MAP kinase phosphatase DUSP1 is overexpressed in obese humans and modulated by physical exercise

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THE PREVALENCE OF OBESITY and its comorbidities, including diabetes, metabolic syndrome, and cardiovascular diseases, has become a major health concern worldwide. The mechanisms underlying obesity are complex, but chronic low-grade inflammation, termed inflammation and muscle wasting, becomes a major health concern worldwide. Our group and others have shown that MAP kinase phosphatase DUSP1 is overexpressed in obese humans and modulated by physical exercise. The mechanisms involved in the regulation of adipose tissue metabolism, response to stress, and inflammation. Originally described as stress or growth factor-induced kinases, it becomes clear that MAPKs are activated in response to a wide range of physiological and pathophysiological stimuli that act in a context-, duration-, and magnitude-dependent manner (42). Therefore, a tight control of their activities is crucial to maintain tissue integrity and physiological homeostasis.

The activity of MAPKs is orchestrated by a complex interplay involving MAPK kinases (MKks) and MAPK phosphatases (MPks) known also as dual-specificity phosphatases (DUSPs) (12). DUSP1 is an archetypical member of the nuclear DUSP family that dephosphorylates MAPKs at threonine, serine, and tyrosine residues (46). It was initially identified as an immediate-early stress response gene induced by oxidative stress (41), but subsequent studies confirmed its multirole in various biological processes, including inflammation and metabolism, and as such it has emerged as key target for treatment of various diseases (46). The role of DUSP1 in the etiology of obesity and metabolism homeostasis was demonstrated in animal models of diet-induced obesity (67). In both mice and rats, DUSP1 is overexpressed following a high-fat diet (HFD) (16, 56). Consistent with this phenotype, DUSP1-deficient mice are resistant to HFD-induced obesity due to enhanced energy expenditure, and this was through MAPKs (67). Recently, DUSP1 was suggested to play a critical role in the switch from oxidative to glycolytic myofibers during HFD feeding by inhibiting p38 MAPK and, indirectly, peroxisome proliferator-activated receptor-γ (PPAR-γ) coactivator 1α (PGC-1α), thus highlighting its central role in the regulation of myofiber composition and obesity (60). In support of these findings, overexpression of DUSP1 attenuated adiponectin-enhanced mitochondrial biogenesis, with signifi-
cantly decreased PGC-1α expression and p38 MAPK phosphorylation both in vitro and in vivo (54, 72). DUSP1 plays also an important role in lipid metabolism, as DUSP1-deficient mice exhibited increased fatty acid oxidation and were protected from the development of hepatic steatosis (23). PPARα, a key mediator of energy burning through lipid oxidation, is another target of p38 MAPK that is overexpressed during brown adipocyte differentiation (53). Because DUSP1 negatively controls p38 MAPK, it could indirectly attenuate PPARα activity (67). Taken together, these studies indicated clearly the critical role of DUSP1 in controlling metabolic homeostasis.

Physical exercise is an important component of a healthy lifestyle widely advocated as a first-line nonpharmacological approach for the management of obesity, insulin resistance, diabetes, and cardiovascular diseases (49, 63). Previous studies attributed the protective effect of physical exercise to improving expression of p38 MAPK and PGC-1α in the skeletal muscle (3). Because DUSP1 negatively controls p38 MAPK, it could indirectly attenuate PPARα, PGC-1α, and insulin signaling by suppressing the activity of protein tyrosine phosphatase 1B (PTP1B) in the brain (15), by increasing expression of p38 MAPK and PGC-1α in the skeletal muscle (3), and by attenuating the JNK signaling pathway in the liver (43). More recently, it was reported that exercise training reduced the expression levels of MKP-3 (DUSP6) protein in the livers of obese mice models (65).

In this study, we compared the expression levels of DUSP1 mRNA and protein between lean and obese nondiabetic human subjects using subcutaneous adipose tissue (SAT) and peripheral blood mononuclear cells (PBMCs) and established the association of these levels with physical, clinical, and biochemical parameters of our study population. We also tested the hypothesis that physical exercise could have an effect on DUSP1 expression. Our main findings indicate that DUSP1 was overexpressed in obese humans and decreased after 3 mo of physical exercise.

MATERIALS AND METHODS

Study population. This investigation was initially carried out on adult male and female human nondiabetic subjects that were lean (BMI = 20–24.9 kg/m²; n = 40) and obese (BMI = 30–40 kg/m²; n = 40) (cohort study 1; Table 1). The finding that DUSP1 has a nuclear and extranuclear localization (Fig. 4A) prompted us to investigate its possible release into the circulation. For this purpose, and based on sample availability, depletion of plasma samples from lean subjects, and enrollment of additional obese subjects, an extra 20 new obese subjects were added to the initial 40 obese subjects of cohort study 1 and used along with the remaining 20 lean subjects from cohort study 1 to form cohort study 2, which consisted of 20 lean and 60 obese subjects (Table 2). Unless stated otherwise, all of the subsequent analyses were done on subjects from cohort study 1. Informed written consent was obtained from all subjects before their participation in the study, which was approved by the Ethical Review Board of Damien Diabetes Institute and carried out in line with the guidelines of the Declaration of Helsinki. Participants that followed any physical exercise within the last 6 mo prior to this study and participants with prior major illness or intake of medications and/or supplements known to influence the body composition or bone mass were excluded from the study. The physical, clinical, and biochemical characteristics of the participating subjects are shown in Tables 1 and 2.

