fisher), *MSCA1* (*ALPL*, Hs01029144_m1, Thermo Fisher), *CKB* (Hs00176484_m1, Thermo Fisher), *ADIPOQ* (forward 5′-GGCCGTGATGGCAGAGAT-3′, reverse 5′-CCTTCAGCCCCGGGTACT-3′, probe 5′-FAM-CGATGTCTCCCTTAGGACCAATAAGACCTGG-TAMRA-3′), *PPARG* (forward 5′-GATCCAGTGG TTGCAGATTACAA-3′, reverse 5′-GAGGGAGTTGG AAGGCTCTTC-3′, probe 5′-FAM-TGACCTGAAACTT CAAGAGTACCAAAGTGCAA-TAMRA-3′), *PGC1A* (forward 5′-CACCAAACCCACAGAGAACA-3′, reverse 5′-GGGTCATTTGGTGACTCTGG-3′, probe 5′-FAM-CGCAGTCACAACACTTACAAGCCAAAAC-TAMRA-3′), *PRDM16* (forward 5′-CCAATAGTGAG ATGAACCAAGCAT-3′, reverse 5′-CCGTCCACGAT CTGCATGT-3′, probe 5′-FAM-AACGCGAACAGAG AAACGGGCG-TAMRA-3′), *UCP1* (forward 5′-ACGA CACGGTCCAGGAGTTC-3′, reverse 5′-ACCAGCTAAAA TCTTGCTTCCTAAAC-3′, probe 5′-FAM-TCACCGCA GGGAAAGAAACAGCACC-TAMRA-3′), *ACTB* (forward 5′-CGAGCGCGGCTACAGCTT-3′, reverse 5′-CCTT AATGTCACGCACGATTT-3′, probe 5′-FAM-ACCACC ACGGCCGAGCGG-TAMRA-3′), *TBP* (forward 5′-TTGTA AACTTGACCTAAAGACCATTGC-3′, reverse 5′-TTCGT GGCTCTCTTATCCTCATG-3′, probe 5′-HEX-AACGCCG AATATAATCCCAAGCGGTTTG-TAMRA-3′), and *HPRT* (forward 5′-GGCAGTATAATCCAAAGATGGTCAA-3′, reverse 5′-GTCTGGCTTATATCCAACACTTCGT-3′, probe 5′-FAM-CAAGCTTGCTGGTGAAAAGGACCCC-TAMRA-3′).

Small Interfering RNA-mediated Knockdown in Simpson Golabi Behmel Syndrome Cells

Small interfering RNA transfections were performed in Simpson Golabi Behmel Syndrome (SGBS) cells using the Neon Transfection System 100 µL Kit (Thermo Fisher). Electroporation was performed at a cell density of 6×10^6 cells/mL at pulse voltage 1300 V, pulse width 20 ms, pulse

number 2. Gene-specific ON-TARGETplus SMARTpool small interfering RNAs and ON-TARGET plus control reagents (Dharmacon, Lafayette, CO, USA) were used at a final concentration of 500 nM. A total of 100 000 transfected SGBS cells per well were seeded in 12-well format and differentiated into mature adipocytes as previously described by Landgraf et al (24).

Mitochondrial Function of Cultivated SVF Cells

For the analysis of mitochondrial function, selected cryopreserved SVF cell samples were cultivated for 4 to 11 passages in culture medium (Gibco Dulbecco's Modified Eagle Medium/ Nutrient Mixture F-12, 10% fetal calf serum, 100 units/mL penicillin-streptomycin) at 37°C and 5% CO_2 before seeding in Seahorse XF24 Cell Culture Microplates (Agilent, Santa Clara, CA, USA) at a density of 60 000 cell/cm². The measurement was performed 48 h later in Assay Medium [XF Base Medium (Agilent), 10 mM glucose (Roth, Karlsruhe, Germany), 2 mM pyruvate (Sigma), 2 mM glutamine (Sigma)] using the Seahorse XF Cell Mito Stress Test Kit (Agilent). The kit components were used in the following concentrations: oligomycin 2 µM, carbonyl cyanide-4 (trifluoromethoxy) phenylhydrazone 3 µM and Rotenone/antimycin A 0.5 µM. Determination of the oxygen consumption rate (OCR) was performed on the Seahorse XFe24 Analyzer (Agilent) together with the Seahorse Wave software and normalized to the cell number as assessed by bicinchoninic acid assay measurement of the amount of protein per well (Thermo Fisher).

Statistical analyses

Data that did not represent a Gaussian distribution were transformed logarithmically before being analyzed. Quantitative variables were analyzed by parametric tests (Student's *t* test, analysis of variance, Pearson correlation analysis), whereas for categorical variables a χ^2 test was performed. For multiple regression analyses, the stepwise forward model was used. Statistical analysis was performed using Statistica 13.3 (StatSoft, Tulsa, OK, USA) and GraphPad Prism 6 (GraphPad Software, Inc, San Diego, CA, USA).

Results

To answer the question whether gene expression of the adipocyte progenitor markers *MSCA1* and *CD36* is associated with obesity in children or related to functional parameters of adipocyte progenitor cells, we investigated AT samples from 59 lean children and 74 children with overweight and obesity of the Leipzig AT Childhood Cohort (21). The general characteristics of the study participants and samples are presented in Table 1. There were no significant differences in sex distribution and height SDS between lean children and children with overweight or obesity, although lean children were about 3 years younger and, hence, presented at an earlier pubertal stage. As described in previous studies, biological indicators of AT dysfunction, such as adipocyte hypertrophy and hyperplasia, increased AT inflammation, and related alterations in metabolic and inflammatory serum parameters, such as increased HOMA IR and hs-CRP levels, were present in the group of children with overweight or obesity (21). Functional AT parameters, such as proliferation and differentiation of SVF cells, were not different between groups (Table 1).

MSCA1 and CD36 Messenger RNA Expression Is Age Dependent and Differs Between SVF cells and Adipocytes

To address potential associations of *MSCA1* and *CD36* expression with obesity and related parameters in children, we first compared freshly isolated SVF cells and adipocytes. We detected significantly higher *MSCA1* expression in SVF cells while *CD36* expression was higher in adipocytes (Fig. 1A).

To assess whether the *MSCA1* and *CD36* expression in SVF cells might be derived from cell populations with potential for adipocyte differentiation, we correlated gene expression levels in SVF cells with those in mature adipocytes. We observed that higher *MSCA1* expression in SVF cells was associated with a higher expression in adipocytes (Fig. 1B). In contrast, we did not find such a relationship for *CD36* (Fig. 1C). Some of the adipocyte progenitor populations within the SVF exhibit both cell surface markers, MSCA1 and CD36 (10). Based on this, we further investigated whether in SVF samples the expression of *MSCA1* is related to *CD36*, indicating the presence of such progenitor cell populations in AT of children. However, we did not detect a correlation between *MSCA1* and *CD36* expression in SVF cells (Fig. 1D), whereas in mature adipocytes, the expression levels of *MSCA1* and *CD36* correlated positively (Fig. 1E).

