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Original Research

Identification of myokines susceptible to improve glucose homeostasis after bariatric surgery

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Abstract

Importance and Objective: The identification of myokines susceptible to improve glucose homeostasis following bariatric surgery could lead to new therapeutic approaches for type 2 diabetes.

Methods: Changes in the homeostasis model assessment (HOMA) test were assessed in patients before and 3 months after bariatric surgery. Changes in myokines expression and circulating levels were assessed using real-time quantitative polymerase chain reaction (RT-qPCR) and enzyme-linked immunosorbent assay (ELISA). Myokines known to regulate glucose homeostasis were identified using literature (targeted study) and putative myokines using RNA-sequencing (untargeted study). A linear regression analysis adjusted for age and sex was used to search for associations between changes in the HOMA test and changes in myokines.

Results: In the targeted study, brain-derived neurotrophic factor (*BDNF*) expression was upregulated (+30%, $P = .006$) while BDNF circulating levels were decreased (−12%, $P = .001$). Upregulated *BDNF* expression was associated with decreased HOMA of insulin resistance (HOMA-IR) (adjusted estimate [95% confidence interval {CI}]: −0.51 [−0.88 to −0.13], $P = .010$). Decreased BDNF serum levels were associated with decreased HOMA of beta-cell function (HOMA-B) (adjusted estimate [95% CI] = 0.002 [0.00002-0.0031], $P = .046$). In the untargeted study, upregulated putative myokines included *XYLT1* (+64%, $P < .001$), *LGR5* (+57, $P < .001$), and *SPINK5* (+46%, $P < .001$). Upregulated *LGR5* was associated with decreased HOMA-IR (adjusted estimate [95% CI] = −0.50 [−0.86 to −0.13], $P = .009$). Upregulated *XYLT1* and *SPINK5* were associated with increased HOMA of insulin sensitivity (HOMA-S) (respectively, adjusted estimate [95% CI] = 109.1 [28.5-189.8], $P = .009$ and 16.5 [0.87-32.19], $P = .039$).

Conclusions: Improved glucose homeostasis following bariatric surgery is associated with changes in myokines expression and circulating levels. In particular, upregulation of *BDNF*, *XYLT1*, *SPINK5*, and *LGR5* is associated with improved insulin sensitivity. These results suggest that these myokines could contribute to improved glucose homeostasis following bariatric surgery.

Study registration: NCT03341793 on ClinicalTrials.gov (https://clinicaltrials.gov/).

Keywords: BDNF, LGR5, muscle, myostatin, secretome, transcriptome, type 2 diabetes, XYLT1

Significance

The identification of myokines susceptible to improve glucose homeostasis following bariatric surgery could lead to new therapeutic approaches for type 2 diabetes. In our study, improved glucose homeostasis following bariatric surgery is associated with changes in myokines expression and circulating levels. In particular, upregulation of *BDNF*, *XYLT1*, *SPINK5*, and *LGR5* is associated with improved insulin sensitivity. Changes in body composition seem to drive most of the impact of weight loss on myokines, in particular on myostatin. These results suggest that several myokines could contribute to improved glucose homeostasis and reflect changes in body composition following bariatric surgery.

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Introduction

Bariatric surgery is effective for both obesity and type 2 diabetes.[1,2] Diabetes remission rates exceeding 50% have led to the concept of metabolic surgery.[2,3] The massive weight loss induced by bariatric surgery results in improved insulin sensitivity and beta-cell function.[2,4] However, the mechanisms underlying diabetes remission remain incompletely understood.[4]

Skeletal muscle is an endocrine organ[5–7] and plays a central role in glucose homeostasis.[8] Indeed, muscle insulin resistance often precedes type 2 diabetes in obese individuals.[9] In addition, some myokines regulate muscle insulin sensitivity (eg, interleukin-6 [IL6]), insulin secretion, and/or beta-cell survival (eg, fractalkine).[10–14] The muscle secretome is modified by factors reducing (eg, palmitate) or improving (eg, exercise) muscle insulin sensitivity.[15–18] On the other hand, myokines secreted by insulin-resistant myotubes negatively influence insulin secretion.[14,19] Finally, preliminary data indicate that bariatric surgery alters circulating levels of some myokines.[20,21] However, the contribution of myokines to improved glucose homeostasis following bariatric surgery is unknown. The identification of these myokines could lead to new therapeutic approaches for obesity and type 2 diabetes.

The primary aim of our study was to test whether myokines known to regulate glucose homeostasis were associated with changes in glucose homeostasis following bariatric surgery (targeted study). The secondary aim was to identify putative myokines associated with changes in glucose homeostasis following bariatric surgery (untargeted study).

Patients and methods

Study design

The MYDIASECRET study is a prospective, longitudinal, and interventional study led from January 2018 through October 2020 (NCT03341793). The study included 62 White patients scheduled for bariatric surgery aged 19-69 years. Eligibility criteria for bariatric surgery consisted of a body mass index (BMI) ≥ 40 kg/m² or between 35 and 39 kg/m² with at least one comorbidity including refractory arterial hypertension, type 2 diabetes, and/or obstructive sleep apnea. Prediabetes and type 2 diabetes were defined according to the American Diabetes Association criteria.[22] Glycated hemoglobin A1c (HbA1c) at inclusion was $\leq$7.5% ($\leq$58 mmol/L) without or with medications, except insulin. Exclusion criteria were cancer history within the last 5 years, pregnancy, breastfeeding, glucocorticoids, type 1 diabetes, history of myopathy, risk of bleeding, insulin use, or previous bariatric surgery.

Patients were evaluated before and 3 months after bariatric surgery, which consisted of either a sleeve gastrectomy (SG) or a Roux-en-Y gastric bypass (RYGB) (Figure 1). Anthropometric parameters included height, weight, BMI, waist, and hip circumferences. Body composition was determined using bioelectrical impedance analysis (Tanita, BC-418 MA). Glucose homeostasis was assessed using the homeostasis model assessment (HOMA) test that estimates steady-state beta-cell function (HOMA-B) and insulin sensitivity (HOMA-S).[23] The HOMA2 calculator was downloaded from the website of the Radcliffe Department of Medicine, Oxford University (https://www.dtu.ox.ac.uk/homacalculator). The HOMA-B and HOMA-S were computed using fasting-specific insulin (pmol/L) and blood glucose (mg/dL). The HOMA of insulin resistance (HOMA-IR) provided by the calculator corresponds to the inverse transformation of HOMA-S. The hyperbolic product was calculated as HOMA-B $*$ HOMA-S to denote the beta-cell function adjusted for an individual's insulin sensitivity.[24] The lipid profile, HbA1c, glucose, and insulin were determined on blood samples using clinical routine methods. Plasma (EDTA) and serum samples were taken to measure circulating levels of myokines. Muscle biopsies were taken from vastus lateralis using BARD® Monopty™ Disposable Core Biopsy Instrument (Bard Peripheral Vascular, Tempe, USA) and immediately frozen in liquid nitrogen for RNA analysis. Anthropometric measurements, blood samples, and muscle biopsies were collected after an overnight fast and after withdrawal of glucose-lowering medications for 7 days in due cases to reduce their effects on metabolic and muscle measurements. Muscle biopsies were taken under general and local anesthesia (Lidocaine 2%, Braun Medical, Machelen, Belgium) before and after surgery, respectively. The study was approved by the institutional review board (Cliniques Universitaires Saint-Luc) and conducted in accordance with the principles of the Declaration of Helsinki as revised in 2008. Patients were enrolled after obtaining written informed consent.

