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Growth and differentiation factor 15 is a biomarker for low back pain-associated disability

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ABSTRACT

The development of low back pain (LBP) is often associated with obesity and sarcopenia. However, the mechanisms of this association remain unclear. To clarify this, we measured circulating levels of a selected panel of soluble factors, presumably involved in obesity and sarcopenia pathogenesis, and correlated them with several LBP-related characteristics, taking into account body composition and other relevant covariates. In the cross-sectional study of 1078 individuals, we collected data on self-reported LBP, body composition (including fat and skeletal muscle mass) assessed by the bioimpedance method and anthropometrically, and measured plasma levels of several cytokines by ELISA. In the statistical analysis, we took into account familial composition of the sample and possible putative genetic effects. We report that LBP-affected individuals were significantly older, with increased obesity and decreased skeletal mass, respectively, compared with the non-affected group. In univariate analyses, plasma concentrations of Growth and differentiation factor 15 (GDF-15), leptin, chemerin and follistatin were found significantly elevated in the LBP-affected groups (with or without sciatic pain) and were highly significantly ($p < 0.001$) associated with other LBP-related phenotypes, specifically, disease duration, disability and physician consults. However, after adjustment for one another, age, sex, body composition and putative genetic factors, the only associations between GDF-15 and LBP disability and medical consulting phenotypes, remained significant. In conclusion, we report for the first time, a significant and independent association between plasma GDF-15 concentrations and LBP-associated disability. Longitudinal studies are needed to determine whether GDF-15 could be a novel therapeutic target for prevention and/or treatment of LBP.

1. Introduction

LBP is a condition affecting most people at some point throughout their life, with consistently increasing prevalence and disability during the past 25 years and will likely increase further with population aging [1]. However, the pathogenesis of this multifactorial condition remains not fully understood. Numerous studies, including our own, suggested the presence of a major genetic component underlying an inter-individual variation of LBP, with heritability estimates often > 30% [2–5]. However, evidence as to the molecular-genetic mechanisms underlying LBP manifestation is limited. Furthermore, the interaction of genetic factors with other intrinsic factors, such as body composition features (fat and skeletal muscle mass) is also not sufficiently investigated. It was shown that fat mass and distribution are associated with LBP intensity and disability, assuming that systemic metabolic factors associated with adiposity play a major role in LBP pathogenesis [6]. However, the others affirm that adjusting for the possible effects of genetic and early-shared environmental factors diminishes significance of associations between obesity and LBP.

This controversy is aggravated by the issue of virtual lack of reliable biomarkers associated with LBP pathogenesis, potentially reflecting underlying molecular mechanisms of the condition. On the other hand, accumulating evidence suggest that two key processes, sarcopenia and adipose tissue dysregulation may play a significant role in LBP pathogenesis. For example, decreased muscle size and radiographic density along with increased muscle-fat infiltration in the multifidus and paraspinal muscles have been detected in LBP patients [7–9]. Moreover, fatty infiltration develops in the areas where most intervertebral disk

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(IVD) degenerative changes occur and the degree of fatty infiltration in the lumbar area multifidus associated with LBP, especially in women, independently of body composition [10]. Fatty acids and their derivatives in the muscles, in turn cause lipotoxic effects, mitochondrial dysfunction and muscle inflammation, thus enhancing sarcopenia [11]. In addition, over-release of free fatty acids and adipokines affect bone metabolism. Considering these data, it is plausible to assume that potential LBP biomarkers might be the biochemical factors involved in muscle, fat and bone pathophysiology.

Recent studies suggest that growth and differentiation factor 15 (GDF-15) and an autocrine glycoprotein follistatin possess the inhibitory and promotional muscle/bone growth activity, respectively [12,13]. Several adipokines, in particular adiponectin and leptin (well-known fat metabolism agonist and antagonist, respectively), and chemerin, in addition to their involvement in inflammation, adipogenesis and regulation of glucose and lipid homeostasis [14], may also affect muscle and bone metabolism [15,16]. However, the attempts to use adiponectin and leptin as LBP biomarkers revealed controversial results [17–19]. To our knowledge, no studies have explored GDF-15, follistatin and chemerin in relation to LBP.