We next analyzed whether *MSCA1* and *CD36* expression in adipocyte progenitor cells is associated with obesity-related parameters already in children. To exclude a potential bias due to the influence of sex and developmental parameters, we first performed multiple regression analyses in the subcohort of lean children. *MSCA1* expression significantly depended on the age of the children. Similarly, *CD36* expression in SVF cells was related to age, whereas *CD36* expression in adipocytes was not affected by any of the investigated variables (Table 2). When we compared expression levels between lean children and children with overweight

Table 1. Characteristics of the Leipzig AT Childhood Cohort (N = 133)

	Lean			Overweight and obese			*P*
	n	Mean ± SEM	Range	n	Mean ± SEM	Range	
Anthropometric parameters							
Male/female (% male)	59	27/32 (45.8)		74	35/39 (47.3)		0.860
Age, years	59	10.4 ± 0.6	1.1 to 18.3	74	13.3 ± 0.3	4.8 to 18.4	**<0.001**
Pubertal stage, PH	50	2 ± 0	1 to 6	64	3 ± 0	1 to 6	**0.003**
BMI SDS	59	0.07 ± 0.11	−1.80 to 1.24	74	2.31 ± 0.07	1.32 to 4.34	**<0.001**
Height SDS	51	−0.04 ± 0.17	−3.18 to 2.31	61	0.35 ± 0.14	−3.16 to 2.37	0.076
AT composition							
Adipocyte diameter, µm	32	114 ± 2	81 to 139	44	127 ± 2	98 to 175	**<0.001**
Total adipocyte number × 10^{9a}	21	24.7 ± 3.2	9.1 to 69.8	31	52.0 ± 4.8	22.7 to 114.4	**<0.001**
Isolated adipocytes per g AT × 10^{6a}	32	2.3 ± 0.1	1.2 to 4.4	45	1.9 ± 0.8	1.0 to 3.1	**0.003**
Isolated SVF cells per g AT × 10^{5a}	27	1.0 ± 0.1	0.0 to 2.4	31	1.2 ± 0.1	0.1 to 4.1	0.841
AT inflammation							
Macrophages per 100 adipocytes[a]	49	9 ± 1	0 to 29	59	16 ± 2	0 to 115	**0.013**
CLS per 100 adipocytes[a]	49	0 ± 0	0 to 2	59	1 ± 0	0 to 8	**<0.001**
hs-CRP mg/L[a]	47	0.7 ± 0.1	0.3 to 3.2	65	2.1 ± 0.3	0.3 to 18.7	**<0.001**
TNF-α pg/mL	44	1.96 ± 0.19	0.68 to 1.78	61	2.03 ± 0.14	0.66 to 5.77	0.698
AT functional parameters							
Doubling time of native cells from the SVF, h[a]	22	142.8 ± 26.6	14.5 to 524.3	29	178.7 ± 34.1	17.8 to 735.9	0.617
Growth rate of proliferating native SVF cells[a]	21	0.016 ± 0.002	0.003 to 0.037	24	0.013 ± 0.001	0.004 to 0.030	0.363
Generation time of proliferating native SVF cells, h[a]	21	72.3 ± 12.7	18.9 to 205.0	24	65.7 ± 8.1	23.3 to 180.7	0.894
Differentiation of cells from the SVF, %	21	28.6 ± 3.6	4.0 to 58.2	27	26.1 ± 3.3	0.2 to 73.2	0.608
Basal lipolysis in adipocytes	15	0.49 ± 0.05	0.20 to 0.83	14	0.36 ± 0.05	0.18 to 0.74	0.071
Isoproterenol-stimulated lipolysis in adipocytes[a]	15	2.07 ± 0.22	0.48 to 3.77	15	1.98 ± 0.34	0.37 to 5.08	0.569
Adipokines and HOMA IR							
Adiponectin, mg/L[a]	45	9.0 ± 1.0	1.6 to 43.8	64	5.7 ± 0.3	1.7 to 15.1	**<0.001**
Leptin, ng/mL[a]	42	7.5 ± 1.2	0.4 to 28.2	65	29.9 ± 2.7	1.3 to 89.1	**<0.001**
HOMA IR[a]	48	1.5 ± 0.2	0.0 to 5.6	63	3.6 ± 0.3	0.3 to 12.7	**<0.001**
MSCA1 and *CD36* expression in AT							
MSCA1 mRNA in SVF cells[a]	59	0.162 ± 0.015	0.031 to 0.648	74	0.176 ± 0.015	0.041 to 0.593	0.509
MSCA1 mRNA in adipocytes[a]	59	0.114 ± 0.013	0.002 to 0.375	74	0.139 ± 0.009	0.022 to 0.363	0.104
CD36 mRNA in SVF cells[a]	55	0.165 ± 0.010	0.053 to 0.292	74	0.124 ± 0.006	0.053 to 0.299	**<0.001**
CD36 mRNA in adipocytes[a]	59	0.233 ± 0.014	0.063 to 0.691	74	0.233 ± 0.012	0.058 to 0.600	0.975

Data are presented as mean with standard error of the mean and range. n indicates the number of subjects. Significant *P*-values ($P < 0.05$) are indicated in bold. Statistical significance for sex distribution was examined by χ^2 test. Statistical significance for differences between lean children and children with overweight and obesity was determined by the Student's *t* test. Basal lipolysis is given as glycerol release in (ng/mL)/1000 adipocytes.
Abbreviations: AT, adipose tissue; BMI, body mass index; *CD36*, cluster of differentiation 36; HOMA IR, homeostatic model assessment-insulin resistance; hs-CRP, high-sensitivity C-reactive protein; mRNA, messenger RNA; *MSCA1*, mesenchymal stem cell antigen 1; SDS standard deviation score; SEM, standard error of the mean; SVF, stromal vascular fraction; TNF-α, tumor necrosis factor α;
[a]Statistical analyses were performed with log-transformed parameters.

and obesity, we did not observe a significant difference for *MSCA1* neither in SVF cells nor in adipocytes. In contrast, we found significantly increased *CD36* expression in SVF cells of lean children, whereas expression in adipocytes did not differ (Table 1). In quantitative analyses, *MSCA1* showed a weak positive association with BMI SDS for expression in SVF cells (R = 0.201; $P = 0.021$) and adipocytes (R = 0.224; $P = 0.009$), which did, however, not withstand adjustment for age (SVF cells: $R_{adjusted} = 0.144$, $P_{adjusted} = 0.138$; adipocytes: $R_{adjusted} = 0.129$, $P_{adjusted} = 0.184$). In contrast, gene expression of *CD36* in SVF cells negatively correlated with BMI SDS (R = –0.360; $P < 0.001$) independently from age of the children ($R_{adjusted} = -0.259$; $P_{adjusted} = 0.008$). There was no significant correlation for adipocyte *CD36* expression and BMI SDS (R = 0.079; $P = 0.366$).

In summary, *MSCA1* and *CD36* are differentially expressed in SVF cells and adipocytes, and in particular, *CD36* expression in SVF cells decreases with obesity in children.

