Potential contribution of myokines

to changes in glucose homeostasis and muscle mass

after bariatric surgery

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LIST OF ABBREVIATIONS

4EBP-1: eIF4E binding protein-1

ADAMTS9: A disintegrin and metalloproteinase with thrombospondin motifs 9

ADP: adenosine diphosphate

AMPK: 5′-adenosine monophosphate-activated protein kinase

ANG: angiogenin

ATGL: adipose triglyceride lipase

ATP: adenosine triphosphate

BACE1: beta-secretase 1

BDNF: brain-derived neurotrophic factor

BMI: body mass index

BTD: biotinidase

CA11: carbonic anhydrase-related protein 11

CD24: signal transducer CD24

COL18A1: collagen alpha-1(XVIII) chain

CPT1: carnitine palmitoyl transferase 1

CTRP: C1q/TNF-related protein

CTSB: cathepsin B

CX3CL1: fractalkine

DXA: dual x-ray absorptiometry

EPO: erythropoietin

ELISA: enzyme-linked immunosorbent assay

FABP3: fatty acid-binding protein 3

FCGR1A: high affinity immunoglobulin gamma Fc receptor I

FGF21: fibroblast growth factor 21

FNDC5: irisin (protein fibronectin type III domain-containing protein 5)

FSTL-1: follistatin-related protein 1

GDE1: glycerophospho-diester phosphodiesterase 1

GLUT4: glucose transporter 4

p70S6K1: p70S6 kinase 1

PDH: pyruvate dehydrogenase

PGC1A: peroxisome proliferator-activated receptor gamma coactivator 1-alpha

PI3K: phosphatidylinositol 3-kinase

PPAR: peroxisome proliferator-activated receptors

RT-qPCR: real-time quantitative polymerase chain reaction

SFRP4: secreted frizzled-related protein 4

SMAD: mothers against decapentaplegic homolog

SPARC: secreted protein acidic and rich in cysteine (or osteonectin)

gp130: glycoprotein 130

GSK3: glycogen synthase 3

GWAS: genome-wide association studies

HbA1c: glycated hemoglobin A1c

HOMA: Homeostasis Model Assessment

HOMA-B: steady state beta cell function estimate

HOMA-IR: Homeostasis Model Assessment of insulin resistance

HOMA-S: steady state insulin sensitivity estimate

HSL: hormone-sensitive lipase

IGF-I: insulin-like Growth Factor I

IL: interleukin

IL-6: interleukin-6

IL-15: interleukin-15

IGLV5-52: immunoglobulin lambda variable 5-52

ILR11A: interleukin-11 receptor subunit alpha

IMAT: intermuscular adipose tissue

IRS-1: insulin-responsive substrate-1

LGR5: leucine-rich repeat-containing G protein-coupled receptor 5

LIF: leukemia inhibitory factor

MAFbx: muscle atrophy F box (or atrogin-1)

MCP-1: monocyte chemoattractant protein-1 (or CCL-2)

MEF2: myocyte enhancer factor 2

Metrnl: meteorin-like protein

MHC: myosin heavy chain

MMP-2: metalloprotease-2

mTORC1: mechanistic target of rapamycin complex 1

MuRF1: muscle RING finger 1

NGS: next-generation sequencing

SPINK5: serine protease inhibitor Kazal type 5

SREBP1c: sterol regulatory element-binding protein 1c

TGF: transforming growth factor
THSD8: thrombospondin type-1 domain-containing protein 8

TIMP2: metalloproteinase inhibitor 2

TNF-a: tumor necrosis factor-a

TNFRSF11B: TNF receptor superfamily member 11b (or osteoprotegerin, OPG)

TRIM72: tripartite motif-containing protein 72 (or MG53, mitsugumin-53)

VEGF: vascular endothelial growth factor

XYLT1: xylosyltransferase 1

I. GENERAL INTRODUCTION

Obesity is a complex chronic disease characterized by excessive fat accumulation that can adversely affect health (World Health Organization, 2023). The global prevalence of obesity has nearly tripled in the last 50 years, making obesity one of the leading epidemics of our time (Blüher, 2019). In 2022, 16% of the world adult population (890 million individuals) was living with obesity (World Health Organization, 2023). The fundamental cause of obesity is a long-term energy imbalance between too many calories consumed and too few calories expended (Blüher, 2019). From an evolutionary perspective, selection pressure most likely contributed to a genotype that favors overfeeding and low energy expenditure, conferring an advantage in surviving periods of undernutrition (Blüher, 2019; Loos et al.; 2017; Rohde et al., 2019). However, changes in population genetics cannot explain the rapid increase in obesity prevalence. In contrast, the rapid environmental and societal changes known as the “Westernization” of lifestyle have fostered an “obesogenic” environment characterized by increased availability of highly palatable, energy-dense food, and reduced physical activity (Blüher, 2019).