Real-time qPCR

Frozen muscle samples were placed in Lysing matrix D tubes (MP Biomedical, France) containing 1 mL of Tripure™ Isolation Reagent (Roche Diagnostics, Germany) on ice and homogenized using the FastPrep-24™ Classic bead beating grinder and lysis system (MP Biomedical, France). Samples were homogenized 3×1 min, at 6 m/s, with 5-min rest on ice between rounds. Total RNA was isolated according to the manufacturer's instructions. One microgram of RNA was reverse transcripted and Sybr Green® real-time quantitative polymerase chain reaction (RT-qPCR) was performed as previously described.[25] Relative gene expression after bariatric surgery was calculated using the comparative Ct method and normalized by the expression of the housekeeping gene GAPDH. Primers used for amplification during RT-qPCR were tested to avoid primer dimers, self-priming formation, or any unspecific amplification (Table S1).[26]

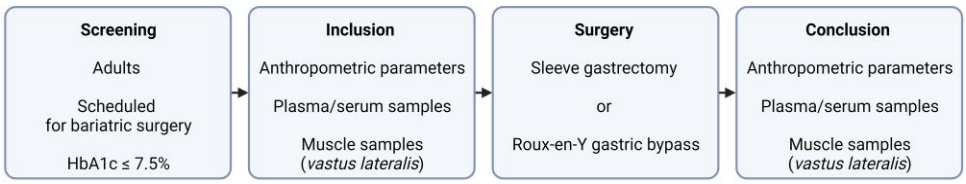

Figure 1. Overview of the study design. Sixty-two patients were evaluated before and 3 months after surgery (ie, sleeve gastrectomy/Roux-en-Y gastric bypass). Plasma and serum samples were taken to quantify circulating levels of myokines. Muscle biopsies were taken from vastus lateralis for RNA analysis. HbA1c, hemoglobin A1c. Created with BioRender.com.

Targeted study

A literature search was performed on PubMed to identify myokines known to regulate glucose homeostasis (Supplementary Methods and Table S2).[26] These myokines included brain-derived neurotrophic factor (*BDNF*),[7,16,17,27] fractalkine (*CX3CL1*),[11,16,17] angiogenin (*ANG*),[7,12,16,17] osteoprotegerin (*TNFRSF11B*),[7,12] a disintegrin and metalloproteinase with thrombospondin motifs 9 (*ADAMTS9*),[10] irisin (*FNDC5*),[28] myostatin (*MSTN*),[6] apelin (*APLN*),[29] interleukin-6 (*IL6*),[6,7,17] tripartite motif-containing protein 72 (*TRIM72*),[7] and secreted frizzled-related protein 4 (*SFRP4*).[6] Relative expression levels of these myokines after surgery were determined using RT-qPCR. A linear regression analysis was performed to search for associations between changes in glucose homeostasis (HOMA test) and changes in myokine expression (fold change). A linear regression analysis was also performed to search for associations between changes in myokine expression (fold change) and changes in body composition after bariatric surgery.

Untargeted study

High-throughput RNA-sequencing (RNA-Seq) was performed on muscle biopsies (Supplementary Methods).[26] Total RNA was extracted from muscle samples as described previously. RNA integrity number (RIN ≥ 7) was checked using Bioanalyzer 2100 (Agilent, USA) and RNA 600 NanoChips before the preparation of libraries. Messenger RNAs (mRNAs) were selected using KAPA mRNA HyperPrep Kit (Roche Sequencing Store, Roche, Switzerland) and reverse transcribed into complementary DNA (cDNA). Deep paired-end sequencing was performed with NovaSeq 6000 S1 system (Illumina, USA) and generated on average 70 million paired-end reads (75 bp) per sample. Bioinformatic methods are detailed in Supplementary Methods.[26] Differential expression of the transcripts was evaluated with the Bioconductor R package DESeq2 (v.1.30.0) for R (v.4.0.4) using a paired design.[30] *P* values were corrected for multiple testing using Benjamini–Hochberg false discovery rate (FDR) and set at $\leq .10$. Genes that were upregulated ($\geq$1.3-fold) or downregulated ($\leq$0.7-fold) were searched among all regulated genes after surgery. Those encoding putative myokines were identified using predicted signal peptide as described elsewhere[31] in combination with annotations from UniProtKB (secreted/extracellular space, https://www.uniprot.org/). An exploratory linear regression analysis was performed to search for associations between changes in glucose homeostasis (HOMA test) and changes in this putative myokine expression (transcriptions per million after/before surgery). Myokines associated with glucose homeostasis ($R^2 \geq 10\%$, $P < .10$) were quantified in RT-qPCR. Linear regression analyses were performed as described previously.

Circulating levels of myokines

Blood samples were centrifuged for 10 min at 4 °C, 2000 *g*. Plasma levels of mature myostatin and fractalkine as well as serum levels of BDNF were measured using quantitative sandwich enzyme-linked immunosorbent assay (ELISA) (DGDF80, DCX310, and DBD00, R&D Systems, Minneapolis, USA). Samples were measured in duplicates, and mean levels were calculated from the standard curves. The mean minimum detectable dose was 2.25 pg myostatin/mL, 0.018 ng fractalkine/mL, and 20.0 pg BDNF/mL, intra-assay coefficients of variation were 3.2%, 2.6%, and 5%, respectively, and inter-assay coefficients of variation were 4.2%, 6.6%, and 9%, respectively. Linear regression analyses were performed as previously described.

Pathways enrichment analysis

Gene set enrichment analysis (GSEA) was performed on the whole transcriptomic data set to identify enriched pathways after bariatric surgery (Figure S1).[26] The analysis was run in R (v.4.0.4), using the Bioconductor R package clusterProfiler (v.3.18.0) and using gene sets from gene ontology (http://geneontology.org/), KEGG (https://www.genome.jp/kegg/kegg.html), and Reactome (https://reactome.org/). *P* values were corrected for multiple tests using FDR and set at <.05.

Supplementary data and data availability[26]

Supplementary data are provided as a separate file.[26] The transcriptomic dataset of the MYDIASECRET study are available online.[30]

Statistical analysis

Continuous variables are reported as median with interquartile range (IQR) and discrete variables as numbers and proportions. Longitudinal differences were calculated using the nonparametric Wilcoxon signed-rank test. For RT-qPCR analysis, differences were calculated on delta Ct. For regression analyses, independent variables were first tested in univariate analysis. All candidate independent variables with $R^2 \geq .10$ and $P < .10$ were introduced into a multivariate model. A backward stepwise selection method was used to select the optimal multivariate model. Multivariate analyses were adjusted for age and sex. A log transformation was used for log-normal response variables. Inverse transformation (HOMA-IR) was used for HOMA-S.[23] Results are expressed as adjusted estimates (95% confidence interval [CI]). Data for body composition were missing in 2 patients and were simply ignored. The sample size was not prespecified given the exploratory nature of the study. Statistical significance was set at $P < .05$ unless otherwise stated. Statistical analyses were performed using SPSS Statistics software (IBM SPSS Statistics, Version 25.0, Armonk, NY, USA), SAS software (version 9.4; SAS Institute Inc., Cary, NC, USA), and R (Version 4.0.5, https://www.r-project.org/). Graphs were made using GraphPad Prism version 8.0.0 for Windows, GraphPad Software, San Diego, California, USA, www.graphpad.com.

Results

Clinical results

Sixty-two patients were included in the study (Table 1). Median weight loss was 22.5 (18.0-26.7) kg (18% of preoperative body weight, $P < .001$). Median fat mass loss was 15.4 (12.4-18.1) kg (28% of preoperative fat mass, $P < .001$). Median fat-free mass loss was 6.6 (4.8-9.4) kg (11% of preoperative fat-free mass, $P < .001$). Insulin resistance was significantly improved with a median HOMA-IR decrease of 53 (35-68) % ($P < .001$). Before surgery, 3 patients with diabetes were treated either with metformin alone or in combination with empagliflozin (Table S3).[26] One patient was still taking metformin after surgery. None of the patients received GLP-1 receptor agonists (neither before nor after surgery). Muscle samples were taken in 39 patients.