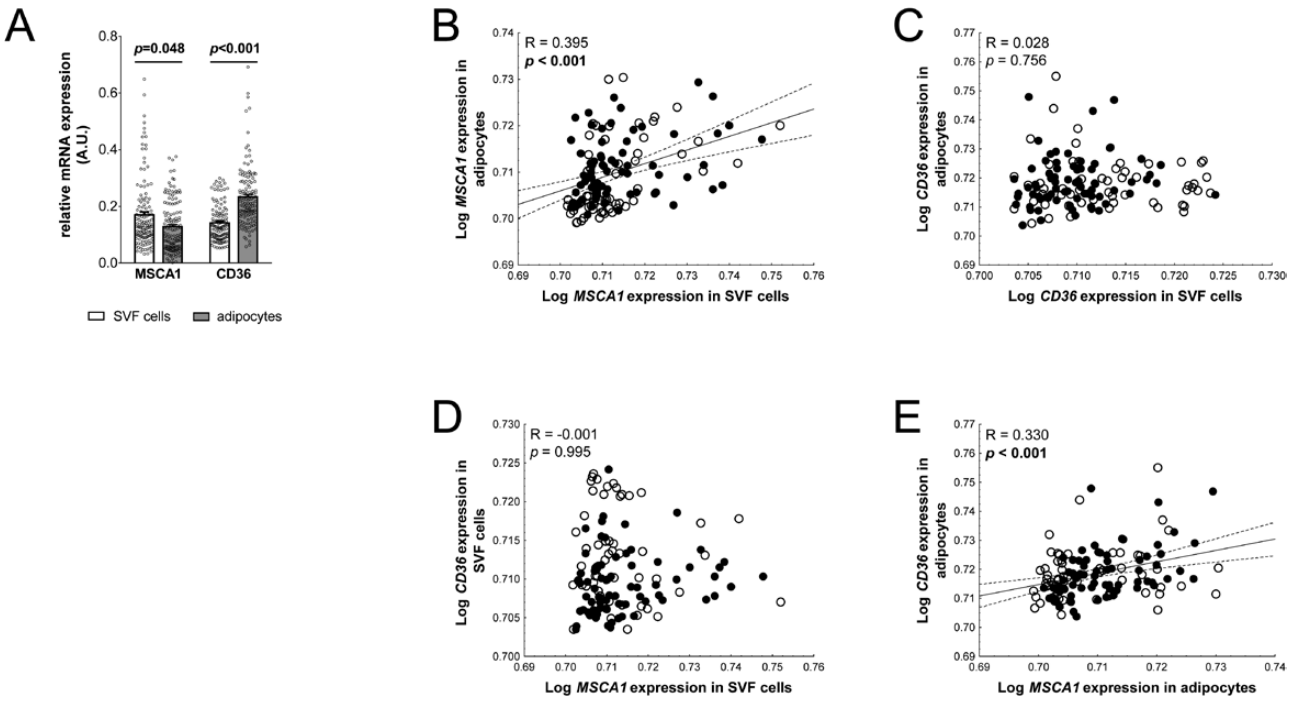

Figure 1. *CD36* (cluster of differentiation 36) and *MSCA1* (mesenchymal stem cell antigen 1) are differentially expressed in SVF cells and adipocytes. (A) *MSCA1* expression is significantly lower and *CD36* expression is significantly higher in adipocytes compared to cells of the stroma vascular fraction (SVF; mean ± standard error of the mean; individual values represented as circles; n = 133). (B) *MSCA1* expression in SVF cells is positively associated with adipocyte *MSCA1* expression. (C) *CD36* expression in SVF cells does not show a correlation with the amount of *CD36* messenger RNA in adipocytes. (D) There is no association between *MSCA1* and *CD36* expression in SVF cells. (E) *MSCA1* correlates with *CD36* gene expression in adipocytes. Pearson correlation coefficient R and *P*-value are given in each scatter plot. Significant *P*-values ($P < 0.05$) are indicated in bold. Lean children are represented as open circles and children with overweight/obesity, as closed circles.

Table 2. Multiple regression analyses for anthropometric parameters in lean children of the Leipzig AT Childhood Cohort

Dependent variable	Independent variable	Step	Parameter	ΔR^2	β ± SEM	*P*	n
MSCA1 mRNA in native SVF cells (R^2=0.086; $P = 0.114$)	Age, sex, height SDS	1	Age	0.064	0.263 ± 0.138	0.063	51
		2	height SDS	0.023	–0.151 ± 0.138	0.281	
MSCA1 mRNA in adipocytes (R^2=0.282; $P < 0.001$)	Age, sex, height SDS	1	Age	0.242	0.507 ± 0.123	**<0.001**	51
		2	height SDS	0.040	–0.201 ± 0.123	0.108	
CD36 mRNA in native SVF cells (R^2=0.174; $P = 0.004$)	Age, sex, height SDS	1	Age	0.174	–0.417 ± 0.135	**0.004**	47
CD36 mRNA in adipocytes	Age, sex, height SDS		ns				51

Significant *P*-values ($P < 0.05$) are indicated in bold.

Abbreviations: *CD36*, cluster of differentiation 36; mRNA, messenger RNA; *MSCA1*, mesenchymal stem cell antigen 1; ns, not significant; SDS, SD score; SVF, stroma vascular fraction.

MSCA1 but Not CD36 Expression Is Associated With Parameters of AT (Dys)function in Children

We next investigated the interrelation of the messenger RNA (mRNA) expression of both genes and obesity-related parameters including AT composition, AT inflammation, and AT function, serum adipokine levels, and HOMA IR as a marker for insulin resistance. For parameters related to AT composition, we observed a positive relationship of *MSCA1* but not *CD36* expression and adipocyte size (Fig. 2A and 2B) and a negative correlation with the number of adipocytes per gram AT (SVF *MSCA1*: R = –0.228, $P = 0.046$; adipocyte *MSCA1*: R = –0.226, $P = 0.048$). However, only the correlation between adipocyte size and *MSCA1* expression in SVF cells remained significant after adjustment for the covariates age and BMI SDS (Table 3). Interestingly, only *CD36* expression in SVF cells showed a significant and BMI-dependent association with total adipocyte number as a measure of hyperplasia with lower *CD36* levels being associated with a higher total adipocyte number (R = –0.408, $P = 0.003$), which was dependent on BMI SDS and age of the children (Table 3). Although *MSCA1* expression in SVF cells is related to adipocyte hypertrophy and this is associated with AT inflammation (21), we did not observe correlations of SVF *MSCA1* expression with macrophage infiltration, crown-like structure (CLS) formation, and tumor necrosis factor α serum levels (Table 3) or *TNFA* expression in AT (data not shown), while there was a significant correlation with hs-CRP serum levels, which we detected similarly for adipocyte *MSCA1* expression (Table 3, Fig. 2C). For *CD36* SVF expression and hs-CRP serum levels, we observed a negative relationship (Fig. 2D), which was secondary to BMI-SDS and age of children (Table 3). Accordingly, *MSCA1* expression is increased with adipocyte hypertrophy and AT inflammation in children, which could not be observed for *CD36*.

Next, we assessed serum parameters indicative for AT dysfunction and early signs of metabolic disease. Mirroring the associations with BMI SDS, we found a positive correlation of SVF as well as adipocyte *MSCA1* expression and leptin serum levels (Fig. 2E, Table 3). For SVF *CD36* expression, we found a negative association with leptin (Fig. 2F). Furthermore, we observed a significant and BMI-independent positive correlation of *CD36* expression in adipocytes and serum leptin levels (Table 3). Expectedly, adiponectin serum levels were significantly associated with *MSCA1* (Fig. 2G) and *CD36* (Fig. 2H) expression levels with correlations showing opposite directions compared to leptin. Similar results were obtained for the association between adiponectin (*ADIPOQ*) expression in AT with *MSCA1* (SVF: R = –0.235, $P = 0.035$; adipocytes: R = –0.397, $P < 0.001$) and *CD36* (SVF: R = 0.496, $P < 0.001$; adipocytes: R = 0.092, $P = 0.414$). In line with this, HOMA IR showed a positive correlation with *MSCA1* mRNA levels in SVF cells and adipocytes as well as a negative correlation with *CD36* mRNA in SVF cells but not in adipocytes (Fig. 2I and J). However, none of the associations with adiponectin and only the association of SVF *CD36* expression and HOMA IR withstood adjusting to BMI SDS and age of children (Table 3). In summary, *CD36* expression in SVF cells is not only associated with obesity in children but also with early signs of metabolic disease.