Exercise protocol. All eligible subjects were enrolled in a supervised exercise program at the Fitness and Rehabilitation Center (FRC) of Damien Diabetes Institute. Prior to exercise prescription, each individual underwent a symptom-limited maximal incremental cardiopulmonary exercise test (CPET) using an electromagnetically braked cycle ergometer (COSMED Quark). The CPET was used primarily to determine the maximum heart rate (max HR) as well as the response to aerobic exercise as measured by the maximum oxygen consumption (VO2max) for each subject. Thereafter, further fitness assessments were performed to determine muscle strength and endurance along with flexibility by performing grip strength (dynamic-
Table 2. Physical, clinical, and biochemical characteristics of the cohort study 2 at baseline

<table>
<thead>
<tr>
<th></th>
<th>Lean (n = 20)</th>
<th>Obese (n = 60)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Anthropometric and physical characteristics</strong></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Age, yr.</td>
<td>39.75 ± 7.83</td>
<td>39.95 ± 8.65</td>
<td>0.77</td>
</tr>
<tr>
<td>Sex (males/females)</td>
<td>8/12</td>
<td>28/32</td>
<td>0.61</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>22.50 ± 2.47</td>
<td>23.95 ± 2.94</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Waist, cm</td>
<td>78.56 ± 17.14</td>
<td>103.31 ± 13.80</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Hip, cm</td>
<td>90.76 ± 16.27</td>
<td>119.04 ± 7.87</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>PBF</td>
<td>27.07 ± 5.32</td>
<td>32.91 ± 4.78</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Resting HR, beats/min</td>
<td>81.15 ± 13.78</td>
<td>79.03 ± 8.49</td>
<td>0.78</td>
</tr>
<tr>
<td>SBP, mmHg</td>
<td>114.30 ± 11.13</td>
<td>128.73 ± 12.22</td>
<td>0.013</td>
</tr>
<tr>
<td>DBP, mmHg</td>
<td>76.81 ± 6.76</td>
<td>81.84 ± 10.02</td>
<td>0.17</td>
</tr>
<tr>
<td>V̇O₂max, ml·kg⁻¹·min⁻¹</td>
<td>22.09 ± 3.95</td>
<td>17.07 ± 5.11</td>
<td>0.023</td>
</tr>
</tbody>
</table>

**Metabolic markers**

- Cholesterol, mmol/l: 5.14 ± 0.80 vs. 5.09 ± 1.02, 0.89
- HDL, mmol/l: 1.46 ± 0.47 vs. 1.13 ± 0.25, 0.006
- LDL, mmol/l: 3.24 ± 0.72 vs. 3.29 ± 0.95, 0.48
- TG, mmol/l: 0.96 ± 0.51 vs. 1.38 ± 0.82, 0.038
- Glucose, mmol/l: 4.88 ± 0.50 vs. 5.37 ± 0.83, 0.014
- Hb A₁c, %: 5.47 ± 0.45 vs. 5.61 ± 1.03, 0.017
- C-peptide, ng/ml: 2.47 ± 1.66 vs. 3.11 ± 2.20, 0.028
- GIP, pg/ml: 140 ± 71 vs. 154 ± 78, 0.12
- Ghrelin, pg/ml: 172 ± 84 vs. 165 ± 102, 0.37
- Glucagon, ng/ml: 0.64 ± 0.09 vs. 0.72 ± 0.13, 0.025
- GLP-1, ng/ml: 2.55 ± 0.86 vs. 2.63 ± 1.40, 0.66
- Insulin, ng/ml: 2.45 ± 1.07 vs. 4.59 ± 2.33, 0.0015
- Resistin, ng/ml: 3.11 ± 1.44 vs. 4.14 ± 1.40, 0.029
- Visfatin, ng/ml: 10.47 ± 4.83 vs. 9.98 ± 8.51, 0.94

**Inflammatory markers**

- IL-6, pg/ml: 5.45 ± 1.89 vs. 5.05 ± 2.06, 0.17
- IP-10, pg/ml: 332 ± 122 vs. 549 ± 211, <0.0001
- MCP-1, pg/ml: 27.78 ± 7.71 vs. 26.23 ± 13.39, 0.72
- RANTES, ng/ml: 9.25 ± 2.91 vs. 9.75 ± 3.98, 0.93

**Oxidative stress markers**

- ROS, nM: 1.35 ± 0.31 vs. 1.51 ± 0.13, 0.12
- TBARS, µM: 1.09 ± 0.49 vs. 1.44 ± 0.39, 0.13

Data are presented as means ± SD. *Lean group was from cohort study 1, as shown in Table 1. #Obese group consisted of 40 subjects from cohort study 1 and 20 new subjects.

overexpression of dusp1 in obese humans

SAT biopsies (−0.5g) were obtained from the periumbilical area by surgical biopsy after a local anesthesia. Once removed, the biopsy was rinsed in cold PBS, divided into four pieces, and stored appropriately until assayed.