Table 1. Patients' characteristics before and 3 months after bariatric surgery ($n = 62$).

Variables	Before surgery	After surgery	*P* value
Females/males (*n*, %)	36/26 (58/42)	/	/
Age (years)	44 [34-54]	/	/
Prediabetes/diabetes (*n*, %)	31/8 (50/13)	/	/
SG/RYGB (*n*, %)	39/23 (63/37)	/	/
Body weight (kg)	124.8 [114.2-136.4]	103.4 [91.7-110.9]	<.001
BMI (kg/m²)	43 [41-44]	35 [32-38]	<.001
Waist circumference (cm)	133 [125-140]	117 [110-125]	<.001
Hip circumference (cm)	128 [121-136]	116 [108-124]	<.001
Fat mass (kg)	54.3 [48.8-64.7]	41.1 [32.8-48.3]	<.001
Fat mass index (kg/m²)	20.0 [16.1-22.7]	15.0 [11.2-17.1]	<.001
Fat-free mass (kg)	64.4 [56.3-83.1]	57.6 [51.9-75.4]	<.001
Fat-free mass index (kg/m²)	23.2 [20.9-26.0]	21.1 [18.9-23.2]	<.001
Systolic BP (mmHg)	140 [130-150]	125 [120-130]	<.001
Diastolic BP (mmHg)	85 [80-90]	80 [75-85]	<.001
Fasting plasma glucose (mmol/L)	5.38 [4.88-5.99]	5.11 [4.66-5.33]	<.001
Fasting insulin (pmol/L)	161.6 [106.3-206.6]	68.6 [50.3-95.1]	<.001
HbA1c (%)	5.7 [5.4-6.2]	5.4 [5.1-5.6]	<.001
HbA1c (mmol/mol)	39 [36-44]	36 [32-38]	<.001
Total cholesterol (mmol/L)	4.84 [4.14-5.49]	4.01 [3.47-4.90]	<.001
LDL cholesterol (mmol/L)	2.90 [2.31-3.29]	2.38 [1.84-2.93]	<.001
HDL cholesterol (mmol/L)	1.19 [0.96-1.42]	1.14 [1.01-1.35]	.26
Triglycerides (mmol/L)	1.30 [1.04-1.72]	1.13 [0.92-1.41]	.001
HOMA-B (%)	190 [141-222]	123 [97-152]	<.001
HOMA-S (%)	32 [26-42]	68 [51-93]	<.001
HOMA-IR	3.2 [2.4-3.8]	1.5 [1.1-2.0]	<.001
Hyperbolic product (%)	59 [44-70]	82 [67-103]	<.001

Data are expressed as medians [IQR]. Hyperbolic product = HOMA-B * HOMA-S. Differences assessed using Wilcoxon signed-rank test. *P* statistically significant < .05. Body composition assessed in 60 patients.
Abbreviations: BMI, body mass index; BP, blood pressure; HbA1c, hemoglobin A1c; HOMA-B, homeostatic model assessment of beta-cell function; HOMA-IR, homeostatic model assessment of insulin resistance; HOMA-S, homeostatic model assessment of insulin sensitivity; RYGB, Roux-en-Y gastric bypass; SG, sleeve gastrectomy.

Targeted RT-qPCR results

RT-qPCR ($n = 39$) showed increased expression of *CX3CL1* (+73%, $P < .001$), *TNFRSF11B* (+64%, $P < .001$), *IL6* (+48%, $P = .004$), *ADAMTS9* (+35%, $P < .001$), *BDNF* (+30%, $P = .006$), *ANG* (+29%, $P < .001$), and *TRIM72* (+22%, $P = .012$) (Figure 2A). RT-qPCR also showed decreased expression of *MSTN* (−45%, $P < .001$), *FNDC5* (−25%, $P < .001$), and SFRP4 (−11%, $P = .04$) (Figure 2B). *APLN* expression was unchanged ($P = .187$) (Figure 2B). *CX3CL1* encoding fractalkine and *MSTN* encoding myostatin were the most upregulated and downregulated genes.

Regression analysis of targeted genes

BDNF, *ADAMTS9*, *TNFRSF11B*, and *ANG* fold changes were statistically associated with changes in glucose homeostasis in univariate analysis (Figure 3A). The multivariate analysis showed that increased *BDNF* expression was significantly associated with decreased HOMA-IR (adjusted estimate [95% CI] = −0.51 [−0.88 to −0.13], $P = .010$) and decreased HOMA-B (adjusted estimate [95% CI] = −7.50 [−14.52 to −0.48], $P = .037$) (Table 2). Regarding body composition, changes in body weight, BMI, fat mass, and/or fat-free mass were statistically associated with *CX3CL1*, *BDNF*, *ADAMTS9*, and *APLN* fold change in univariate analysis (Figure 3B). In multivariate analysis, fat mass loss, expressed as an absolute decrease in fat mass index, was associated with increased *APLN* expression while fat-free mass loss, expressed as relative change in fat-free mass index, was associated with increased *CX3CL1* and *ADAMTS9* expression (Table 2). No significant association was found between myokines and type of surgery, changes in blood glucose, and changes in HbA1c.

Circulating levels of myokines

BDNF levels were significantly decreased (−12%, $P = .001$) after surgery (Figure 4A). Fractalkine levels were significantly increased (+7%, $P = .001$) (Figure 4B) while myostatin levels were significantly decreased (−32%, $P < .001$) (Figure 4C). No statistically significant association was found between fractalkine or myostatin levels and glucose homeostasis in univariate analysis (Figure 4D). However, absolute decrease in BDNF serum levels was associated with absolute decrease of HOMA-B (adjusted estimate [95% CI] = 0.002 [0.00002-0.0031], $P = .046$) (Table 2). Regarding body composition, no statistically significant association was found between body composition and fractalkine levels in univariate analysis (Figure 4E). In contrast, changes in body composition were associated with changes in myostatin and BDNF levels (Figure 4E). In the multivariate analysis, absolute decrease in fat-free mass was statistically associated with absolute decrease of plasma myostatin levels (adjusted estimate [95% CI] = 113.1 [43.4-182.5], $P = .002$) (Table 2). The association was no longer significant for BDNF (not shown).

Changes in the muscle transcriptome

RNA-sequencing was performed in 12 patients (Table S4).[26] A total of 74 285 transcripts of 22 936 single genes were sequenced. Most single genes (94%) were unchanged after bariatric surgery. Among the 1363 regulated genes, 711 were downregulated and 652 were upregulated (Figure 5A). At