Finally, we investigated a potential relationship of *MSCA1* and *CD36* expression in AT and functional AT parameters (ie, proliferation and differentiation of native SVF cells in vitro as well basal and isoproterenol stimulated lipolytic activity of adipocytes). In this regard, only adipocyte *MSCA1* expression was related to functional characteristics of native SVF cells in quantitative analyses. In particular, we detected a significant and age and BMI SDS independent association with growth rate and generation time in vitro (Table 3). Furthermore, we detected an inverse and BMI-dependent correlation of *MSCA1* expression with basal lipolysis as well as a BMI-independent correlation of SVF *MSCA1* expression with isoproterenol stimulated lipolysis (Fig. 2K, Table 3). There was no association between *CD36* expression in AT and lipolytic function of isolated adipocytes (Fig. 2L, Table 3). Taken together, *MSCA1* but not *CD36* expression is related to parameters of AT function.

CD36 mRNA Expression Correlates With Adipocyte Progenitor Cell Differentiation In Vitro

To analyze a possible relationship between *MSCA1* and *CD36* gene expression and adipogenesis in vitro in more detail, we made use of 23 cryopreserved SVF samples derived from 7 lean and 16 children with overweight or obesity.

First, we investigated the in vitro proliferation and differentiation potential of the prepassaged SVF cells. Our data showed no difference in the proliferation rate or the ability of the cells to differentiate into mature adipocytes between lean children and children with overweight or obesity (Fig. 3A).

When we compared mRNA expression during the differentiation process, we observed an increase in *MSCA1* and *CD36* expression (Fig. 3B), and for both, mRNA expression before differentiation correlated with expression after differentiation (Fig. 3C). Next, we studied whether *MSCA1* and *CD36* expression in native SVF cell samples might be predictive for their in vitro proliferation and/or differentiation capacity after several passages of cell culture. There was no correlation between the native SVF expression of *MSCA1* (R = 0.309, $P = 0.152$) or *CD36* (R = 0.324, $P = 0.141$) and generation time as a parameter

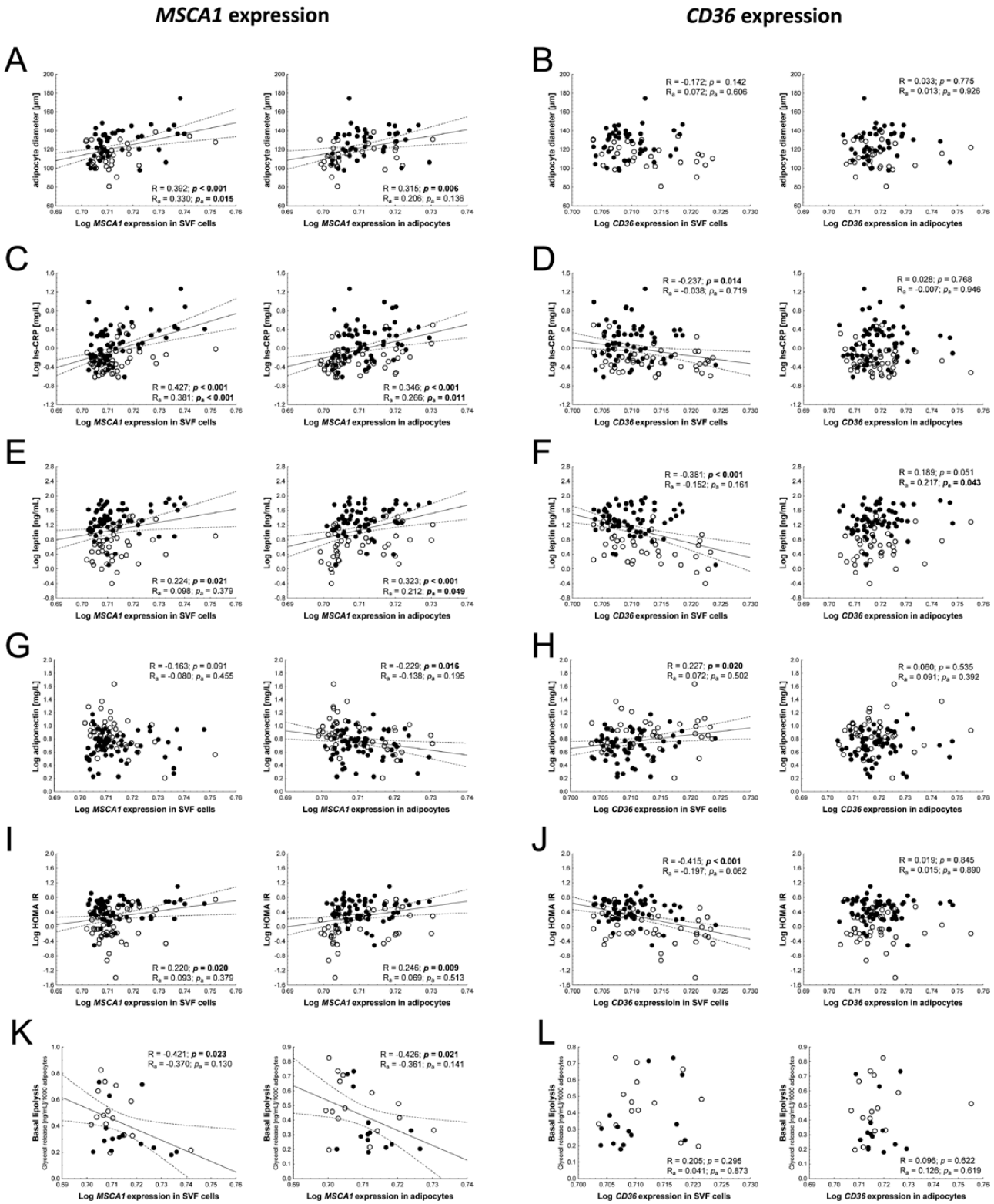

Figure 2. *MSCA1* (mesenchymal stem cell antigen 1) expression correlates with adipocyte size and parameters of adipose tissue (dys)function. (A) High *MSCA1* expression in stroma vascular fraction (SVF) cells and adipocytes is associated with larger adipocytes in adipose tissue, while (B) *CD36* (cluster of differentiation 36) expression is not correlated to adipocyte size. (C)The low-grade system inflammation marker high-sensitivity C-reactive protein (hs-CRP) is increased with increasing *MSCA1* expression levels. (D) *CD36* expression in SVF cells shows a negative association with hs-CRP. (E) *MSCA1* expression in both SVF cells and adipocytes is positively associated with leptin serum levels whereas (F) *CD36* expression in SVF cells shows a negative correlation. (G) *MSCA1* expression in adipocytes shows a negative correlation with adiponectin serum levels whereas there is no correlation for expression in SVF cells. (H) In contrast, a positive association exists between *CD36* expression in SVF cells and adiponectin serum