2. Patients and methods

2.1. Sample

The population examined was from an ethnically Arab population in Israel, which is characterized by a stable family structure with traditional relationships, shared similar living, economic and professional conditions, in addition having access to modern medical facilities. The data were collected from 01/2014 until 01/2016. Assessment of the families was performed according to our detailed protocol and conducted by certified and experienced nurses. The research was approved by the International Review Board -Helsinki Committee (Helsinki number 042/2013k), Meir Medical Center, Kfar Saba, Israel and the Ethics Committee of Tel Aviv University. Each individual signed an informed consent form written in Arabic before participation and data collection.

The families were selected via probands suffering from LBP confirmed by an orthopedist (with onset at < 50 years of age) and at least one first degree relative diagnosed with a similar condition. Our data were collected from 1078 individuals belonging to 28 complex families, encompassed 99 nuclear families, with 1 to 11 siblings per family. However, twenty-six of the recruited individuals were excluded due to severe heart problems or pregnancy and were < 17 years of age. Elderly people (> 80 years old) were not included due to their physical inability. Participants attended an assessment that included a nurse led interview and a number of clinical and laboratory tests. As part of the study, all individuals participating in the study, regardless of their LBP status, completed a standardized questionnaire detailing their lifetime history of LBP at their visits to the clinic. The questionnaire followed the format used in the Medical Research Council (MRC) Nurses' Study [20], which has been widely used in other contemporary population studies of LBP. The questionnaire was translated into Arabic for the present study and was validated.

2.2. LBP-phenotypes

The definition of LBP was illustrated on a mannequin and located between the 12th rib and the gluteal folds. The following characteristics of LBP were examined: (1) LBP-defined as a binary phenotype (e.g. 1 = affected *vs* 0 = non-affected), when the patient answered the question "*During the previous year, did you have LBP that continued for > 1 day?*"; (2) Age-dependent continuous phenotype LBP_{AGE} was defined by response to: "*How old were you when your first back pain appeared and lasted more than a day*". Similarly, (3) sciatic pain (SCP) was defined by answer to: "*Has your back pain ever spread to your legs" (yes = 1 vs. no = 0)*, and (4) SCP_{AGE} was defined by positive answer to: "*If your pain had spread down your leg(s) or below the knee(s), how old were you when this first happened?*"; (5) Duration of the disease: $LBP_{DURATION}$ was defined as "the duration of pain during the past 10 years (Gr1 – up to 12 months, and Gr2 ≥ 12 months); (6) Disability associated with the disease, $LBP_{DISABLE}$ was defined as pain associated with disability lasting > 1 day during the previous 12 months, including eight statements specifically related to physical functions. Disability was defined as an impairment resulting in restrictions in any of the following activities: walking around the house, standing for 15 min, getting up from a low chair, getting out of the bathtub, getting in and out of a car, going up and down stairs, putting on socks and/or tights and cutting one's toenails. The individuals were divided in three groups: (A) $LBP_{NOT\text{-}DIFFICULT}$, average score = "0", (B) $LBP_{DIFFICULT}$, $0 > LBP_{DISABLE} \leq 1$" (C) $LBP_{IMPOSSIBLE}$, average score > 1"; (7) Consulting the doctor about the pain, $LBP_{CONSULT}$ phenotype was defined according to answer to "*During the past 12 months, have you consulted the doctor about your back pain?* (Yes/No)".

2.3. LBP-covariates

Potential confounding variables were recorded at the interview, including data relating to age, gender, anthropometric measurements, and body composition. Anthropometric measurements were taken according to the World Health Organization recommendations and included body weight in kg (WT), height in cm (HT), waist in mm (WC), hip circumference in mm (HC), body mass index (BMI) in kg/m^2 and waist to hip ratio (WHR). Body composition was assessed by the bioimpedance (BIA) method using the BIA101 device (https://www.smt-medical.com/en/products/akernbioimpedance-measuring-devices/bia-101.html), which calculates body fat as function of sex, height, weight and electrical impedance [21]. The rationale for employing this measurement is that the BIA accurately measures the amount of fat mass and fat free mass in the human body [22] and is safe for patients equipped with implantable cardioverter-defibrillators [23]. Our study focused on relative fat mass (FM/WT) and skeletal muscle mass (SMM/WT). We also examined the body cell mass (BCM/WT), one of the basic measurements evaluated by BIA and because of its fundamental physiological significance [24].