**Anthropometric measurements and blood biochemistry.** Anthropometric measurements were taken at the baseline and after 3 mo of exercise. Whole body composition was determined by a dual-energy radiographic absorptiometry device (Lunar DEXA; Lunar Radiation, Madison, WI). Glucose and lipid profiles were measured on the Siemens Dimension RXL chemistry analyzer (Diamond Diagnostics, Holliston, MA). Hb A₁c was determined using the Variant device (Bio-Rad, Hercules, CA). Plasma levels of inflammatory and metabolic markers were measured using bead-based multiplexing technology. Median fluorescence intensities were collected on a Bioplex-200 system using Bio-plex Manager software version 6 (Bio-Rad). Lipid peroxidation was assessed by measuring plasma levels of malonaldehyde using TBARS Assay Kit (Cayman Chemical, Ann Arbor, MI). Serum levels of reactive oxygen species (ROS) were determined using the Oxiselect ROS Assay Kit (Cell Biolabs, San Diego, CA). All of the above assays were carried out according to the instructions of the manufacturers.

**Quantitative real time-PCR.** Total RNA was extracted from frozen SAT and PBMCs isolated from lean and obese subjects in cohort study 1. RNeasy Lipid Tissue Mini Kit and AllPrep RNA/Protein Kit (Qiagen, Valencia, CA) were used to prepare total RNA from subcutaneous adipose tissue and PBMCs, respectively. The cDNA was synthesized from total RNA sample using High Capacity cDNA Reverse Transcription Kits (Applied Biosystems, Foster City, CA). Quantitative real time-PCR (qRT-PCR) was performed on a Rotor-Disc 100 system using SYBR Green (Qiagen). Primers used in this study for qRT-PCR consisted of DUSP1 forward (5’-TCTTCTTCATCAAGGAGGATAGC-3’) in conjunction with DUSP1 reverse (5’-GTGGGACTTGAGGGTAAGCTG-3’), PGC-1α forward (5’-CCAGTGCCATCATCATC-3’) and PGC-1α reverse (5’-AACAACCGCAAGGACCGCAGT-3’), PPARα forward (5’-GAGGACACACCGGAGGACT-3’) and PPARα reverse (5’-AACAACCGCAAGGACCGCAGT-3’), and PAPD forward (5’-AATCTTCTGAGTGGAGGAGG-3’) and PAPD reverse (5’-TGTGAGGGAGAGGCTCACGTG-3’). Relative expression was assessed by using the ΔΔCT method (47), and GAPDH was used as internal control for normalization.

**Western blot analysis.** Western blots were carried out on PBMCs isolated from lean and obese subjects of cohort study 1. Whole cell extracts were prepared in RIPA buffer (50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 1% Triton X-100, 1 mM EDTA, 0.5% sodium deoxycholate, and 0.1% SDS). Cytosolic and nuclear extracts were prepared from PBMCs using the ReadyPrep protein extraction kit according to the manufacturer’s instructions (Bio-Rad, Hercules, CA). Protein concentration was determined by the Bradford method using γ-globulin as a standard, and 20 μg of protein was resolved on 12% SDS-PAGE gels. Proteins were then transferred onto PVDF membranes, blocked with 5% nonfat dried milk in Tris-buffered saline containing 0.05% Tween 20 (TBST) for 1 h at room temperature (rt), and then probed with the primary antibody for overnight at 4°C. After washing, the membranes were incubated with horseradish peroxidase-conjugated secondary antibody for 2 h at rt, and finally, protein bands were visualized by chemiluminescence and the images captured by using the Versadoc 5000 system (Bio-Rad). The primary antibodies used in this study were raised against DUSP1 (LifeSpan Biosciences, Seattle, WA), p38 MAPK, total p38 MAPK, p-INK, total INK, p-ERK1/2, total ERK1/2 (Cell Signaling Technology, Danvers, MA), and GAPDH (Millipore, Temecula, CA). Unless indicated otherwise in the figure legends, GAPDH was used as internal control for monitor for protein loading. To measure changes in the levels of phosphorylated MAPKs, the membranes were stripped and reprobed with antibodies against total MAPKs. Fold change of each of the phosphorylated MAPK was calculated after normalization with the corresponding total MAPK, as indicated in the Fig. 2 legend. For densito-
metric analysis, the intensity of the bands was determined using Quantity One Software (Bio-Rad).

Immunohistochemistry and immunofluorescence studies. Formalin-fixed, paraffin-embedded SAT sections from lean and obese subjects in cohort study 1 were deparaffinized and rehydrated prior to antigen retrieval by boiling in the unmasking solution (Dako, Glostrup, Denmark). The endogenous peroxidase was quenched using 3% H2O2 for 1 h at rt. Sections were blocked with 5% fat-free milk for 1 h at rt, followed by 1% BSA for another hour, and then incubated at 4°C for overnight with primary antibodies. The primary antibodies used in immunohistochemistry (IHC) staining were raised against DUSP1 (LifeSpan Biosciences), p-p38 MAPK, p-JNK, and p-ERK1/2 (Cell Signaling Technology) and PPARα and PGC-1α (Santa Cruz Biotechnology, San Diego, CA). Staining with horseradish-conjugated secondary antibody (Dako) was done for 1 h at rt. Colors were developed using a DAB kit (Dako), and sections were counterstained with hematoxylin (Sigma Aldrich, St. Louis, MO). All slides were scanned at ×20 magnification, and the quantification of the IHC data was done using Aperio ImageScope software version 11.1 (Aperio, Vista, CA). The Algorithm Positive Pixel Count (version 9 of this software) provided the percentage of positive staining (no. of stained pixels) compared with the whole slide. Finally, each annotated picture was checked manually against the original slide picture to ensure the correct match between the positive staining and the software annotation.