for cell proliferation. Similarly, differentiation potential of prepassaged SVF cells did not show an association with the native expression of *MSCA1* (R = –0.244, *P* = 0.263) or *CD36* (R = 0.218, *P* = 0.329). Considering the changing composition of cell types during cultivation of SVF cells in vitro, we further investigated the relationship between in vitro proliferation and differentiation potential of the prepassaged cells with their intrinsic *MSCA1/CD36* gene expression levels. We did not detect a correlation of *MSCA1* or *CD36* expression levels in undifferentiated prepassaged SVF cells and their in vitro proliferation rate (Fig. 3D). Furthermore, we did not observe a correlation between *MSCA1* gene expression and in vitro differentiation efficiency measured by the percentage of differentiated cells (Fig. 3E and 3F) or the Oil Red O absorbance on day 8 of adipocyte differentiation (R = –0.183, *P* = 0.402). In contrast, we observed that in vitro differentiation capacity of SVF cells was increased in cells with higher *CD36* mRNA levels already before adipogenic induction as well as in differentiated cells (*CD36* mRNA and Oil Red O absorbance on day 8: R = 0.605, *P* = 0.002) (Fig. 3E and 3G). Since *PPARG* is the master regulator of adipogenesis, and since there is evidence that *CD36* expression is activated by peroxisome proliferator-activated receptor γ (25), we assessed a potential association of these genes. We observed that there is a significant positive correlation between the expression of *CD36* and *PPARG*, in prepassaged SVF cells before and after adipocyte differentiation (Fig. 3H), which was even more pronounced in freshly isolated native SVF cells (R = 0.732, *P* < 0.001). For *MSCA1*, we did not observe any association with *PPARG*, neither in native SVF (R = –0.096, *P* = 0.390) cells nor in prepassaged SVF cells before (R = 0.352, *P* = 0.099) and after (R = 0.221, *P* = 0.311) differentiation, which is line with the observation that there is no association of *MSCA1* with the in vitro adipogenic potential of SVF cells. To examine the relationship between *CD36* and *PPARG* in more detail, we performed small interfering RNA-mediated knockdown experiments in SGBS cells and confirmed downregulation of *CD36* expression after knockdown of *PPARG* (Fig. 3I). To confirm this finding, we went back into the analyses of native SVF cells of the Leipzig AT Childhood cohort and grouped the samples into high and low differentiation for comparison of *PPARG* and *CD36* expression. Indeed, we observed that both *PPARG* and *CD36* expression was elevated in native SVF cells with high differentiation capacity (Fig. 3J), hence indicating that *CD36* expression and associated regulation of adipocyte differentiation might be linked to processes of adipocyte hypertrophy/hyperplasia in AT with obesity development in children (Fig. 3K).

MSCA1 mRNA Levels in Native SVF Cells Are Correlated With Parameters of Mitochondrial Respiration

Particularly, MSCA1-positive progenitor cells are characterized by a higher mitochondrial content and show an increased potential for adipogenesis in beige adipocytes (9). Therefore, we additionally measured functional parameters of the mitochondrial respiratory chain in undifferentiated prepassaged SVF cells using Seahorse technology. We did not observe any differences in the metabolic activity of the mitochondria between lean children and children with overweight and obesity (Fig. 4A). We then investigated whether mitochondrial function is associated with *CD36* and *MSCA1* gene expression in SVF cells and stratified samples into low-and high-expressing examples according to mean expression in native or prepassaged SVF cells, respectively. We did not detect an association between parameters of the mitochondrial respiratory chain and *CD36* expression, neither in native nor in prepassaged SVF cells (Table 4). *MSCA1* expression in prepassaged SVF cells did also not show a correlation with mitochondrial activity. In contrast, when samples were stratified for *MSCA1* expression in native SVF cells, we observed that the mean OCR of cells with high *MSCA1* expression was elevated compared to cells with low expression (Fig. 4B, Table 4). In addition, high *MSCA1* expression was associated with higher OCR values for maximum respiration and the corresponding spare capacity as well as increased nonmitochondrial OCR (Table 4). In line with this, in quantitative analyses, we detected a significant positive correlation between *MSCA1* expression levels in native SVF cells and several parameters of mitochondrial function, such as basal respiration, proton leak, maximal respiration, and spare capacity (Fig. 4C). To look at this relationship in more detail, we performed gene expression analysis of marker genes characterizing beige/brown adipogenesis (*PRDM16*, *PGC1A*, *UCP1*) as well as *CKB* as a regulator of the futile creatine cycle, which has been recently implicated in MSCA1-mediated regulation of thermogenic activity in AT of mice (18), in the prepassaged SVF cells in which mitochondrial activity was

Figure 2: continued

levels but not with *CD36* expression in adipocytes. (I) Similar to leptin, the homeostasis model assessment-insulin resistance shows a positive correlation with *MSCA1* expression and (J) a negative association with *CD36* expression in SVF cells. (K) *MSCA1* expression is negatively associated with basal lipolytic activity of adipocytes (L) but shows no correlation with *CD36* expression. Pearson correlation coefficient are given unadjusted (R and *P*-value) and adjusted (R_a and P_a-value) in each scatter plot. Significant *P*-values (*P* < 0.05) are indicated in bold. Lean children are represented as open circles and children with overweight/obesity, as closed circles.

Table 3. Age- and BMI SD score–adjusted correlation analyses between *MSCA1*/*CD36* and obesity-related adipose tissue and serum parameters

	MSCA1 mRNA in SVF cells[a]			*MSCA1* mRNA in adipocytes[a]			*CD36* mRNA in SVF cells[a]			*CD36* mRNA in adipocytes[a]		
	n	R_{adj}	P_a	n	R_{adj}	P_{adj}	n	R_{adj}	P_{adj}	n	R_{adj}	P_{adj}
AT composition												
Adipocyte diameter, µm	76	0.330	0.004	76	0.206	0.079	74	0.072	0.549	76	0.013	0.913
Total adipocyte number × 109[a]	52	−0.223	0.119	52	−0.133	0.356	50	−0.129	0.389	52	0.345	0.014
Isolated adipocytes per g AT × 106[a]	77	−0.157	0.177	77	−0.138	0.239	75	0.039	0.742	77	0.217	0.061
Isolated SVF cells per g AT × 105[a]	57	0.047	0.735	57	0.228	0.094	56	−0.202	0.14	57	0.014	0.919
AT inflammation												
Macrophages per 100 adipocytes[a]	108	0.137	0.161	108	0.100	0.310	105	−0.119	0.232	108	−0.070	0.476
CLS[a]	108	0.032	0.745	108	0.174	0.075	105	−0.005	0.959	108	−0.081	0.407
hs-CRP, mg/L[a]	112	0.381	<0.001	112	0.266	0.005	108	−0.038	0.698	112	−0.007	0.941
TNF-α, pg/mL[a]	105	0.056	0.797	105	−0.064	0.521	101	0.114	0.262	105	0.114	0.251
Adipokines and HOMA IR												
Adiponectin, mg/L[a]	109	−0.080	0.414	109	−0.138	0.157	105	0.072	0.472	109	0.091	0.349
Leptin, ng/mL[a]	107	0.098	0.319	107	0.212	0.030	104	−0.152	0.128	107	0.217	0.026
HOMA IR[a]	111	0.093	0.335	111	0.069	0.474	107	−0.197	0.045	111	0.015	0.879
AT functional parameters												
Doubling time of native cells from the SVF, h[a]	51	0.133	0.362	51	0.035	0.811	50	0.207	0.159	51	−0.037	0.802
Growth rate of proliferating native SVF cells[a]	45	−0.061	0.699	45	−0.301	0.050	44	−0.089	0.576	45	0.038	0.808
Generation time of proliferating native SVF	45	0.014	0.932	45	0.336	0.028	44	0.024	0.879	45	−0.098	0.533
Differentiation of native cells from the SVF, %	48	−0.079	0.601	48	−0.255	0.088	47	0.177	0.244	48	0.025	0.870
Basal lipolysis of adipocytes	29	−0.370	0.057	29	−0.361	0.064	28	0.041	0.844	29	0.126	0.532
Isoproterenol-stimulated lipolysis of adipocytes	30	−0.417	0.027	30	−0.069	0.727	29	−0.003	0.990	30	0.182	0.354

R indicates the Pearson correlation coefficient. Significant *P*-values are indicated in bold. R_{adj}, and P_{adj} analyses are adjusted for age and BMI SDS.

Abbreviations: AT, adipose tissue; BMI, body mass index; CLS, crown-like structure; HOMA IR, homeostatic model assessment-insulin resistance; hs-CRP, high-sensitivity C-reactive protein; SVF, stromal vascular fraction; TNF-α, tumor necrosis factor α; SDS, SD score.