2.4. Soluble biomarker analysis

Venous blood samples obtained by venipuncture from overnight-fasting individuals underwent centrifugation within 1 h after collection according to the standard protocol. Plasma samples were separated and stored in aliquots at −80 °C until usage. Following our working hypothesis presented in Fig. 1, circulating levels of five soluble markers were detected by ELISA using the DuoSet kits (R&D systems, Minneapolis, MN, USA). The detection limits were as follows: 7.8 pg/ml for GDF-15, 46.9 pg/ml for follistatin, 16.7 pg/ml for chemerin, 31.2 pg/ml for leptin, and 62.5 pg/ml for adiponectin. The intra- and inter-assay coefficients of variation were between 2.3 and 8.1%. Due to the significant deviation of the respective distribution from the normality assumptions, the original GDF-15 and leptin measurements were subjected to log-normal transformation to approximate normality prior to analysis.

2.5. Statistical analysis

Preliminary statistical analysis using the STATISTICA 7.0 package for Windows (Statsoft, Inc., USA) included basic descriptive statistics, testing for normality of the distributions of the study quantitative variables, identification of the outliers and selection of the potential covariates for LBP, such as sex, age, body composition phenotypes, etc. For each quantitative trait measurement, we calculated the mean and standard errors. All quantitative variables were compared between

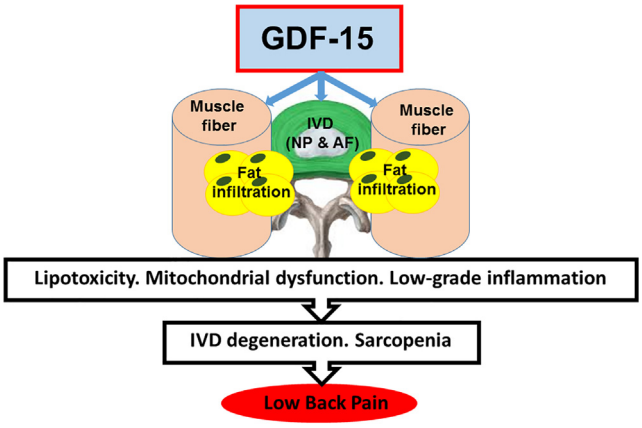

Fig. 1. Proposed hypothetical mechanisms of GDF-15 participation in the development of LBP. The diagram suggests that LBP development is associated with two key processes – IVD degeneration and sarcopenia of lumbar multifidus and paraspinal muscles; the latter is induced mainly by lipotoxicity provided by ectopic adipose infiltration in both muscles and bone. Ectopic fat accumulation in skeletal muscle induces a recruitment of various immune cells capable of producing pro-inflammatory cytokines, thus establishing and supporting both local (muscle/bone) and systemic low-grade inflammation also promoting sarcopenia and, presumably, IVD degeneration. We suggest that these events can be provided by GDF-15, shown to reduce food intake and BMI, and improve metabolic profile [26,32,33,35,49,50]. Described in the present study independent association of elevated GDF-15 circulating levels with detrimental LBP phenotypes confirms this assumption. Abbreviations: BMI, body mass index; IVD, intervertebral disk; NP, nucleus pulposus; AF, annulus fibrosus.

dichotomy of LBP phenotypes, using a standard one-way analysis of variance. The affected groups were defined according to the LBP-related phenotype (see above), which was always compared to the group of control, unaffected individuals. Once the potential risk variables were identified, their simultaneous association with the dichotomous LBP phenotype was examined using a multiple logistic regression analysis.

2.6. Statistical-genetic analysis

Once the association between the LBP phenotypes and above covariates was detected, to take into account familial composition of the sample and possible putative genetic effects, we conducted quasi-variance component analysis [5]. This analysis is based on Falconer's concept of heritability of threshold characters [25], which assumes the existence of the unobserved normally distributed in the random population liability to the disease (binary condition), caused by independent effects of the additive genetic factors and environmental covariates. The liability determines the threshold above, which individuals considered affected. The implemented analysis simultaneously estimates the extent of association of the covariates with LBP-related phenotypes, controlling for the genetic effects.