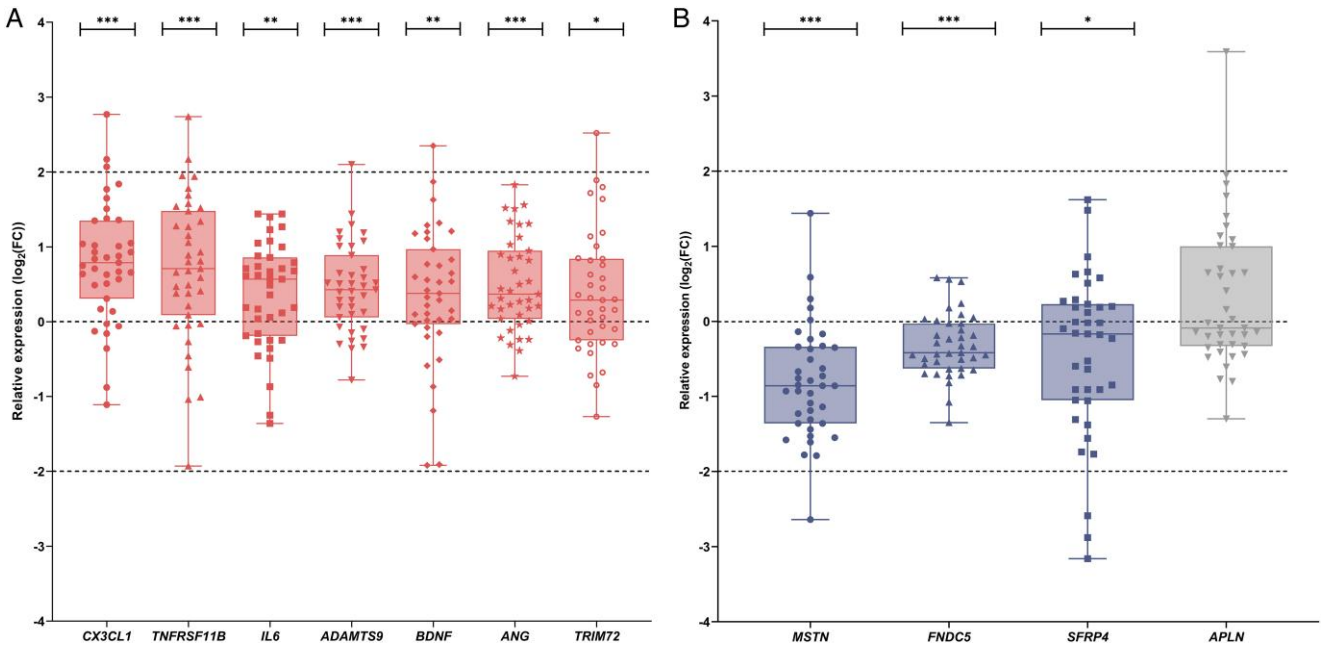

Figure 2. Targeted study: changes in the expression of myokines by RT-qPCR (A) Upregulated genes and (B) downregulated and unchanged genes. $n = 39$, results were presented as median $\log_2$(FC) (min, max). Preoperative expression (control value) arbitrary set at 0. Wilcoxon signed-rank test performed on ΔCT to assess relative mRNA expression between presurgery and postsurgery visits. $^*P < .05$, $^{**}P < .01$, and $^{***}P < .001$. FC, fold change; RT-qPCR, real-time quantitative polymerase chain reaction.

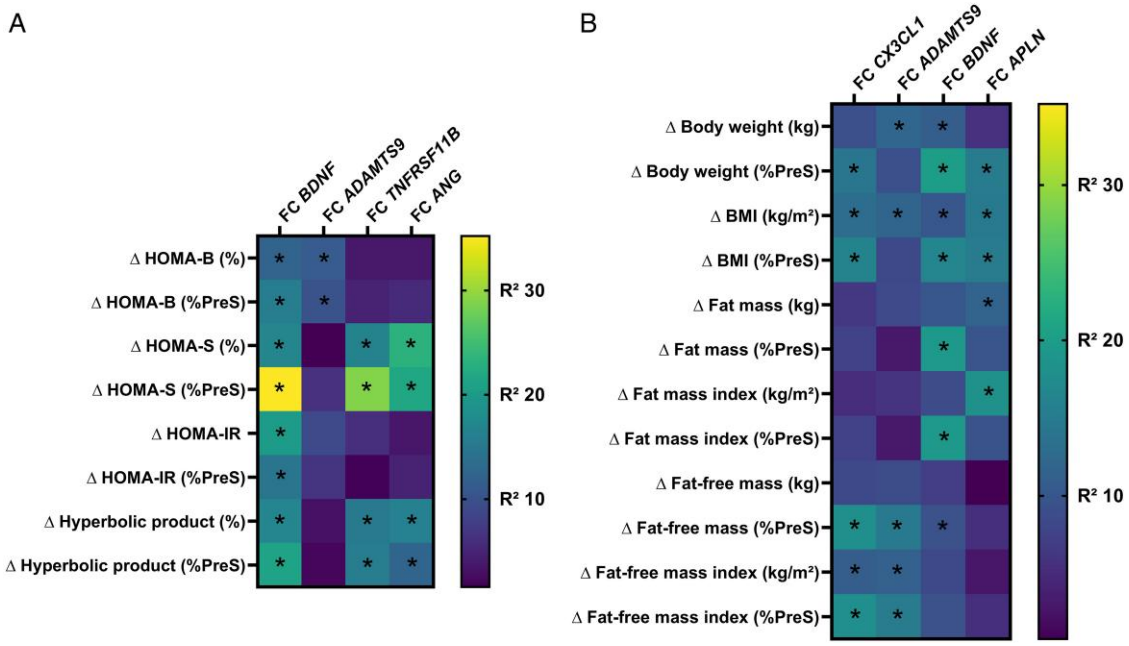

Figure 3. Associations between myokines expression, changes in glucose homeostasis, and changes in body composition after bariatric surgery. (A) Univariate linear regression between changes in myokines expression (fold change) and changes in glucose homeostasis (HOMA test). (B) Univariate linear regression between changes in body composition and changes in myokines expression (fold change). $n = 39$; scale represents R^2. $^*P < .10$. %PreS, change relative to presurgery value; BMI, body mass index; Δ, change from presurgery value; HOMA, homeostasis model assessment; HOMA-B, homeostatic model assessment of beta-cell function; HOMA-IR, HOMA insulin resistance; HOMA-S, HOMA insulin sensitivity.

the pathways level, the most upregulated pathways were related to ribosomes, translation, and protein localization to the endoplasmic reticulum and membrane suggesting that bariatric surgery alters protein dynamics (Figure 5B). Interestingly, *MSS51* and *DDIT4*, which are 2 inhibitors of mTORC1, were among the most downregulated genes (Figure 5A). These data suggest an attempt to preserve protein homeostasis after bariatric surgery. On the other hand, the most downregulated pathways were related to inflammation and immune cells (Figure 5C). Glucose homeostatic pathways (eg, glucose transport) were not enriched after surgery (not shown). However, some mitochondrial pathways including

Table 2. Targeted study: associations between glucose homeostasis, body composition, and myokines (multivariate linear regression analyses, adjustment for age and sex).

Dependent variables	Independent variables	N	Unadjusted estimate (95% CI)	Adjusted estimate (95% CI)	*P*-value
Changes in myokines associated with changes in glucose homeostasis					
Myokines expression					
HOMA-IR (absolute change)	*BDNF* (fold change)	39	−0.58 (−0.96 to −0.19)	−0.51 (−0.88 to −0.13)[a]	.010
HOMA-B (relative change)	*BDNF* (fold change)	39	−8.62 (−15.24 to −2.00)	−7.50 (−14.52 to −0.48)[a]	.030
Myokines circulating levels					
HOMA-B (absolute change)	Serum BDNF, absolute change	62	0.0019 (0.0004-0.0033)	0.002 (0.00002-0.0031)[a]	.040
Changes in body composition associated with changes in myokines					
Myokines expression					
CX3CL1 (fold change)	Relative change in fat-free mass index	38	0.930 (0.882-0.979)	0.920 (0.868-0.975)[b]	.007
ADAMTS9 (fold change)	Relative change in fat-free mass index	38	0.952 (0.915-0.990)	0.944 (0.904-0.986)[b]	.010
APLN (fold change)	Absolute change in fat mass index	38	0.811 (0.697-0.944)	0.806 (0.689-0.942)[b]	.008
Myokines circulating levels					
Myostatin plasma levels (absolute change)	Absolute change in fat-free mass	60	114.3 (60.7-168.0)	113.1 (43.4-182.5)[a]	.002

Body composition was assessed in 60 patients. Muscle biopsies were taken in 39 patients.
Abbreviations: 95 CI, 95% confidence interval; BDNF, brain-derived neurotrophic factor; HOMA-B, homeostatic model assessment of beta-cell function; HOMA-IR, homeostatic model assessment of insulin resistance.
[a]Adjusted estimate should be interpreted as the mean change in the dependent variable for 1 unit of change in the independent variable.
[b]Adjusted estimate should be interpreted as (estimate − 1) × 100 = percentage change in the dependent variable (log transformed) for 1 unit increase in the independent variable.

the respiratory electron transport (R-HAS-611105, NES −1.63, FDR = 0.029, not shown) and the citric acid cycle (R-HAS-1428517, NES −1.55, FDR = 0.048, not shown) were downregulated, suggesting changes in energy or lipid metabolism after bariatric surgery.