[a]Statistical analysis was performed for log-transformed parameters.

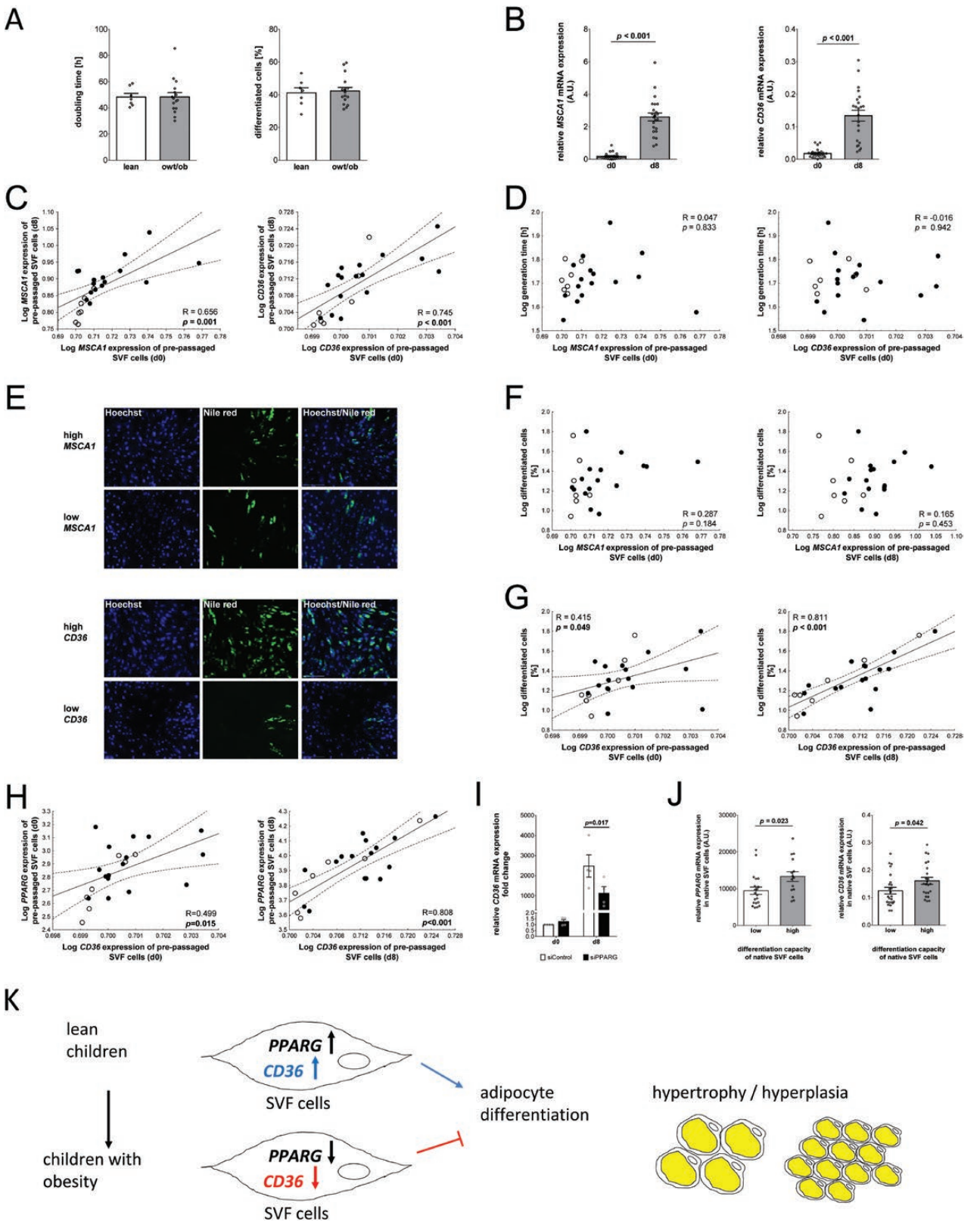

Figure 3. *CD36* (cluster of differentiation 36) gene expression is related to differentiation capacity of stroma vascular fraction (SVF) cells in vitro. (A) Body weight groups are not indicative for in vitro proliferation or differentiation capacity of prepassaged SVF cells. Quantitative data of n = 23 children samples are presented as mean ± standard error of the mean (SEM). (B) *MSCA1* (mesenchymal stem cell antigen 1) and *CD36* messenger RNA (mRNA) levels of prepassaged SVF cells increase during adipocyte differentiation (bar plots show quantitative data of n = 23 SVF samples; mean ± SEM). (C) SVF mRNA level of *MSCA1* and *CD36* before differentiation correlate with gene expression after differentiation. (D) There is no

measured. We did not detect a clear association of *MSCA1* expression with expression of the analyzed regulators of beige and brown adipocyte function or the futile creatine cycle. However, there was a nominal significant tendency toward a correlation of high *MSCA1* expression in native SVF cells with increased *PGC1A* expression ($P = 0.059$) and with decreased *CKB* expression ($P = 0.075$). In summary, our findings indicate a potential role of *MSCA1* in beige adipogenesis in children.

Discussion

Here we investigated the relevance of the adipocyte progenitor marker genes *MSCA1* and *CD36* for AT biology during physiological and obesity-related AT accumulation in children and assessed a potential link to functional properties of SVF cells in vitro.

We show that AT mRNA levels of *MSCA1* but not *CD36* increase with age in lean children. MSCA1 has been shown to be involved in processes related to growth in several tissues, including the bone (26), the brain (27), and the AT (12), where it promotes cellular growth and differentiation. One might hence speculate that MSCA1 plays a role in AT accumulation during childhood potentially by regulating AT function. Supporting this assumption, we observed a positive association of *MSCA1* expression and parameters of AT composition, in particular adipocyte size. Interestingly, a similar association between MSCA1 and hypertrophy has been described for bone marrow-derived mesenchymal stromal cells (MSCs) before showing that MSCA1 levels were increased in larger cells and that the MSCA1-positive subpopulation exhibited higher levels of hypertrophic markers (28). In previous studies, we have shown that adipocyte hypertrophy is associated with an increase in macrophage infiltration and the presence of CLS in AT already during childhood (21). Although we could not find a direct relationship between *MSCA1* and the number of macrophages within the AT, we observed positive associations with other inflammatory parameters related to hypertrophy in AT, such as hs-CRP serum levels, a marker for systemic low-grade inflammation, and the number of CLS in AT, especially for *MSCA1* mRNA in adipocytes. This is in line with results from previous studies that indicate a connection between *MSCA1* and inflammatory processes in AT (eg, inflammatory cytokines affecting the expression and activity of MSCA1) (9).

We observed that *MSCA1* expression increases during adipogenic differentiation of unsorted prepassaged SVF cells in vitro, and Estève et al described a similar effect for CD45-/CD34+/CD31– selected adipocyte progenitor cells (9). They further showed a positive association between *MSCA1* and adipogenic potential of adipocyte progenitor cells (9,29), which we could not confirm in our experimental setup. Unfortunately, we were limited by the often small amount of AT we obtained from children during elective surgeries, which results in the fact that we often do not have sufficient native cells available for cell selection by fluorescence-activated cell sorting. Hence, our results are not entirely comparable to those of Estève et al. In this regard, a previous study has shown that lipid production during adipogenesis in unsorted cells was more similar to that of MSCA1 negative cells, while MSCA1 positive cells showed a higher adipogenic potential (29).