3. Results

3.1. Sample characteristics

Demographic characteristics and basic descriptive statistics of the study population are summarized in Table S1 (supplementary material). The study included 489 males and 589 females, with non-significant ($p = 0.68$) age differences between men (42.80 ± 13.90) and women (43.20 ± 13.70). In 556 LBP-complaining participants, the higher prevalence was observed in women sample (56.0% *vs* 46.0%, $p < 0.0001$). However, the average age of LBP onset was similar in men and women (33.78 ± 11.0 years *vs* 34.67 ± 11.2, $p = 0.35$). Likewise, there was no sex difference in the age of SCP onset (40.30 ± 12.14 years *vs* 38.44 ± 11.51, in men and women, respectively, $p = 0.104$). However, SCP was more prevalent among women (267, 45.3%) in comparison to men (180, 36.8%), $p = 0.036$, one-sided *t*-test. Prevalence of $LBP_{DURATION} \geq 12$ months (G2 phenotype) was significantly more common in women (38%, 222/589) in comparison to men (32%, 155/489), $p = 0.008$, one-sided *t*-test. In the total sample, 351 individuals (32.5%) have been consulted by a physician during the past 12 months ($LBP_{CONSULT}$ phenotype).

The ranges of variation of the body composition measures and levels of the circulating biochemical factors are shown in Table S2. Their variations were not fully independent (Table S3). Significant correlations were observed between the obesity measures (BMI, WHR and FM/WT) and all soluble factors except adiponectin. The latter demonstrated a weak but significant ($p = 0.01$) negative correlation with BMI. These trends were similar in men and women. Notably, the levels of leptin, chemerin and GDF-15 disclosed the strongest positive correlations with the obesity parameters. Skeletal muscle mass phenotypes (SMM/WT and BCM/WT) displayed significant negative correlations with leptin, chemerin and GDF-15 levels ($R = -0.30$–0.69, $p < 0.01$), the correlation of follistatin levels with body composition phenotypes were weak, and adiponectin did not correlate with SMM/WT.

Table 1
Body composition parameters and plasma levels of soluble markers in individuals with low back pain (LBP) only or with LBP combined with sciatic pain (SCP), compared with control subjects.

Characteristics	Control (N = 522)	LBP (N = 556)	P_1	SCP (N = 447)	P_2
Age (y)	39.58 ± 0.595	46.25 ± 0.558	5	47.41 ± 0.612	2
BMI (kg/m^2)	27.32 ± 0.232	28.99 ± 0.225	5	29.35 ± 0.256	5
WHR	0.89 ± 0.003	0.91 ± 0.003	5	0.92 ± 0.004	5
FM/WT	0.30 ± 0.003	0.33 ± 0.003	5	0.33 ± 0.004	5
SMM/WT	0.33 ± 0.002	0.31 ± 0.002	5	0.31 ± 0.003	5
BCM/WT	0.36 ± 0.002	0.34 ± 0.003	5	0.33 ± 0.003	5
GDF-15*	2.58 ± 0.009	2.65 ± 0.009	5	2.66 ± 0.010	5
Leptin*	9.51 ± 0.044	9.76 ± 0.044	5	9.79 ± 0.050	5
Chemerin (ng/ml)	87.08 ± 1.175	91.67 ± 1.258	5	93.46 ± 1.416	4
Follistatin (pg/ml)	498.85 ± 17.455	582.83 ± 18.428	4	581.46 ± 19.584	3
Adiponectin (μg/ml)	3.87 ± 0.072	4.05 ± 0.075	NS	4.07 ± 0.821	NS

Data presented as mean, stranded error; N, sample size; LBP, low back pain; SCP, sciatic pain; Age, in years; BMI, body mass index; WHR, waist-hip-ratio; FM/WT, fat mass/weight ratio; SMM/WT, skeletal muscle mass/weight ratio; BCM/WT, body cell mass/weight ratio; P_1 and P_2 show significance levels achieved upon comparison control individuals with LPB- and SCP-affected individuals respectively: 1 ≤ 0.05; 2 ≤ 0.01; 3 ≤ 0.001; 4 ≤ 0.0001; 5 ≤ 0.00001; NS, non-significant.

* The original measurements were in pg/ml (see Supplemental Table S2). Due to the significant deviation of the respective distributions from the normality assumptions, the original measurements were subjected to log-normal transformation to approximate normality prior to analysis.

Table 2
Body composition parameters and plasma levels of soluble markers (covariates) in individuals with different LBP-related phenotypes compared with control subjects.