Observational studies have consistently reported adverse associations between higher body mass index (BMI), morbidity, and mortality (Padwal et al., 2011). Excess weight *per se* has many health consequences, ranging from musculoskeletal diseases (e.g., osteoarthritis due to mechanical overload) to

psychosocial burden, including reduced quality of life and unemployment (Blüher, 2020). Specifically, a proportionally greater amount of fat in the upper body (i.e., visceral fat distribution) compared to the lower body (i.e., gluteo-femoral fat distribution) is strongly associated with metabolically unhealthy obesity, chronic low-grade inflammation, insulin resistance, and metabolic syndrome (Blüher, 2020; Fazakerley et al., 2019). Overall, obesity is an established risk factor for cardiometabolic diseases including type 2 diabetes, metabolic dysfunction-associated steatotic liver disease, atherogenic dyslipidemia, hypertension, atherosclerotic cardiovascular diseases, and some cancers (Klein et al., 2022; Blüher, 2020; Padwal et al., 2011). These obesity-related diseases contribute to premature death, such that obesity has replaced tobacco as the leading lifestyle-related risk factor (Blüher, 2019).

Most people living with type 2 diabetes are overweight or obese (Ahmad et al., 2022). However, the mechanisms that lead from excessive fat accumulation to type 2 diabetes are not fully understood (Ahmad et al., 2022; Kahn, 2003; Roden et al., 2019). Adipose tissue is a central metabolic and endocrine organ in the regulation of whole-body energy homeostasis, storing energy in the form of triglycerides (Klein et al. 2022). Insulin exerts many actions on glucose and lipid metabolism in adipose tissue that are essential for whole-body glucose homeostasis, particularly the inhibition of lipolysis (Santoro et al., 2021; Fazakerley et al., 2019). The anti-lipolytic action of insulin regulates the flux of fatty acids

to the liver and skeletal muscle, preventing ectopic fat accumulation (Santoro et al., 2021). However, chronic overfeeding causes adipose tissue to expand through adipocyte hypertrophy and hyperplasia (Klein et al., 2022; Santoro et al., 2021). When adipose tissue can no longer expand, ectopic fat accumulates in distant organs, including insulin-sensitive tissues such as the liver and skeletal muscle, as well as in the pancreas (Santoro et al., 2021). In addition, the expansion of adipose tissue induces adipocyte hypoxia because local blood vessels do not expand adequately to meet the increased oxygen demand (Klein et al., 2022). Dead adipocytes stimulate macrophage chemotaxis, adipose tissue fibrogenesis and inflammation, resulting in the sustained release of pro-inflammatory adipokines (e.g., interleukin-6) and toxic bioactive lipids (e.g., diacylglycerol, ceramides) (Klein et al., 2022; Santoro et al., 2023). Together, ectopic fat accumulation and adipose tissue inflammation cause lipotoxicity and chronic low-grade inflammation, which are important drivers of multi-organ insulin resistance and beta cell dysfunction (Czech, 2017; Roden et al., 2019; Santoro et al., 2021; Klein et al., 2022). Interestingly, only a few genes contributing to type 2 diabetes risk are associated with insulin resistance (e.g., *ADAMTS9*), while most are associated with beta cell dysfunction (e.g., *TCF7L2*) (Graae et al., 2019; Bonnefond et al., 2015). Thus, excessive fat accumulation primarily promotes the onset of multi-organ insulin resistance, which exacerbates a genetically

determined propensity for beta cell dysfunction, ultimately leading to chronic hyperglycemia and type 2 diabetes (Kahn, 2003).

Bariatric surgery is effective in treating obesity and type 2 diabetes, leading to the concept of metabolic surgery (Nguyen et al., 2017; ElSayed et al., 2023; Mingrone et al., 2021). Weight loss after bariatric surgery is achieved mainly through caloric restriction and is likely the predominant factor in improving glucose homeostasis (Yoshino et al., 2020; Miras et al., 2013; Bradley et al. 2012; Madsbad et al., 2014; Sandoval et al., 2023). Reduction in total and visceral adipose tissue, inflammatory markers, ectopic fat accumulation, and restoration of anti-inflammatory adipokines (e.g., adiponectin) after bariatric surgery contribute to improved insulin sensitivity and beta cell function (Sandoval et al., 2023; Madsbad et al., 2014). In addition, the observation that hepatic insulin sensitivity and glucose control in people with type 2 diabetes improve within days of bariatric surgery has prompted the hypothesis of weight loss-independent mechanisms, including changes in gastrointestinal hormones (e.g., increased postprandial glucagon-like peptide-1 secretion), bile acids, branched-chain amino acids, and gut microbiota (Sandoval et al., 2023; Miras et al., 2013; Bradley et al., 2012; Klein et al., 2023). Roux-en-Y gastric bypass and sleeve gastrectomy are the most performed procedures (Nguyen et al., 2017). Roux-en-Y gastric bypass is similarly to or slightly more effective than sleeve gastrectomy in inducing weight loss and type 2 diabetes remission (Courcoulas