Previous studies in human adults indicated that MSCA1 supports beige adipogenesis (9). Studies in mice indicated that obesity-related diseases, such as diabetes, are associated with a reduced mitochondrial activity in AT (30). In contrast, we did not detect any differences in mitochondrial activity between SVF samples derived from lean children and children with overweight and obesity, which might be due to the fact that children with obesity represent early stages of disease progression and mitochondrial dysfunctions may not have developed yet. However, we could show that a high level of *MSCA1* in native SVF cells is associated with higher oxygen consumption rates in mitochondrial respiration. Surprisingly, we could not detect this for intrinsic *MSCA1* expression of prepassaged SVF cells. Reason for this might be that gene expression in prepassaged cells may differ from that in native SVF cells due to the altered composition of cell types and associated cell-cell interactions. Furthermore, several studies showed that during the cultivation of SVF cells there is a loss of surface receptor

Figure 3: continued

association between *MSCA1*/*CD36* expression in prepassaged SVF cells and generation time of proliferating cells. (E) Cells were grouped for high and low *MSCA1* or *CD36* expression at baseline (d0) and adipocyte differentiation was analyzed. Representative images of differentiated SVF cells (d8) after Hoechst and Nile Red staining (scale bar 200 µm). *MSCA1* gene expression is not related to (F) the percentage of differentiated cells while (G) high *CD36* expression is associated with higher percentages of differentiated cells. (H) In accordance with this, there is a positive correlation between *PPARG* expression and *CD36* expression, before and after differentiation into mature adipocytes. (I) Small interfering RNA knockdown of *PPARG* in Simpson Golabi Behmel Syndrome cells results in decreased *CD36* expression. (J) High differentiation capacity of native SVF cells (grouped according to the median of the samples) is associated with higher *PPARG* and *CD36* mRNA expression in native SVF cells (bar plots show quantitative data of n = 47 SVF samples; mean ± SEM). (K) Schematic overview of the proposed role of *CD36* mRNA in adipose tissue of children. Pearson correlation coefficient R and *P*-value are given in each scatter plot. Significant *P*-values ($P < 0.05$) are indicated in bold. Lean children are represented as open circles and children with overweight/obesity (owt/ob), as closed circles. Abbreviation: *PPARG*, peroxisome proliferator-activated receptor gamma.

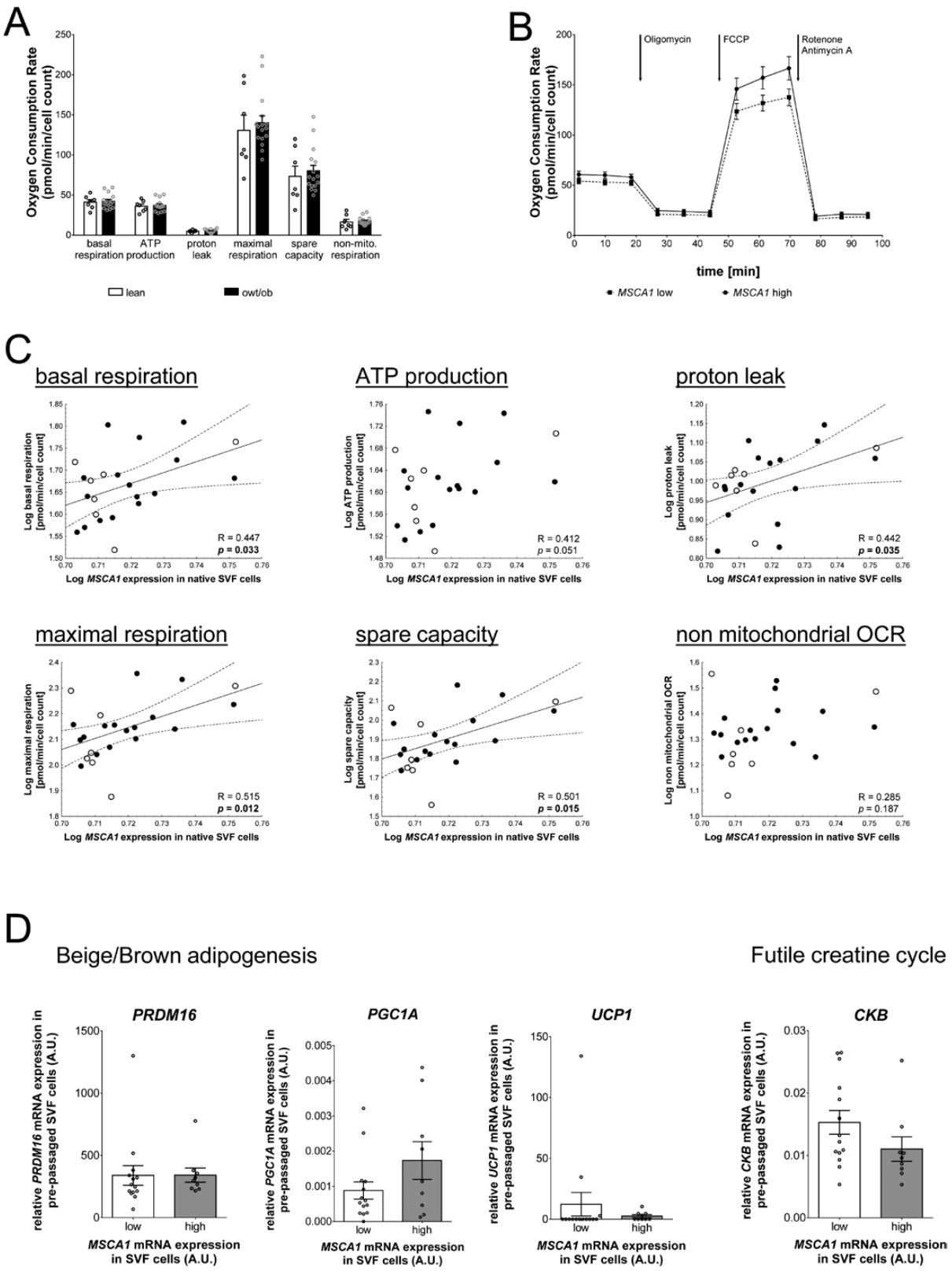

Figure 4. *MSCA1* (mesenchymal stem cell antigen 1) expression in native SVF cells is associated with cellular respiration *in vitro*. (A) The OCR of the investigated specific parameters of the mitochondrial respiratory chain did not differ between pre-passaged SVF cells of lean children and children with overweight/obesity (owt/ob). Bar plots show quantitative data of n = 23 children (mean ± SEM). (B) High *MSCA1* expression in native SVF cells is associated with a higher OCR in pre-passaged cells. Graph depicts the averaged seahorse measurement (mean ± SEM) of n = 9 SVF cell samples with high compared to n = 14 samples with low *MSCA1* expression. (C) *MSCA1* expression in native SVF cells correlates with specific parameters

Table 4. Correlation analyses between functional parameters of mitochondrial respiration and *CD36* or *MSCA1* gene expression in SVF cells