For immunofluorescence (IF) staining studies, the tissue sections from lean and obese subjects of cohort study 1 were incubated with anti-DUSP1 antibody conjugated with Alexa Fluor 488 (Biosis, Woburn, MA). DAPI was used at 0.05% for nuclear staining. The sections were analyzed with a Zeiss LSM 710 confocal laser-scanning microscope, and the fluorescent images of the representative areas of the adipose tissue were photographed using a ×40 objective.

Quantification of circulating DUSP1 protein by ELISA. Circulating levels of DUSP1 protein were determined by ELISA method using the human DUSP1 ELISA kit (EIAab, Wuhan, China). The assay was carried out on plasma samples from lean and obese subjects in cohort study 2 according to the instructions of the manufacturer.

Statistical analysis. Statistical analyses were performed with SAS version 9.2 (SAS Institute, Cary, NC). Unless stated otherwise, all descriptive statistics for the variables in the study were reported as means ± SD. For skewed data, nonparametric Mann-Whitney test was used to determine significance of difference in means between the two groups, as indicated in the figure legends. Spearman’s correlation coefficients were estimated to determine associations between DUSP1 concentrations and anthropometric, clinical, and biochemical variables. To assess the difference in categorical variables between lean and obese subjects, a chi-squared test was used. Logistic regression analysis was performed to estimate odds ratios adjusted for covariates. Differences were considered statistically significant at P values of <0.05.

RESULTS

Baseline characteristics of the study population. The physical clinical and biochemical characteristics of the participating subjects are shown in Tables 1 and 2. Both cohort studies displayed similar baseline characteristics. The difference in mean age between lean and obese subjects was not statistically significant. As expected, BMI, waist and hip circumferences, and percent body fat (PBF) were all significantly higher in the obese group (P < 0.0001). The obese group had higher systolic blood pressure (SBP; P < 0.05) and lower capacity of VO2max (P < 0.05). The levels of HDL were significantly lower in the obese group (P = 0.0001 for cohort study 1 and P = 0.0006 for cohort study 2), but the levels of triglycerides (TG) were higher in the obese group (P = 0.0008 and P = 0.038 for cohort studies 1 and 2, respectively). Although our selected population is not diabetic, fasting blood glucose, Hb A1c, insulin, and C-peptide were higher in obese subjects than in the lean group (P < 0.05). Obese subjects had altered metabolic profiles, as indicated by increased levels of leptin (P < 0.0001) as well as glucagon and plasminogen activator inhibitor-1 (PAI-1; P < 0.05). The level of inflammatory chemokines IFNγ-induced protein 10 kDa (IP-10) and regulated upon activation, normal T cell expressed and secreted (RANTES) were also higher in the obese group compared with the lean group (P < 0.001). However, there was no significant difference in the rest of the inflammatory mediators and oxidative stress markers (Tables 1 and 2 and data not shown).

DUSP1 mRNA and protein are upregulated in obese nondiabetic subjects. Studies in mice clearly indicated a role for DUSP1 in obesity and energy expenditure (67). In obese humans, however, there was no study that investigated the expression pattern of DUSP1 or whether this pattern correlated with physical, clinical, and biochemical profiles. Therefore, we compared the expression levels of DUSP1 between lean and obese subjects in PBMCs and adipose tissue. As shown in Fig. 1, qRT-PCR data indicated that obese subjects displayed significant increases in DUSP1 mRNA in both PBMCs and SAT (P < 0.05; Fig. 1A). To confirm these findings at the protein level, we carried out a series of Western blot and IHC analyses using both PBMCs and SAT, and the results are shown in Fig. 1, B and C. As displayed, Western blot results performed on PBMCs indicated a significant increase in DUSP1 protein in the obese group (P = 0.04; Fig. 1B) that was also confirmed by IHC analysis using the adipose tissue from the obese group (P = 0.006; Fig. 1C). Taken together, our findings clearly indicate increased expression of DUSP1 mRNA and protein in obese subjects.

The levels of p-p38 MAPK and PGC-1α are reduced in obese subjects. DUSP1 is a specific phosphatase that acts upstream of the three MAPKs JNK, ERK, and p38 MAPK (50). We next investigated the phosphorylation status of these enzymes by Western blots and IHC using PBMCs and SAT from lean and obese participants. As shown in Fig. 2A, the levels of p-JNK and p-ERK were higher in PBMCs from obese subjects compared with lean subjects. Conversely, the levels of p-p38 MAPK were markedly reduced in obese subjects (Fig. 2A). Consistent with this pattern, IHC data carried out on the SAT from the two groups confirmed the increased levels of both p-JNK and p-ERK (P < 0.05; Fig. 2B) and the reduced levels of p-p38 MAPK (P = 0.004; Fig. 2B).

PGC-1α has emerged as one of the targets for metabolic disorders (22, 53). Based on the role of DUSP1 in energy expenditure and metabolic homeostasis (67), and given that PGC-1α is a downstream target of p38 MAPK (7, 44), we compared its expression in the SAT obtained from lean and obese subjects. Figure 3 indicates that obese subjects had reduced levels of PGC-1α mRNA (P = 0.018; Fig. 3A) and protein (P = 0.03; Fig. 3B). Under the same conditions, there was no significant difference in the expression of PPARα mRNA or protein (Fig. 3, A and B).