Covariate	$LBP_{DURATION}$				$LBP_{DISABLE}$				$LBP_{CONSULT}$			
	GR-1 (N,133)	P	GR-2 (N,377)	P#	Difficult (N,240)	P	Impossible (N,140)	P#	No (N,204)	P	Yes (N,351)	P
Age	40.58 ± 1.086	NS	49.40 ± 0.639	2	44.03 ± 0.818	5	53.15 ± 1.064	2	44.18 ± 0.894	5	47.40 ± 0.708	5
BMI	27.79 ± 0.220	NS	29.65 ± 0.423	5	28.64 ± 0.327	3	31.28 ± 0.472	5	28.34 ± 0.356	1	29.45 ± 0.287	5
WHR	0.89 ± 0.007	NS	0.92 ± 0.004	5	0.90 ± 0.005	3	0.97 ± 0.008	5	0.90 ± 0.005	1	0.92 ± 0.005	5
FM/WT	0.31 ± 0.007	NS	0.33 ± 0.004	5	0.33 ± 0.005	5	0.35 ± 0.008	5	0.32 ± 0.005	4	0.33 ± 0.005	5
SMM/WT	0.32 ± 0.005	NS	0.30 ± 0.003	5	0.31 ± 0.004	5	0.29 ± 0.005	5	0.31 ± 0.004	4	0.31 ± 0.003	5
BCM/WT	0.35 ± 0.006	NS	0.33 ± 0.003	5	0.34 ± 0.004	5	0.31 ± 0.006	5	0.34 ± 0.004	5	0.33 ± 0.004	5
GDF-15*	2.60 ± 0.017	NS	2.68 ± 0.011	5	2.57 ± 0.010	NS	2.83 ± 0.019	5	2.59 ± 0.013	NS	2.68 ± 0.012	5
Leptin*	9.63 ± 0.917	NS	9.82 ± 0.532	5	9.75 ± 0.066	3	9.98 ± 0.086	5	9.70 ± 0.067	1	9.80 ± 0.057	5
Chemerin	87.71 ± 2.523	NS	94.26 ± 1.501	5	86.14 ± 1.826	NS	105.63 ± 2.486	5	87.68 ± 1.972	NS	95.74 ± 1.612	5
Follistatin	613.8 ± 43.062	3	589.9 ± 21.609	4	580.8 ± 29.381	2	645.7 ± 34.614	4	538.2 ± 26.971	NS	626.4 ± 24.465	5
Adiponectin	4.00 ± 0.150	NS	4.04 ± 0.921	NS	4.04 ± 0.116	NS	3.88 ± 0.135	NS	4.16 ± 0.121	NS	4.00 ± 0.095	NS

Data presented as mean, standard errors; N, sample size; Age, in years; BMI, body mass index; WHR, waist-hip-ratio; FM/WT, fat mass/weight ratio; SMM/WT, skeletal muscle mass/weight ratio; BCM/WT, body cell mass/weight ratio; P# show significance levels achieved upon comparison of each LBP-related phenotype with control group; GR-1, duration between 1 and 12 months; GR-2, duration ≥12 months; 1 ≤ 0.05; 2 ≤ 0.01; 3 ≤ 0.001; 4 ≤ 0.0001; 5 ≤ 0.00001; NS, non-significant.

3.2. Comparison between LBP-affected and control groups

Table 1 shows that LBP and SCP individuals were significantly older and heavier with significantly reduced skeletal muscle mass. The plasma concentrations of GDF-15, leptin, chemerin and follistatin were significantly elevated in LBP-affected group with no difference in adiponectin levels. Aggravation of LBP manifestations (GR-2 *vs* GR-1 in the $LBP_{DURATION}$ category, $LBP_{IMPOSSIBLE}$ *vs* $LBP_{DIFFICULT}$ in the $LBP_{DISABLE}$ category, and those who visited the doctor *vs* non-visited in the $LBP_{CONSULT}$ category) was accompanied by significantly enhanced levels of obesity measures and reduced levels of the skeletal muscle mass measures (Table 2). Notably, the highest and lowest obesity and skeletal muscle mass measures were registered in the $LBP_{IMPOSSIBLE}$ phenotype (all $p < 0.001$, Table 2). In addition, the worsening of all three studied LBP phenotypes was accompanied by a significant elevation in the plasma levels of GDF-15, leptin, chemerin and follistatin (but not adiponectin) (Table 2). Elevation in the plasma levels of all studied soluble markers (except adiponectin) was highest in the $LBP_{IMPOSSIBLE}$ phenotype as compared to other LBP phenotypes (all $p < 0.001$, Table 2).