Untargeted identification of putative myokines

Genes encoding putative myokines were searched among the 228 downregulated genes with fold change ≤ 0.7 and the 228 upregulated genes with fold change ≥ 1.3 (Figure 6A). Fifty-six downregulated genes and 41 upregulated genes encoded putative myokines (Table S5).[26] Associations were searched between these genes and changes in glucose homeostasis (Figure S2).[26] This analysis identified 12 myokines associated with changes in glucose homeostasis including *FNDC5*, previously reported (Table 3). These myokines were quantified by RT-qPCR in the whole cohort ($n = 39$). Increased expression of *XYLT1* (+64%, $P < .001$), *LGR5* (+57%, $P < .001$), *SPINK5* (+46%, $P < .001$), KDR (+39%, $P < .001$), *ADGRB2* (+31%, $P < .001$), and *CD6* (+13%, $P = .012$) after surgery was confirmed (Figure 6B). Decreased expression of *LIPG* (−47%, $P < .001$) and *DLK1* (−32%, $P < .001$) was also confirmed. *PF4* expression was unchanged ($P = .819$) (Figure 6C). *FCRL1* and *ELFN2* were unmeasurable in RT-qPCR and were excluded from further analysis.

Regression analysis of untargeted genes

XYLT1, *LGR5*, and *SPINK5* fold changes were statistically associated with changes in glucose homeostasis in univariate analysis (Figure 7A). The multivariate analysis showed that increased *XYLT1* expression was associated with relative increase of HOMA-S (adjusted estimate [95% CI] = 109.1 [28.5-189.8], $P = .009$) and with relative increase of the hyperbolic product (adjusted estimate [95% CI] = 43.8 [13.5-74.1], $P = .006$) (Table 4). Increased *SPINK5* expression was also associated with absolute increase of HOMA-S (adjusted estimate [95% CI] = 16.5 [0.87-32.19], $P = .039$). Increased *LGR5* expression was associated with absolute decrease of HOMA-IR (adjusted estimate [95% CI] = −0.50 [−0.86 to −0.13], $P = .009$). Changes in body weight, BMI, and fat mass were statistically associated with *XYLT1* and *SPINK5* fold changes (Figure 7B). In the multivariate analysis, weight loss, expressed as absolute decrease in BMI, was associated with increased *SPINK5* expression (adjusted estimate [95% CI] = 1.14 [1.01-1.29], $P = .033$) while fat mass loss, expressed as relative change in fat mass, was associated with increased *XYLT1* expression (adjusted estimate [95% CI] = 0.98 [0.97-0.99], $P = .029$) (Table 4). No significant association was found between *XYLT1*, *LGR5*, and *SPKINK5* and type of surgery, changes in blood glucose, and changes in HbA1c.

Discussion

Our study first shows that changes in glucose homeostasis after bariatric surgery are indeed associated with changes in myokines known to regulate glucose homeostasis. In particular, *BDNF* upregulation is associated with improved insulin sensitivity. Our study also shows that putative myokines are associated with changes in glucose homeostasis after bariatric surgery. In particular, *XYLT1*, *LGR5*, and *SPINK5* upregulation is associated with improved insulin sensitivity. Taken together, these results suggest that myokines could play a role in improved glucose homeostasis following bariatric surgery. In addition, some myokines such as myostatin could be involved in other pathways altered by bariatric surgery, namely, the dynamics of protein synthesis and mitochondrial pathways.

BDNF is secreted by muscle cells and induced by muscle contraction.[5,27,32] In line with this, increased BDNF expression in human muscle has been reported after physical exercise.[33] In our study, we have also demonstrated an increase in BDNF expression after bariatric surgery. In contrast, BDNF circulating levels were decreased after surgery in our study, as already reported by others.[34,35] Muscle BDNF is thought to act locally while circulating BDNF is mainly released by platelets and brain.[27,36] This means that changes