Nuclear and extranuclear localization of DUSP1 protein. DUSP1 was reported initially as a nuclear phosphatase that dephosphorylates and inactivates p38 MAPK, JNK, and ERK (68). The finding that p-p38 MAPK, but not p-JNK or p-ERK, was reduced in obese subjects (Fig. 2) prompted us to inves-
tigate whether there was no change in the subcellular localization of DUSP1 in human subjects. To this end, we performed IF staining analysis to investigate the localization of DUSP1 in SAT using confocal microscopy. As shown in Fig. 4A, our results confirmed the increased expression of DUSP1 in obese subjects compared with lean subjects, and this was consistent with the IHC data (Fig. 1C). Unexpectedly, these results revealed a nuclear and extranuclear localization of DUSP1 in the SAT (Fig. 4A). In PBMCs, we investigated the expression of DUSP1 by Western blot using nuclear and cytoplasmic fractions, and we found that DUSP1 was expressed in both compartments (data not shown).

Circulating levels of DUSP1 protein are higher in obese subjects. It has been shown recently that circulating DUSP1 protein can independently predict the risk for postoperative morbidities after coronary bypass grafting (31). On the other hand, based on our finding that indicated a nuclear and extranuclear localization of DUSP1 (Fig. 4A), we postulated that this extranuclear localization might play a direct or indirect role in its release into the circulation. For this purpose, we measured the plasma levels of DUSP1 protein in lean and obese subjects from cohort study 2 using ELISA method. As shown in Fig. 4B, there was a significant increase in the release of DUSP1 protein in obese subjects compared with lean subjects ($P = 0.006$).

Correlation analysis of DUSP1 levels with physical, clinical, and biochemical parameters. To understand the possible relationships between increased expression of DUSP1 and the baseline characteristics of our study population, we applied Spearman’s rank test to correlate between circulating levels of DUSP1 and the physical, clinical, and biochemical parameters of the participating subjects from cohort study 2. Results shown in Table 3 indicate a positive correlation between the levels of DUSP1 protein and the BMI ($P = 0.003$), waist and hip circumferences ($P = 0.03$ and $P = 0.01$, respectively), and PBF ($P = 0.003$) and a negative correlation with $\dot{V}O_{2\text{max}}$ ($P = 0.039$) and the HDL levels ($P = 0.04$). Other positive correlations were found between DUSP1 protein and the glucose levels ($P = 0.014$), TG ($P = 0.009$), glucagon ($P = 0.016$), insulin and leptin ($P < 0.0001$ and $P = 0.005$, respectively), and PAI-1 ($P = 0.026$). No other correlations were found between circulating DUSP1 protein and the remaining parameters measured. Interestingly, most of these correlations were confirmed at the level of DUSP1 mRNA (Table 4).

We next performed a logistical regression analysis showing that, out of these correlations, the BMI was significantly
associated with the least square means of DUSP1 and leptin proteins after adjustment for age and sex (Table 5). Indeed, DUSP1 and leptin had higher odds of having higher BMI ($P = 0.0272$ and $P = 0.0055$, respectively; Table 5). Furthermore, when adjusted for age, sex, and leptin, the least square mean of DUSP1 protein remained significantly higher in obese compared with lean subjects ($P = 0.037$; Table 6).

**Physical exercise reduced DUSP1 and increased PGC-1α expression levels.** Physical exercise as a nonpharmacological intervention is known for its anti-inflammatory and anti-stress properties (28). We tested whether it has modulating effects on the expression of DUSP1 in obese subjects with concomitant improvement of the outcomes. Previously, we reported the effectiveness of our physical exercise protocol on improving the physical, clinical, and metabolic parameters on obese subjects (2). Accordingly, although there was no significant change in the BMI or waist and hip circumferences after 3 mo of moderate exercise, there was a significant reduction of PBF and SBP and an increase in $V_{O_2\text{max}}$ along with a decrease in TBARS levels (9). To investigate whether physical exercise has an impact on the endogenous expression of DUSP1, qRT-PCR, Western

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**Fig. 2. MAPK phosphorylation levels in PBMCs and adipose tissue.** A: total proteins were extracted from PBMCs of lean and obese participants and subjected to Western blot using the indicated antibodies. Fold change of each of the phosphorylated MAPKs was calculated after normalization with the corresponding total MAPK, and the data are presented as a ratio of phospho-MAPK/total MAPK. An arbitrary value of 1 was used for lean subjects. The blots shown are representative of 3 experiments with consistent results. B: IHC staining using SAT biopsies from lean and obese subjects using phospho-MAPK-specific antibodies. Aperio software was used to quantify positive staining, as detailed in MATERIALS AND METHODS, and data are presented as fold of changes. For each experiment, the sample size for each group is indicated by $n$. $P$ value was determined using Mann-Whitney test.
DISCUSSION

In the present study, we investigated whether obesity triggers an aberrant expression of DUSP1 in human subjects, and if so, does physical exercise restore its normal expression? The main findings of the study are that 1) the expression levels of DUSP1 mRNA and protein were significantly increased in obese subjects both in PBMCs and SAT, 2) there was a release of DUSP1 protein in the plasma that was more pronounced in obese subjects both in PBMCs and SAT, 3) 3 mo of physical exercise was sufficient to restore the normal expression of DUSP1 in obese subjects with a concomitant improvement of clinical outcomes and, 4) DUSP1 levels were inversely proportional to p-p38 MAPK and PGC-1α levels. Our findings demonstrating for the first time increased expression of DUSP1 in obese humans add further evidence that the MAPK signaling pathway plays an important role in the pathophysiology of obesity.