A binary multiple regression analysis was implemented to test simultaneously the associations of LBP phenotypes with plasma levels of GDF-15, leptin, chemerin and follistatin in comparison with controls. Associations between GDF-15 levels and $LBP_{DISABLE}$ (Table 3) and $LBP_{CONSULT}$ (Table 4) phenotypes remained significant after adjustment for age and sex as well as after additional adjustment for obesity (WHR and FM/WT) and body cell mass (BCM/W) measures ($p < 0.001$ for $LBP_{DISABLE}$ and $p < 0.003$–0.004 for $LBP_{CONSULT}$ phenotypes). Association between GDF-15 and $LBP_{DURATION}$ phenotype was diminished after adjustment for age, sex and other covariates ($p > 0.05$, Table S4). Similarly, associations of leptin, chemerin and follistatin plasma levels with different LBP phenotypes attenuated after adjustment for age, sex and obesity and skeletal muscle measures ($p > 0.05$, Tables 3 and 4).

An association between GDF-15 and severe LBP phenotypes was assessed also by an analysis taking into account familial composition of the sample and possible putative genetic effects. We examined the extent of the GDF-15 association with $LBP_{DISABLE}$ and $LBP_{CONSULT}$ phenotypes in two separate quasi-variance component analyses [5], each of which took into account possible genetic effect on the inter-individual variation of the liability scores to LBP phenotype (Table 5). Additional covariates retained in these analyses were sex, age and WHR, the same variables that appeared as significant covariates in the above multiple logistic regression analyses (Tables 3 and 4). The results fully confirmed our conclusion: by likelihood ratio test, both LBP-related phenotypes were significantly ($p = 0.0001$–0.000007) associated with GDF-15 plasma levels, despite the adjustment for familial composition, putative genetic factors and other covariates.

4. Discussion

Although the pathogenesis of LBP remains poorly understood, accumulating evidence suggests its close association with obesity and sarcopenia of paraspinal muscles [3,4,8]. One of the major questions in our study was therefore whether we could detect a significant association between the LBP-related phenotypes and circulating levels of selected biochemical factors presumably involved in pathogenesis of obesity and sarcopenia. While in the univariate analyses all the selected biomarkers, except adiponectin, showed clear associations with LBP-phenotypes, only GDF-15 was consistently significant in a variety of multivariable analyses. Thus, we report here the novel finding that higher GDF-15 concentrations are significantly associated with the intense of LBP manifestations, independently of covariates such as age, sex and obesity and skeletal muscle measures. The results of association

Table 3
A binary multiple logistic regression analysis exploring relationships between soluble markers and $LBP_{DISABILE}$ phenotype.

Dependent variable: $LBP_{DISABLE}$ during past 12 months									
Independent variable	Model 1 adjusted for age, sex and WHR			Model 2 adjusted for age, sex, and FM/WT			Model 3 adjusted for age, sex, and BCM/WT		
	Odd ratio (95% CI)	SE	P	Odd ratio (95% CI)	SE	P	Odd ratio (95% CI)	SE	P
GDF-15	1.76(1.45–1.64)	0.09	< 0.001	1.74(1.43–2.11)	0.10	< 0.001	1.72(1.42–2.10)	0.10	< 0.001
Leptin	0.90(0.73–1.12)	0.10	0.38	0.97(0.78–1.20)	0.11	0.80	0.91(0.74–1.14)	0.11	0.44
Chemerin	1.21(0.96–1.51)	0.11	0.09	1.19(0.96–1.49)	0.11	0.10	1.19(0.95–1.50)	0.11	0.11
Follistatin	1.03(0.84–1.26)	0.10	0.77	1.02(0.83–1.26)	0.10	0.79	1.02(0.83–1.25)	0.10	0.81

Dependent variable: $LBP_{DISABLE}$ during past 12 months. Data reported as odds ratio with 95% confidence intervals [OR (95% CI)] with corresponding standard errors (SE) and P values. Variables were log-transformed to approximate normality prior to analysis; all variables were standardized prior analysis.

Table 4
A binary multiple logistic regression analysis exploring relationships between soluble markers and $LBP_{CONSULT}$ phenotype.