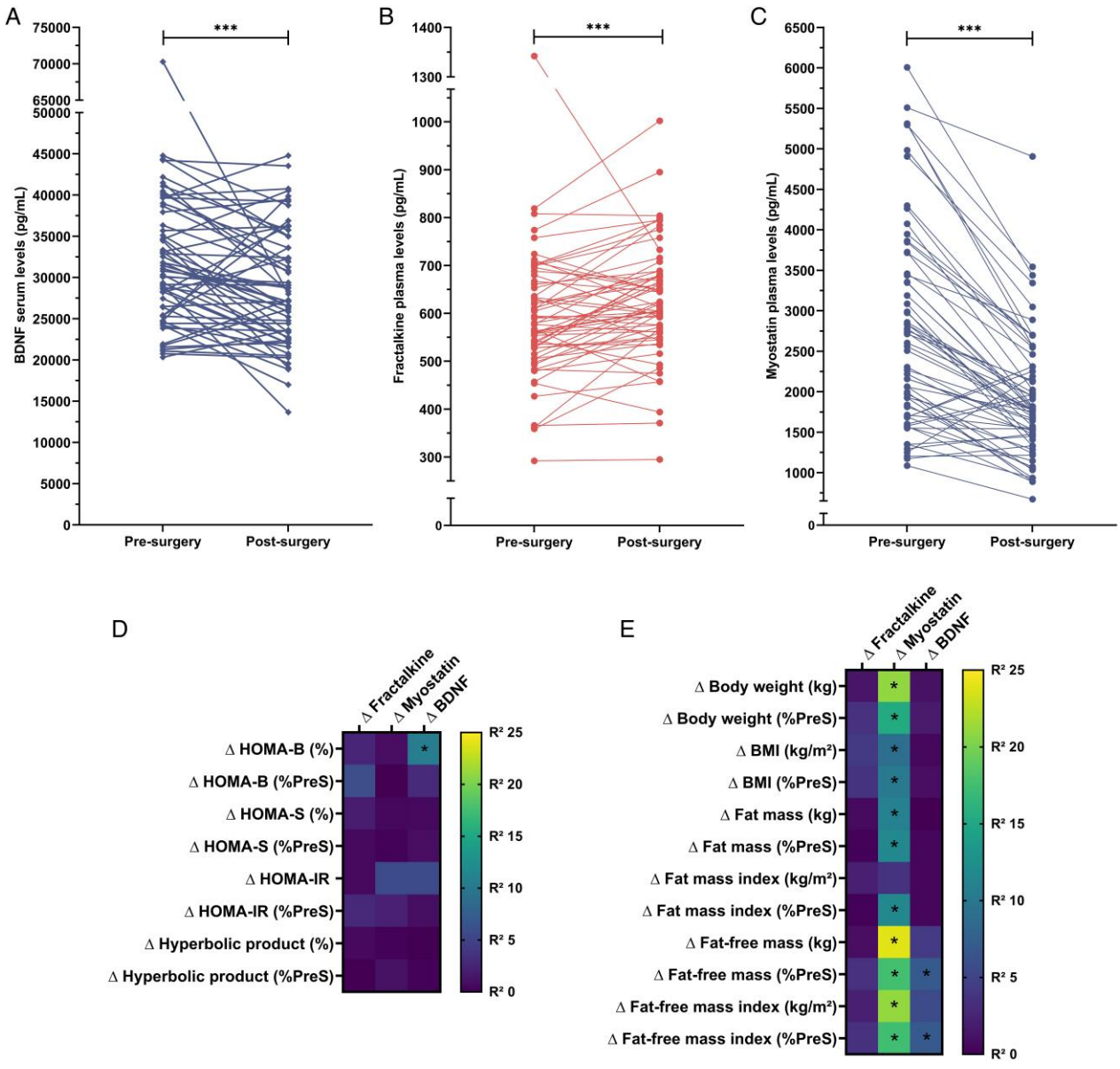

Figure 4. Changes in circulating levels of myokines and their associations with changes in glucose homeostasis and body composition after bariatric surgery. (A) BDNF serum levels. (B) Fractalkine plasma levels. (C) Myostatin plasma levels. $n = 62$, Wilcoxon matched-pairs signed-rank test. $***P < .001$. (D) Univariate linear regression between changes in myokines expression (fold change) and changes in glucose homeostasis (HOMA test). (E) Univariate linear regression between changes in body composition and changes in myokines expression (fold change). $n = 60$; scale represents R^2. $*P < .10$. %PreS, change relative to presurgery value; BDNF, brain-derived neurotrophic factor; HOMA, homeostasis model assessment; HOMA-B, homeostatic model assessment of beta-cell function; HOMA-IR, HOMA insulin resistance; HOMA-S, HOMA insulin sensitivity; Δ change from presurgery value.

in BDNF expression in muscle may not translate into changes in circulating levels. Moreover, muscle BDNF has been shown to improve insulin sensitivity while circulating BDNF has been shown to enhance glucose-stimulated insulin secretion.[27,37] Therefore, changes in BDNF reported in our study, although occurring in opposite directions at the gene and circulating levels, could contribute to improved insulin sensitivity and decreased compensatory hyperinsulinemia. Myostatin and fractalkine were the most downregulated and upregulated genes encoding myokines known to regulate glucose homeostasis. Myostatin is a member of the transforming growth factor beta (TGF-beta) superfamily, which negatively regulates muscle mass and insulin sensitivity.[38] Myostatin expression and circulating levels are increased in type 2 diabetes and/or obesity and correlate negatively with insulin sensitivity.[39,40] Inhibition of myostatin has been reported to improve glucose homeostasis.[41] Fractalkine is a chemokine protecting glucose-stimulated insulin secretion and preserving beta cells against pro-inflammatory cytokines or fatty acids.[11,42] Mice treated with a fractalkine analog show improved glucose tolerance, insulin secretion, and beta-cell survival.[43] In humans, data are conflicting regarding the circulating levels and muscle expression of fractalkine in type 2 diabetes and obesity.[44] Based on these data, myostatin and fractalkine could contribute to improved glucose homeostasis after bariatric surgery, although we did not find any association between these myokines and changes in glucose homeostasis in our study.

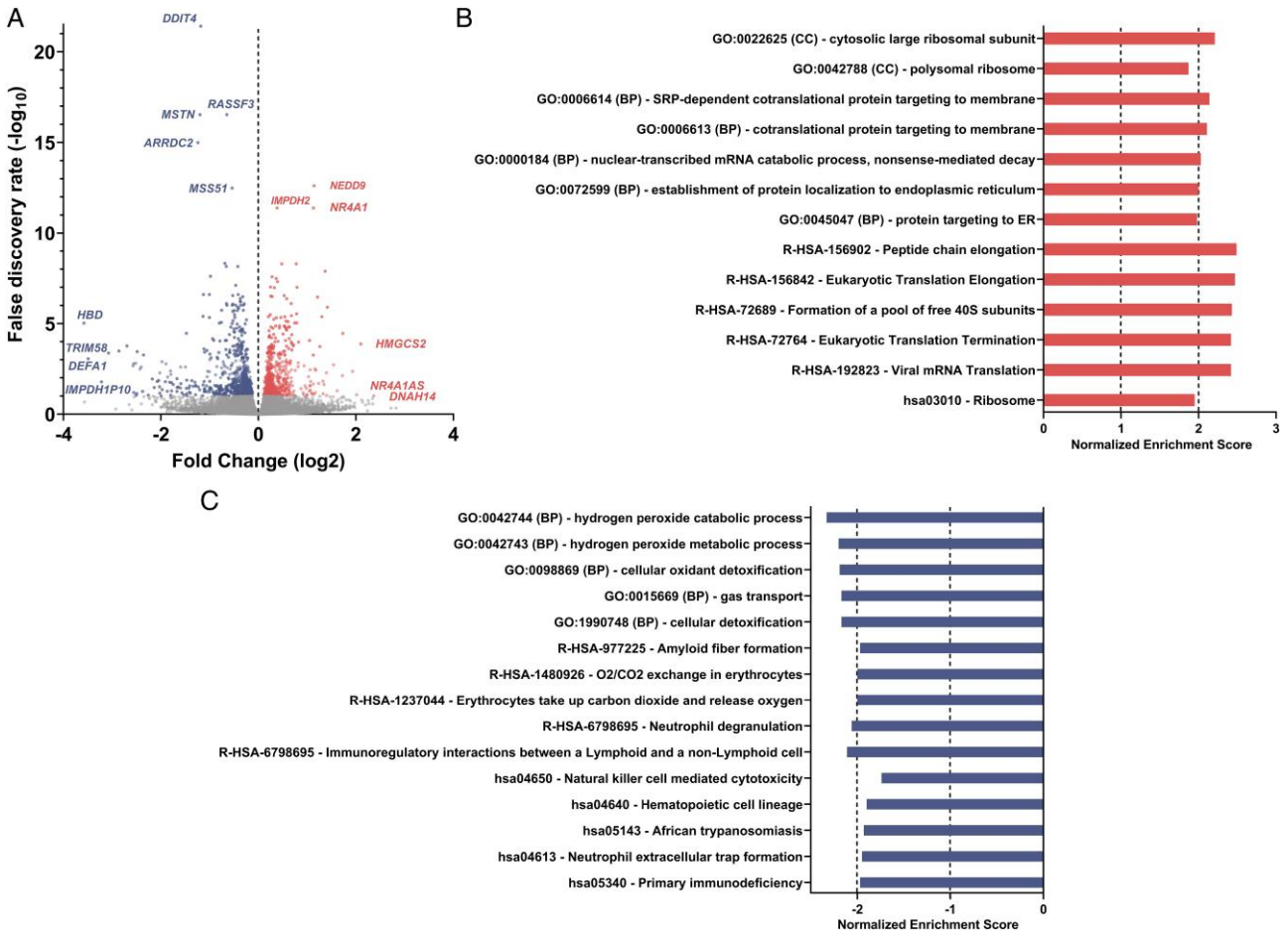

Figure 5. Changes in muscle transcriptome induced by bariatric surgery. (A) RNA-Seq: all regulated single genes after bariatric surgery (FDR ≤ 0.10). Most upregulated and downregulated genes are identified by their Hugo Gene Nomenclature Committee (HGNC) symbol including *MSS51* and *DDIT4*, 2 mammalian target of rapamycin (mTOR) inhibitors. (B) GSEA: top upregulated enriched pathways. (C) GSEA: top downregulated enriched pathways. FDR, false discovery rate; GSEA, gene set enrichment analysis; HOMA, homeostasis model assessment; RNA-Seq, RNA-sequencing

In the untargeted study, we have identified XYLT1, LGR5, and SPINK5, as three putative myokines that could contribute to improved glucose homeostasis after bariatric surgery. *XYLT1* is expressed in many tissues including pancreas, muscle, and adipose tissue and encodes xylosyltransferase 1 involved in the biosynthesis of glycosaminoglycan chains.[45] *LGR5* is highly expressed in skeletal muscle and encodes leucine-rich repeat-containing G protein–coupled receptor 5 involved in the canonical Wnt signaling pathway.[45] Finally, *SPINK5* encodes serine peptidase inhibitor Kazal type 5, which is a serine protease inhibitor involved in anti-inflammatory protection.[45] Few data are available on these myokines and glucose homeostasis. Nevertheless, *XYLT1* and *LGR5* variants have been reported as associated with type 2 diabetes in genome-wide association studies (GWAS).[46–48] Further studies are needed to investigate their role in the regulation of glucose homeostasis.