There is considerable interest in studying the role and implication of protein phosphatases in metabolic diseases as they start to emerge as key therapeutic targets for obesity and insulin resistance (6). For instance, PTP1B is one of the phosphatases that was initially linked to obesity, and its role in metabolic homeostasis was confirmed both in vitro and vivo (69). Likewise, a recent study on MKP-3 (DUSP6)-deficient animals demonstrated its role in diet-induced obesity (21). DUSP1 is another target candidate that elicited great interest by virtue of its dual specificity toward serine/threonine and tyrosine residues and hence, its large spectrum effect on its downstream targets (59). Similar to PTP1B and DUSP6, the effect of DUSP1 on obesity and metabolic homeostasis was clearly established in animal models of high-fat diet-induced obesity using DUSP1-deficient mice (67). Accordingly, these animals were protected from diet-induced obesity; however, they were still vulnerable to glucose intolerance and hyperinsulinemia caused by high-fat feeding (67). These findings indicate that DUSP1 may regulate body mass independently of the regulation of glucose homeostasis (67). In agreement with this study, we provide in the current investigation evidence that DUSP1 expression was increased in obese humans at the mRNA and protein levels and in both PBMCs and SAT, with a significant increase in DUSP1 protein in the systemic circulation. Recently, a large cohort study has associated severe obesity with epigenetic modification of DUSP1, although the impact of such modification on the expression and/or activity of DUSP1 remains to be elucidated (30).

There are 25 DUSPs that have been reported so far (36), out of which 10 DUSPs were classified as typical MAPK phosphatases (i.e., having the kinase-interacting motif) that act as direct upstream regulators of various MAPKs in response to both physiological and pathological stimuli (14). However, this regulation is context, duration, magnitude, and spatiotemporal dependent (14). In the case of DUSP1, early studies demonstrated preferential regulation of the all three MAPKs by DUSP1 in the following order, p38 > JNK > ERK (25, 29), but subsequently, these MAPKs were shown to be dephosphorylated to the same extent by DUSP1 (11). It is generally accepted that DUSP1 is a critical node in MAPK signaling because it has the capacity to dephosphorylate all MAPKs and
serves as channel through which positive and negative signals are transmitted to control different processes required to maintain cellular homeostasis (46, 50). One of the prominent questions that came out of this study was to evaluate the functional consequence of increased DUSP1 expression on the activity of its downstream targets, namely p38 MAPK, JNK, and ERK. It is worth mentioning one previous study that compared the expression/activation of various MAPKs between omental adipose tissue and SAT in severely obese women (8). In this study, the authors showed clearly a significant increase in the protein and mRNA levels of p38 MAPK, JNK1, ERK2, and IKKβ/H9252 in omental adipose tissue vs. SAT (between 1.5- and 2.5-fold increase). They also reported a similar increase in the p-p38 MAPK, p-JNK1, and p-IKKβ in omental vs. SAT. More importantly, they added a lean women group to their study and compared the levels of these kinases as well as their phosphorylated forms. They did not detect any difference in ERK2 and IKKβ expression and phosphorylation in either omental or SAT between the two groups; however, the levels of p38 MAPK, JNK1, and their phosphorylated forms were elevated specifically in omental adipose tissue. In the SAT, the levels of p38 MAPK, JNK1, and their phosphorylated forms remained comparable with the lean group (8). They concluded that obesity-induced stress response is more pronounced in the omental tissue than the SAT. They suspected that these expression differences could be due to the intrinsic heterogeneity between the two fat depots (8). In our context, we used SAT and PBMCs, and in both cases, whereas the levels of p-JNK and p-ERK were increased significantly in obese subjects (Fig. 2), the p38 MAPK pathway was impaired as assessed by decreased levels of p-p38 MAPK (Fig. 2). There are many possible explanations for the discrepancy between the study by Bashan et al. (8) and our study. First, in our study we had access only to the SAT, and thus our data are not directly comparable with the Bashan et al. (8) study. Second, we used mixed sexes of nondiabetic lean and obese subjects (average BMI for obese ≈34.8 kg/m²), and the average age of our study population was ≈39 yr for lean and ≈40 yr for obese. In the Bashan et al. (8) study, the average age was ≈50 yr, they were all severely obese women, and some were diabetics (average BMI >41 kg/m²). Third, it is possible that aging also has an effect on the physiology of the two fat depots and. And fourth, it is also possible that the SAT behave differently according to the BMI.
Our data that showed that reduced levels of p-p38 MAPK are consistent with a recent study reported by another group (33). They demonstrated that Shp2, another nonreceptor tyrosine phosphatase, acts by promoting adipogenesis through inhibition of p38 MAPK phosphorylation (33). They also showed a decrease in the levels of p-p38 MAPK both in a mouse model of HFD-induced obesity and in obese humans both in the adipose tissue and isolated adipocytes (33), highlighting the critical role of p38 MAPK in adipogenesis. By measuring the levels of p-p38 and p38 MAPK according to the BMI, these authors found a negative correlation between the relative p-p38/p38 MAPK and the BMI. Likewise, the pattern that we obtained on p-JNK in our study population is also in agreement with another study carried out on healthy individuals (BMI = 25 ± 4 kg/m²), and those authors found increased levels of p-JNK in the SAT that was linked to insulin resistance (64).