Dependent variable: $LBP_{CONSULT}$									
Independent variable	Model 1 adjusted for age, sex and WHR			Model 2 adjusted for age, sex, and FM/WT			Model 3 adjusted for age, sex, and BCM/WT		
	Odd ratio (95% CI)	SE	P	Odd ratio (95% CI)	SE	P	Odd ratio (95% CI)	SE	P
GDF-15	1.34(1.09–2.13)	0.10	0.004	1.36(1.11–1.67)	0.10	0.003	1.35(1.10–1.66)	0.10	0.004
Leptin	0.95(0.78–1.17)	0.10	0.67	1.03(0.85–1.25)	0.09	0.73	0.96(0.78–1.17)	0.10	0.70
Chemerin	1.11(0.91–1.37)	0.10	0.28	1.09(0.89–1.33)	0.10	0.39	1.08(0.88–1.33)	0.10	0.41
Follistatin	1.17(0.96–1.42)	0.10	0.11	1.13(0.93–1.38)	0.10	0.19	1.15(0.93–1.38)	0.10	0.19

Dependent variable: $LBP_{CONSULT}$ data reported as odds ratio with 95% confidence intervals [OR (95% CI)] with corresponding standard errors (SE) and P values. Variables were log-transformed to approximate normality prior to analysis; all variables were standardized prior analysis.

Table 5
Maximum likelihood parameter estimates in best fitting and most parsimonious quasi variance component analysis of $LBP_{DISABLE}$ and $LBP_{CONSULT}$ in the study sample.

Parameters	Parameters estimates by LBP phenotype	
	$LBP_{DISABLE}$	$LBP_{CONSULT}$
a_{0m}	−0.244	−0.153
a_{0f}	−0.082	−0.018
b_{1m} (age)	0.208 ± 0.092	−0.235 ± 0.155
b_{1f} (age)	0.260 ± 0.107	0.033 ± 0.061
b_{2m} (WHR)	0.239 ± 0.099	0.250 ± 0.144
b_{2f} (WHR)	0.197 ± 0.077	0.043 ± 0.077
b_{3f} (GDF-15)	0.245 ± 0.079	0.219 ± 0.151
b_{3f} (GDF-15)	0.373 ± 0.081	0.395 ± 0.110
V_{AD}	0.109 ± 0.138	0.252 ± 0.236

Parameter estimates shown in model, a_{0m}, a_{0f} are sex-specific intercepts (m-male, f-female); b1m, b1f - regression coefficients for age; b2m, b2f - regression coefficients for WHR; b3m, b3f - regression coefficients for GDF-15, V_{AD}- additive genetic component.

were in particular well seen when we considered most severe cases of LBP-related disability, namely, $LBP_{IMPOSSIBLE}$ and $LBP_{CONSULT}$ phenotypes. At this cutoff score, GDF-15 added significant prognostic information in patient risk to have LBP regardless of sex and independent of age and body composition. Moreover, the results of our analyses virtually did not change when we implemented quasi variance component analysis, which relates to a familial structure of the sample and accounts for the putative genetic effects (Table 5).

Cytokine GDF-15, a member of TGFβ superfamily, is implicated in a variety of physiological and pathological processes, including cancer, chronic inflammation, obesity and related disorders [26,27]. Recent discovery of its receptor, GFRAL, signaling through its co-receptor RET [28–31], identified a new brain-associated pathway, presumably capable of regulating cellular metabolism disturbed in obesity and cachexia [32]. Circulating GDF-15 concentrations were found to be increased in obese animals in comparison with age-matched lean controls [33]. In human studies, elevated circulating GDF-15 levels were found to be positively correlated with obesity measures and resultant comorbidities [34]. However, chronic administration of recombinant GDF-15 resulted in significantly reduced adiposity and correction of the metabolic dysfunction in obese animals [35]. Moreover, some studies have reported a protective rather than detrimental role of GDF-15 in obesity-associated conditions [36,37]. These findings suggest that elevated GDF-15 levels may reflect a physiological response aimed at reestablishing metabolic homeostasis, albeit insufficiently. This may explain why administration of GDF-15 to obese mice improved their metabolic phenotype.

It is well established that in obesity, excessive lipids in the form of free fatty acids outflow the adipose tissue and accumulate ectopically in skeletal muscle. This leads to mitochondrial dysfunction, disturbed β-oxidation of fatty acids, and enhanced reactive oxygen species production, leading to lipotoxicity and insulin resistance, resulted in sarcopenia and muscle pain [38]. Notably, elevated inter- and intramyocellular lipid concentrations in the multifidus muscle have been reported in LBP patients in correlation with age, obesity, atrophy of the multifidus muscle and LBP intensity [39]. In addition, IVD cell death, particularly apoptosis and autophagy, was found to be closely associated with mitochondrial dysfunction, significantly contributing to IVD degeneration [40]. Given the fact that elevated GDF-15 levels were detected in our LBP individuals in close correlation with obesity parameters (Table S3), it is plausible to suggest that GDF-15-mediated IVD and paraspinal muscle mitochondria dysfunctions might be one of the mechanisms underlying LBP aggravation (Fig. 1). However, whether GDF-15 is a causative mediator or a risk biomarker of obesity and LBP remains uncertain.