	MSCA1 expression					
	Native SVF cells			Prepassaged SVF cells		
Functional parameters of mitochondrial respiration chain	Low (≤0.228) n = 14	High (>0.228) n = 9	*P*	Low (≤0.180) n = 15	High (>0.180) n = 8	*P*
Basal respiration, pmol/min/cell count	39.3 ± 2.1	48.6 ± 3.6	0.060	42.0 ± 2.3	41.9 ± 2.9	0.978
ATP production, pmol/min/cell count	34.7 ± 1.8	40.3 ± 2.1	0.057	37.1 ± 1.9	36.5 ± 2.4	0.835
Proton leak, pmol/min/cell count	4.7 ± 0.4	5.8 ± 0.8	0.187	4.9 ± 0.5	5.5 ± 0.7	0.530
Maximal respiration, pmol/min/cell count	120.5 ± 7.9	163.4 ± 12.8	**0.006**	140.0 ± 12.0	132.2 ± 7.0	0.657
Spare capacity, pmol/min/cell count	65.9 ± 5.5	96.9 ± 10.5	**0.009**	80.6 ± 8.8	73.4 ± 6.5	0.585
Non-mitochondrial OCR, pmol/min/cell count	15.3 ± 1.5	20.4 ± 1.9	**0.044**	17.4 ± 1.7	16.9 ± 1.8	0.856
	CD36 expression					
	Native SVF cells			Prepassaged SVF cells		
Functional parameters of mitochondrial respiration chain	Low(≤0.120) n = 14	High (>0.120) n = 9	*P*	Low (≤0.018) n = 13	High (>0.018) n = 10	*P*
Basal respiration, pmol/min/cell count	43.8 ± 2.7	39.2 ± 1.2	0.219	42.2 ± 2.6	41.7 ± 2.4	0.891
ATP production, pmol/min/cell count	38.3 ± 2.3	34.7 ± 1.1	0.230	37.2 ± 2.2	36.6 ± 1.9	0.844
Proton leak, pmol/min/cell count	5.4 ± 0.6	4.6 ± 0.5	0.301	5.1 ± 0.5	5.1 ± 0.7	0.915
Maximal respiration, pmol/min/cell count	146.1 ± 12.2	123.7 ± 6.8	0.183	143.9 ± 11.8	128.7 ± 10.5	0.366
Spare capacity, pmol/min/cell count	84.6 ± 9.1	67.9 ± 5.6	0.189	84.3 ± 8.9	69.9 ± 7.6	0.251
Nonmitochondrial OCR, pmol/min/cell count	17.7 ± 1.5	16.5 ± 2.3	0.645	17.4 ± 1.6	17.1 ± 2.2	0.927

Significant *P*-values ($P < 0.05$) are indicated in bold. Gene expression of n = 23 samples is grouped into high (>mean expression) and low (≤mean expression).
Abbreviations: ATP, adenosine triphosphate; *CD36*, cluster of differentiation 36; *MSCA1*, mesenchymal stem cell antigen 1; OCR, oxygen consumption rate; SVF, stroma vascular fraction.

expression, which might reflect differences in gene expression (7,9). Nevertheless, we see that *MSCA1* expression tends to be related to *PGC1A* expression. Since *PGC1A* is a marker gene for beige/brown adipocytes with higher thermogenic potential and higher mitochondrial content than white adipocytes, this correlation could explain the association between *MSCA1* and mitochondrial activity of SVF cells. However, without further experimental investigations, such suggestions remain speculative. Taken together, we provide evidence that *MSCA1* expression is related to mitochondrial function of SVF cells in vitro. Future studies investigating a potential link to obesity-related adipocyte hypertrophy and AT dysfunction in children would be of interest, as this may be related to the development of obesity-associated disease.

Gao et al discovered that MSCA1 positive adipocyte progenitor cells separate into further subpopulations characterized by presence or absence of the fatty acid translocase and membrane glycoprotein CD36 (10). Several studies indicated that CD36 plays a role in the regulation of lipid storage and lipolysis (10, 31) and in inflammatory processes in AT (32-34) and that its expression is upregulated in AT of adults with obesity (35, 36). Surprisingly, we detected a downregulation of *CD36* expression in native SVF cells with increasing BMI SDS and no correlation in adipocytes. Furthermore, there were no prominent BMI-independent associations of *CD36* expression with adipocyte hypertrophy or inflammatory processes in AT of children. In contrast to most previous studies in humans, we measured gene expression in the separated fractions of

Figure 4: continued

of the mitochondrial respiratory chain, ie, basal respiration, proton leak, maximal respiration and spare capacity. (D) The gene expression of marker genes for beige/brown adipogenesis (*PRDM16*, *PGC1A*, *UCP1*) and the regulator of the futile creatine cycle *CKB* showed no significant correlation with *MSCA1* expression in native SVF cells. Pearson correlation coefficient R and *p* value are given in each scatter plot. Significant *p* values ($P < 0.05$) are indicated in bold. SVF samples from lean children are represented as open circles and children with overweight/obesity as closed circles. Abbreviations: ATP, adenosine triphosphate; *CKB*, creatine kinase B; FCCP, carbonyl cyanide-4 (trifluoromethoxy) phenylhydrazone; OCR, oxygen consumption rate; *PGC1A*, Peroxisome proliferator-activated receptor gamma coactivator 1-alpha; *PRDM16*, PR domain containing 16; *UCP1*, uncoupling protein 1.

SVF cells and adipocytes instead of intact AT, which allows a more differentiated insight into processes occurring in AT with development of obesity.

Apart from this, analyses in preadipocytes of mice have shown that *CD36* expression increases during adipocyte development once the differentiating cells are able to uptake and store fatty acids (19,37). We observed a similar *CD36* regulation during adipocyte differentiation of prepassaged SVF cells of children. Previous studies provided evidence that CD36 promotes adipocyte differentiation in vitro (38) and that the MSCA1+/CD36+ adipocyte progenitor population of the SVF shows an increased capability for white adipogenesis (10). Interestingly, in mature adipocytes of children the expression levels of *MSCA1* and *CD36* correlated positively. One might speculate that this association reflects the presence of differentiated adipocytes derived from MSCA1+/CD36+ adipose progenitor cells. Furthermore, we show here that the link between *CD36* expression levels and the adipogenic potential of SVF cells is already present in children with high *CD36* expression being associated with increased adipocyte differentiation. In addition, we could show that *CD36* expression in SVF cells of children is closely linked to expression of *PPARG*, which is the master regulator of adipogenesis, and that SVF cells with high adipogenic potential are characterized by in increased expression of both *PPARG* and *CD36*. Based on our data showing an inverse relationship between *CD36* expression in SVF cells and obesity, inflammation, and insulin sensitivity, one might speculate that, similar to *PPARG*, *CD36* expression is protective in terms of metabolic health. Furthermore, the fact that SVF *CD36* expression is inversely correlated to total adipocyte number might indicate a potential role of *CD36* in the regulation of hypertrophic *vs* hyperplasic processes in AT with early obesity development.

One strength of our study is the analyses of AT samples of children, while most previous studies focusing on obesity-related AT expansion have been performed in adults. Due to the fact that obesity manifests in early childhood and is already associated with changes in AT (21), studies in children might allow better insight into the early processes related to obesity development. Moreover, children present early stages of the disease and the influence of drug treatments or diets on AT biology is reduced compared to adults. However, in our experimental analyses of functional AT parameters, we are limited by the amount of AT, which is dependent on the site and nature of surgery and on the age of children. Because of this subsamples are available for different measures of AT function, which did not allow more mechanistic analyses. Nevertheless, to the best of knowledge the Leipzig AT Childhood Cohort is unique in both sample size and the functional comprehensive characterization of AT biology in children.

In conclusion, we provide evidence that both *MSCA1* and *CD36* are associated with obesity-related alterations in AT of children as well as functional properties of SVF cells in vitro. While *MSCA1* is more related to metabolic and mitochondrial function of AT, *CD36* expression predicts adipogenic differentiation potential of SVF cells, indicating that it might play a role in the regulation of adipocyte hyperplasia and hypertrophy with obesity development in children.

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Disclosure Summary: The authors have nothing to disclose.

Data Availability: The datasets generated during and/or analyzed during the current study are not publicly available but are available from the corresponding author on reasonable request.

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