Another question that was raised from this study was based on the observation that both JNK and ERK were activated in obese subjects, whereas p-38 MAPK was inactivated. In this context, how does DUSP1 select its substrates? In addition, our results showing a parallel increase in the levels of DUSP1 along with increased activity of the JNK and ERK pathways are inconsistent with the role of DUSP1 as archetypical member of DUSPs family and need further investigation. DUSPs are characterized by different subcellular localization (nuclear, cytosolic, or dual location). For DUSP1, it was classified initially as a nuclear protein (68), but subsequent studies reported its localization in the cytoplasm and mitochondria (10, 45, 58). As a note of caution, the molecular mechanism by which DUSP1 is selecting and controlling the activity of its downstream targets and their pertinence to obesity is still poorly elucidated (19, 61, 67). More specifically, the role of the MAPK signaling in obesity is still not yet completely understood. For example, the activation of JNK is considered to be one of the critical events that lead to insulin resistance and diabetes (34), but its role in adipogenesis needs to be deciphered (12). Likewise, the inhibition of p38 MAPK can interfere or promote adipogenesis (5, 20). In the SAT, confocal immunofluorescence results confirmed the nuclear localization of DUSP1, but more importantly, these results revealed the extranuclear localization of DUSP1 in the SAT. Because DUSP1 is known to dephosphorylate JNK in the nucleus, one possible explanation of our results is that DUSP1 is inactive in the nucleus. In our study, we did not investigate the status of DUSP1 activity in our study population, and this represents one of the limitations of the current investigation. Alternatively, it is possible that obesity triggers an excessive activation of the upstream regulators of JNK, such as MKK proteins that need to be unraveled in future followup studies.

Modulation of PGC-1α expression, a key factor involved in the regulation of energy expenditure, calorie restriction, and metabolism, was also investigated in this study to confirm our data, as its induction is attributed in large part to the activation of p38 MAPK (3, 4, 22). In the white adipose tissue of obese, insulin-resistant, and diabetic subjects, the expression of PGC-1α is reduced (32, 52, 62). In contrast to the adipose tissue, PGC-1α is highly expressed in human skeletal muscle, and this expression is further enhanced by exercise in part through activation of the p38 MAPK signaling pathway (66). In agreement with

![Table 3. Correlation between circulating DUSP1 protein and physical, clinical, and biochemical parameters](image)

<table>
<thead>
<tr>
<th>Marker</th>
<th>r²</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>0.19</td>
<td>0.128</td>
</tr>
<tr>
<td>BMI</td>
<td>0.35</td>
<td>0.003</td>
</tr>
<tr>
<td>Waist</td>
<td>0.27</td>
<td>0.030</td>
</tr>
<tr>
<td>Hip</td>
<td>0.31</td>
<td>0.010</td>
</tr>
<tr>
<td>PBF</td>
<td>0.35</td>
<td>0.003</td>
</tr>
<tr>
<td>V̇O2max</td>
<td>−0.22</td>
<td>0.039</td>
</tr>
<tr>
<td>Glucose</td>
<td>0.30</td>
<td>0.014</td>
</tr>
<tr>
<td>Hb A1c</td>
<td>0.14</td>
<td>0.259</td>
</tr>
<tr>
<td>Cholesterol</td>
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<td>0.521</td>
</tr>
<tr>
<td>TG</td>
<td>0.32</td>
<td>0.009</td>
</tr>
<tr>
<td>HDL</td>
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<td>0.042</td>
</tr>
<tr>
<td>LDL</td>
<td>0.13</td>
<td>0.303</td>
</tr>
<tr>
<td>C-peptide</td>
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<td>0.078</td>
</tr>
<tr>
<td>Glucagon</td>
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<td>0.016</td>
</tr>
<tr>
<td>GLP-1</td>
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<td>0.203</td>
</tr>
<tr>
<td>Insulin</td>
<td>0.45</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Leptin</td>
<td>0.33</td>
<td>0.005</td>
</tr>
<tr>
<td>PAI-1</td>
<td>0.26</td>
<td>0.026</td>
</tr>
<tr>
<td>IP-10</td>
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<td>0.981</td>
</tr>
<tr>
<td>RANTES</td>
<td>0.81</td>
<td>0.207</td>
</tr>
</tbody>
</table>

The correlation was assessed by Spearman’s rank correlation coefficient and based on the ΔACt method using lean (n = 14) and obese (n = 17) subjects from cohort study 1.