Weight loss is considered as the main driver of metabolic benefits of bariatric surgery.[2] Fat mass loss contributes the most to weight loss after bariatric surgery, but some degree of fat-free mass loss, particularly of skeletal muscle, is also expected.[49] As a consequence, changes in body composition may contribute to changes in myokines after surgery. Indeed, we showed that increased expression of myokines such as *CX3CL1*, *BDNF*, *ADAMTS9*, and *SPINK5* was associated with the degree of weight loss. More specifically, increased expression of *CX3CL1*, *BDNF*, and *ADAMTS9* was associated with the degree of fat-free mass loss. Moreover, fat-free mass loss was found as an independent predictor of decreased circulating myostatin levels, as reported by others.[50] Therefore, fat-free mass loss seems to drive most of the impact of weight loss on the expression and circulating levels of certain myokines.

Glucose homeostatic pathways were not enriched after bariatric surgery. However, changes in other metabolic pathways could result from changes in myokines, particularly myostatin. Indeed, the stimulation of pathways involved in protein homeostasis (ribosomal pathways for example) as well as the inhibition of *DDIT4* (or *REDD1*), a powerful inhibitor of mTORC1 activity,[51] can result from a decrease in myostatin and could represent a counter-regulatory mechanism to preserve muscle mass after bariatric surgery.[50] Additionally, the decline in *MSS51*, an effector of myostatin on metabolic processes including fatty acid oxidation, glycolysis, and oxidative phosphorylation,[52,53] should result in increased mitochondrial function, as often expected after bariatric surgery.[54,55] However, our data showed downregulation of mitochondrial pathways 3 months after bariatric surgery, in agreement with others.[56] This decrease in mitochondrial activity could result from a dramatic drop in energy intake and may contribute to a decrease in energy expenditure, as observed at

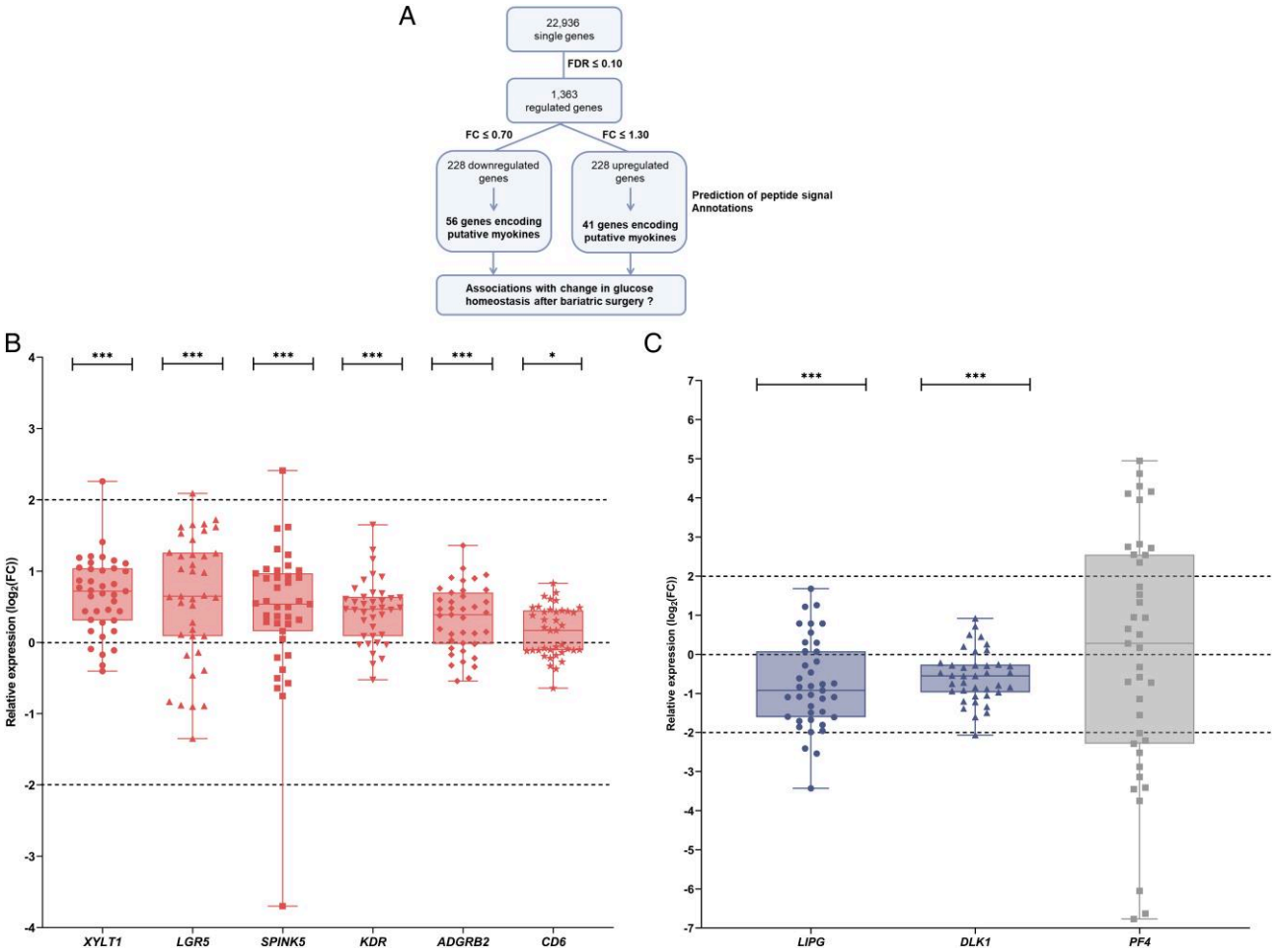

Figure 6. Untargeted study: identification of regulated genes encoding putative myokines. (A) Workflow for the identification of regulated genes encoding putative myokines. (B) Upregulated genes. (C) Downregulated and unchanged genes. $n = 39$ (RT-qPCR), results presented as median $\log_2$(FC) (min, max). Preoperative expression (control value) arbitrary set at 0. Wilcoxon signed-rank test performed on ΔCT to assess relative mRNA expression between presurgery and postsurgery visits. $^*P < .05$ and $^{***}P < .001$. FC, fold change; FDR, false discovery rate; RT-qPCR, real-time quantitative polymerase chain reaction.

Table 3. Regulated genes encoding putative myokines.

Ensembl gene ID	Gene symbol	Protein name	TPM T0	TPM T3	FC	FDR
ENSG00000166897	*ELFN2*	Protein phosphatase 1 regulatory subunit 29	.020	.077	2.51	7.46E−2
ENSG00000133710	*SPINK5*	Serine protease inhibitor Kazal type 5	.267	.539	1.94	2.74E−02
ENSG00000103489	*XYLT1*	Xylosyltransferase 1	.193	.338	1.55	3.84E−02
ENSG00000121753	*ADGRB2*	Adhesion G protein–coupled receptor B2	.733	1.112	1.50	3.48E−02
ENSG00000139292	*LGR5*	Leucine-rich repeat-containing G-protein–coupled receptor 5	5.719	9.016	1.50	1.07E−02
ENSG00000128052	*KDR*	Vascular endothelial growth factor receptor 2	1.607	2.016	1.31	4.02E−02
ENSG00000160097	*FNDC5*	Fibronectin type III domain-containing protein 5 (= irisin)	319.9	225.3	.69	9.76E−07
ENSG00000185559	*DLK1*	Protein delta homolog 1	18.29	12.10	.63	7.11E−09
ENSG00000013725	*CD6*	T-cell differentiation antigen CD6	.804	.319	.46	1.55E−02
ENSG00000101670	*LIPG*	Endothelial lipase	.116	.039	.31	5.50E−02
ENSG00000163534	*FCRL1*	Fc receptor-like protein 1	.160	.022	.21	8.89E−2
ENSG00000163737	*PF4*	Platelet factor 4	3.188	.720	.19	5.50E−02

T0 and T3 refer to before surgery and after surgery results, respectively.
Abbreviations: FC, fold change; FDR, false discovery rate; TPM, transcripts per million.

early times after bariatric surgery.[57,58] Taken together, these data indicate that myokines such as myostatin could be involved in altered metabolic pathways after bariatric surgery.