Another potential metabolic pathway connecting GDF-15 to LBP might be related to brain-located expression of GFRAL and the changes occurred in the brain of patients with chronic pain. Thus, binding of GDF-15 to GRFAL/RET receptor complex on the cell surface of neurons within the brainstem was suggested to trigger downstream intracellular signaling that affects yet to be defined neuronal circuitry. It may include brain structures, such as nucleus of the solitary tract, lateral parabrachial nucleus, central amygdala, and hypothalamus, presumably involved in the regulation of food intake [32]. GDF-15, excessively produced in LBP, can enhance this pathway. Moreover, GDF-15 expression is detected in hippocampus [41], and brain imaging studies revealed some structural changes in the dorsolateral prefrontal cortex and thalamus, temporal lobes, insula and the primary somatosensory cortex in individuals with chronic back pain compared with healthy controls [42]. Whether these brain-associated anatomical and functional alterations are casual to the GDF-15/GFRAL axis and LBP relations remain unclear and require further study.

Body composition analysis revealed a strong association between exacerbation of LBP manifestations with increased obesity and decreased skeletal muscle characteristics (Table 2). Cross-sectional studies showed that obesity increases the risk of LBP [43]. This conclusion has been confirmed in a recent prospective study of 5058 participants, in which fat mass and distribution positively associated with LBP intensity and disability at 12 years after adjustment for potential confounders [6]. However, the relationships between LBP and obesity seem to be not clear-cut. Indeed, it has been shown that not all obese individuals even with high BMI ($> 40\,kg/m^2$) have LBP and a significant decrease in BMI value in patients suffered from LBP not always accompanied by a substantial reduction in pain [44]. Nevertheless, recently described an obesity-associated multifidus muscle-destroying effect of infiltrated fatty acids might be one of the mechanisms connecting obesity and LBP manifestations [9]. Reported in our study a significant and independent association of WHR with LBP risk and disability supports this suggestion.

Since we suggest that sarcopenia and adipose tissue dysregulation play an important role in LBP pathogenesis, plasma levels of the relevant molecules (chemerin, leptin, adiponectin, and follistatin) known to be involved in the musculoskeletal and obesity-associated pathology

[13–15,45–47], have also been measured in our study. As we hypothesized previously [11,13] these factors, except adiponectin, demonstrated in our study significant correlations with obesity and skeletal mass measures. They showed also significant correlations with severe LBP phenotypes, in a univariate analyses. However, no correlation between LBP phenotypes and their plasma levels remained statistically significant after adjustment for age, sex, obesity and skeletal muscle mass measures. Among possible explanations of dropped associations between chemerin, leptin, and follistatin and LBP phenotypes after adjustment for various covariates might be special properties of our study population such as the prevalence of obese individuals, especially in females, and significant correlations of the plasma levels of soluble markers with age and adiposity measures. These circumstances may mask the input of elevated circulating levels of chemerin, leptin, and follistatin in LBP pathogenesis, thus leaving non-defined their role in the condition. The data also suggest that these factors may play a less significant role in the pathology of LBP in comparison to GDF-15, since after adjustment for several covariates only GDF-15 remained significant.

4.1. Limitations

This study has some limitations. Its cross-sectional design prevents from drawing a conclusion about the causality of the associations found. Longitudinal studies are required to establish the cause-and-effect relationships between LBP, measured body mass composition parameters and soluble factors (especially GDF-15), and evaluate their predictive nature. Obviously, IVD and paraspinal muscle specimens for measurement of LBP biomarkers would be preferable, but implausible in general population studies. However, determination of serum/plasma levels of various cytokines and other soluble molecules has been successfully used for monitoring the initiation, intensity and progression of LBP [17,19,48], and our data confirm this statement.

In conclusion, this is the first study providing statistically significant evidence that GDF-15 circulating levels are independently associated with detrimental LBP phenotypes. These observations provide new insights into the LBP pathogenesis and highlight the need for larger longitudinal studies to determine if GDF-15 could be a novel therapeutic target for prevention and/or treatment of LBP.

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Conflict of interest

The authors declare that they have no conflict of interest.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cyto.2019.01.011.

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