![Table 4. Correlation between DUSP1 mRNA expression and physical, clinical, and biochemical parameters at baseline](image)

<table>
<thead>
<tr>
<th>Marker</th>
<th>r²</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI</td>
<td>0.65</td>
<td>0.0002</td>
</tr>
<tr>
<td>Waist</td>
<td>0.61</td>
<td>0.001</td>
</tr>
<tr>
<td>Hip</td>
<td>0.64</td>
<td>0.001</td>
</tr>
<tr>
<td>PBF</td>
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<tr>
<td>SBP</td>
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</tr>
<tr>
<td>DBP</td>
<td>0.55</td>
<td>0.047</td>
</tr>
<tr>
<td>TG</td>
<td>0.35</td>
<td>0.046</td>
</tr>
<tr>
<td>C-peptide</td>
<td>0.40</td>
<td>0.033</td>
</tr>
</tbody>
</table>

![Table 5. Logistical regression model for BMI association](image)

<table>
<thead>
<tr>
<th>Marker</th>
<th>OR (95% CI)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Circulating DUSP1</td>
<td>2.831 (1.125–7.125)</td>
<td>0.0272</td>
</tr>
<tr>
<td>Circulating leptin</td>
<td>6.676 (1.745–25.533)</td>
<td>0.0055</td>
</tr>
</tbody>
</table>

CI, confidence interval. *Data were adjusted for age and sex difference. Analysis was done on all of the subjects from cohort study 2.
these studies, we showed reduced expression of PGC-1α mRNA and protein in the SAT in obese subjects, which was concomitant with increased expression of DUSP1 and impairment of the p38 MAPK signaling pathway. Our results also corroborate a recent finding showing that excess dietary fat enhances DUSP1 expression in muscle and promotes loss of the oxidative myofibers by inhibiting p38 MAPK and indirectly suppressing PGC-1α (60).

One of the interesting findings of our investigation is the localization of DUSP1 in the SAT and its possible impact on the release of DUSP1. Our confocal immunofluorescence data (Fig. 4A) indicate a positive staining corresponding to DUSP1 inside and outside the nucleus membrane, and it was more pronounced in obese subjects. This could presumably explain the presence of DUSP1 in the blood, as it might represent a critical step that leads to its release into the circulation. This finding prompted us to postulate whether DUSP1 is released or not into the circulation, and ELISA data confirmed the existence of DUSP1 protein in the plasma that was much higher in obese subjects than in lean subjects (Fig. 4B). In a previous study, it has been shown that circulating DUSP1 protein can independently predict the risk for postoperative morbidities after coronary bypass grafting (31). In our study population, logistical regression analysis showed a highly significant asso-

Fig. 6. Physical exercise modulated p-JNK and p-ERK but not p-p38 MAPK in obese subjects. A: IHC staining using SAT biopsies from obese participants before and after exercise. Aperio software was used to quantify positive staining as detailed in MATERIALS AND METHODS. B: p38 MAPK phosphorylation levels using Western blot and PBMCs from obese participants before and after exercise. The bands were quantified as described in MATERIALS AND METHODS. Quantified data are presented as fold of change. For each experiment, the sample size for each group is indicated by n. P value was determined using paired t-test.
cation of the BMI with the least square means of DUSP1 and leptin proteins, as they had higher odds of having higher BMI (Tables 5 and 6).

Of particular interest is that our study emphasized the role of physical exercise in modulating the expression of DUSP1. It is well established that exercise reduces the risk of chronic metabolic and cardiorespiratory diseases by modulating the inflammatory and stress responses (28, 49, 57) in part by reduction in the visceral fat mass (39). Our data showed for the first time that physical exercise significantly reduced the expression of DUSP1 in the adipose tissue in a manner that was concomitant with decreased PBF and improved cardiopulmonary performance as monitored by decreased SBP and increased \( \dot{V}O_2_{\text{max}} \). The reduced expression of DUSP1 by physical exercise was also consistent with attenuated inflammatory response, as indicated by a decrease in the endogenous levels of TNF\( \alpha \), IL-6, and RANTES in the SAT (9). Other strategies used to manage obesity and its related complications were associated with a downregulation of DUSP1 in mice and humans. They included bariatric surgery in obese patients (18) and intake of antiobesity natural extract from peucedanum japonicum thumb in obese diabetic mice (48). Reduced levels of DUSP1 have also been observed in patients with metabolic syndrome following intake of phenol-rich virgin olive oil (13).

Physical exercise is also known to act differentially on MAPKs. Indeed, it was shown that exercise inhibits metabolic stress in part by dephosphorylating and thus inactivating JNK (17, 43, 51), whereas it induces p38 MAPK and ERK activities as well as the expression of PGC-1\( \alpha \) (27, 71). In our case, although the effect of physical exercise on the increase of PGC-1\( \alpha \) was highly significant, it had only a marginal effect on p-p38 MAPK. In our study population, the exercise effect was also associated with decreased levels of TBARS and increased levels of ROS in the circulation, and this was consistent with other studies (1, 70). Given the lack of a drastic effect of physical exercise on p-p38 MAPK, the increase in PGC-1\( \alpha \) levels after physical exercise could be attributed to the increase of ROS, as reported previously (22, 66).

However, the current study has some limitations that deserve considerations. For instance, what is the status of DUSP1 activity in obese subjects? What are the molecular mechanisms leading to the overexpression of DUSP1 in obese humans and its attenuation by physical exercise? Our data suggest that p38 MAPK is a bridge between DUSP1 and PGC-1\( \alpha \), but the lack of a strong effect on physical exercise on the activation p38 MAPK does not support this suggestion. What is the role, if any, of secreted DUSP1, and how is this role achieved? However, despite these limitations, we provided ample evidence that the endogenous and circulating levels of DUSP1 are higher in obese humans and that there was a differential effect of DUSP1 on its downstream targets. We also showed that physical exercise significantly reduced the levels of DUSP1 mRNA and protein in the SAT. These findings add further evidence to the importance of physical exercise in attenuating metabolic stress linked to metabolism.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

REFERENCES