Our study has several limitations. Our cohort includes both SG and RYGB. Although RYGB may be slightly more effective than SG for early improvement of glucose homeostasis,[59] SG and RYGB have the same time course for changes in body weight, muscle insulin sensitivity, and muscle transcriptome.[60] In addition, we found no significant association between myokines and type of surgery. We chose a unique early time point while patients are in a dynamic phase of weight loss and catabolism. Our data may therefore not be

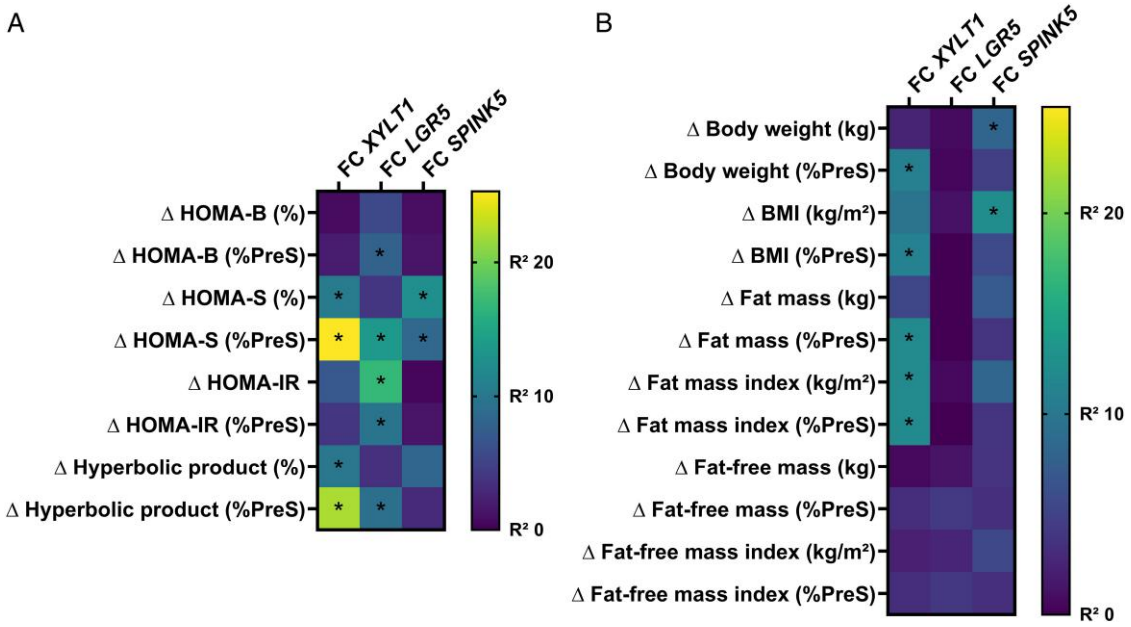

Figure 7. Associations between putative myokines expression, changes in glucose homeostasis, and changes in body composition after bariatric surgery. (A) Univariate linear regression between changes in myokines expression (fold change) and changes in glucose homeostasis (HOMA test). (B) Univariate linear regression between changes in body composition and changes in myokines expression (fold change). $n = 39$; scale represents R^2. *$P < .10$. %PreS, change relative to presurgery value; HOMA, homeostasis model assessment; HOMA-B, homeostatic model assessment of beta-cell function; HOMA-IR, HOMA insulin resistance; HOMA-S, HOMA insulin sensitivity; Δ, change from presurgery value.

Table 4. Untargeted study: associations between glucose homeostasis, body composition, and myokines (multivariate linear regression analyses, adjustment for age and sex).

Dependent variables	Independent variables	*N*	Unadjusted estimate (95% CI)	Adjusted estimate (95% CI)	*P*-value
Changes in myokines associated with changes in glucose homeostasis					
Myokines expression					
HOMA-S (absolute change)	*SPINK 5* (fold change)	39	17.8 (2.1-33.6)	16.5 (0.87-32.19)[a]	.030
HOMA-S (relative change)	*XYLT1* (fold change)	39	113.23 (48.57-177.90)	109.1 (28.5-189.8)[a]	.009
HOMA-IR (absolute change)	*LGR5* (fold change)	39	−0.52 (−0.91 to −0.14)	−0.50 (−0.86 to −0.13)[a]	.009
HOMA-B × S (relative change)	*XYLT1* (fold change)	39	44.0 (16.5-71.5)	43.8 (13.5-74.1)[a]	.006
Changes in body composition associated with changes in myokines					
Myokines expression					
SPINK5 (fold change)	Absolute change in BMI	38	1.13 (1.01-1.27)	1.14 (1.01-1.29)[b]	.030
XYLT1 (fold change)	Relative change in fat mass	38	0.986 (0.974-0.998)	0.98 (0.97-0.99)[b]	.020

Body composition was assessed in 60 patients. Muscle biopsies were taken in 39 patients.
Abbreviations: 95 CI, 95% confidence interval; BMI, body mass index; HOMA-B, homeostatic model assessment of beta-cell function; HOMA-IR, homeostatic model assessment of insulin resistance; HOMA-S, homeostatic model assessment of insulin sensitivity .
[a]Adjusted estimate should be interpreted as the mean change in the dependent variable for one unit of change in the independent variable.
[b]Adjusted estimate should be interpreted as follows (estimate − 1) × 100 = percentage change in the dependent variable for one unit increase in the independent variable.

representative of later time points. We however consider that a 3-month assessment is valid given that significant weight loss and improved glucose homeostasis were observed in our patients. The hyperinsulinemic euglycemic clamp and/or the oral glucose tolerance test would have provided a better assessment of glucose homeostasis than the HOMA test. However, we expect that muscle insulin resistance was improved in our patients since improved muscle insulin resistance has been reported in patients with similar weight loss 3 months after bariatric surgery using gold-standard methods (ie, hyperinsulinemic euglycemic clamp and 3-stage hyperinsulinemic euglycemic pancreatic clamp).[2,61] Muscle gene expression is influenced by contraction and insulin.[18,62,63] We could have missed myokines whose basal expression levels are low at rest and after an overnight fast. Increased physical activity after bariatric surgery could be a confounding factor. Indeed, exercise also improves insulin sensitivity and alters myokine expression.[17,18,62,64] The RNA analysis does not allow us to determine which muscle cell populations contribute the most to gene expression. Finally, some myokines could be influenced by sex hormones.[65] Nevertheless, multivariate regression analyses were adjusted for sex. We are convinced that the longitudinal design and the number of patients in whom muscle biopsies were taken are the main strength of our study.

In conclusion, our study found that changes in myokines including BDNF as well as XYLT1, LGR5, and SPINK5, three putative myokines, are associated with improved glucose

homeostasis after bariatric surgery. Our study also showed that myokines such as myostatin could be involved in metabolic processes altered by bariatric surgery such as the dynamics of protein synthesis and mitochondrial pathways.

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Supplementary material

Supplementary material is available at *European Journal of Endocrinology* online.

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Conflicts of interest: None declared.

Data availability

Some or all data sets 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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