et al., 2024; Buchwald et al., 2004; Hofsø et al., 2019). Overall, the remission rate of type 2 diabetes is generally related to the amount of weight loss, regardless of the surgical procedure (Nguyen et al., 2017). The remission rate of type 2 diabetes is estimated at 51% at 1 year and 20% at 7 years using the consensus definition of type 2 diabetes remission after bariatric surgery (i.e., HbA1c < 6.5% without glucose-lowering medications for ≥ 3 months) (Courcoulas et al., 2024). Bariatric surgery leads to a global improvement in obesity- and diabetes-related diseases, quality of life, and long-term survival (Nguyen et al., 2017; Sjöström et al., 2014). The risks of laparoscopic procedures are now considered acceptable in relation to the health benefits (Nguyen et al., 2017). However, caloric restriction and weight loss inevitably lead to a loss of skeletal muscle mass and a decrease in energy expenditure, effects that could hinder weight loss and maintenance (Madsbad et al., 2014).

Skeletal muscle is the largest tissue in the human body. Skeletal muscle accounts for 30-50% of body weight and 50-75% of total body proteins in healthy people (Sartori et al., 2021). Skeletal muscle mass and strength are important determinants of health, survival, and quality of life (Cruz-Jentoft et al., 2019; Donini et al., 2022). While skeletal muscle is essential for vital functions such as posture, locomotion, and breathing, it is also a dynamic metabolic tissue involved in energy metabolism, nutrient storage, and thermogenesis (Sartori et al., 2021). Specifically, skeletal muscle plays a central role in whole-

body glucose homeostasis as the primary site of glucose uptake in response to insulin (Sylow et al., 2021; DeFronzo et al.,1981). In addition, skeletal muscle is an endocrine tissue that secretes myokines, some of which are involved in the regulation of whole-body glucose homeostasis, muscle mass and function by acting in an auto/paracrine or endocrine fashion (Barlow et al., 2018; Eckardt et al., 2014; Lee et al, 2019). Obesity, physical inactivity, and aging alter skeletal muscle metabolic and functional homeostasis, promoting type 2 diabetes and sarcopenia (Cruz-Jentoft et al., 2019; Donini et al., 2022; Sylow et al. 2021; Park et al., 2009). In addition, obesity and sarcopenia can reinforce each other in a vicious cycle of muscle fat infiltration and inflammation, muscle fatigue, decreased physical activity and energy expenditure, and further weight gain promoting sarcopenic obesity (Donini et al. 2022; Prado et al., 2024). Paradoxically, muscle insulin sensitivity and function improve significantly after bariatric surgery, even though muscle mass is reduced (Yoshino et al., 2020; Donini et al., 2022; Davidson et al., 2018). The mechanisms by which bariatric surgery improves muscle metabolism and function while causing muscle mass loss are incompletely understood. In particular, the potential contribution of myokines to changes in glucose homeostasis, muscle mass and function after bariatric surgery is not established.

1. The skeletal muscle

1. 1. Skeletal muscle structure

Skeletal muscle is organized at several structural levels (Figure 1). First, skeletal muscle is surrounded by a fibrous connective tissue called the epimysium, which in turn is covered by a fascia that defines and maintains the entire muscle. Second, each muscle contains several bundles of muscle fibers grouped into fascicles and bounded by the perimysium. The muscle fibers, surrounded by the endomysium, are the constitutive elements of skeletal muscle. Muscle fibers, also called myofibers or muscle cells, are long, multinucleated, cylindrical cells. Their cytoplasm, called sarcoplasm, is bounded by a plasma membrane (sarcolemma) and contains hundreds to thousands of myofibrils composed of successive functional contractile units called "sarcomeres". The sarcomere is composed of thick and thin filaments composed of myosin and actin, respectively, that slide over each other to produce muscle contraction (Frontera et al., 2015). Myofibrillar proteins, mainly composed of actin and myosin, are the predominant protein pool in muscle tissue, accounting for 60-65% of all muscle proteins (Paulussen et al., 2021).

In addition to muscle fibers, the skeletal muscle bed consists of satellite cells (myogenic precursors), as well as fibroadipogenic progenitors and myo-endothelial cells, as well as the adjacent vascular and motor nerves and,

finally, immune cells (Sylow et al., 2021). Together, these diverse cell types are involved in the maintenance of skeletal muscle integrity and plasticity, and their interactions affect muscle metabolism and functions as well as all measurements made on the intact tissue (e.g., RNA analysis). Interactions between progenitor cells, immune cells, nerve endings and capillaries are critical for integrated functions such as growth, regeneration and senescence, but also influence muscle response to insulin and contraction (Sylow et al., 2021). Fibroadipogenic progenitor cells contribute to the integrity of the extracellular environment. However, these cells promote the deposition of intramuscular and intermuscular adipose tissue in obesity and type 2 diabetes (Sylow et al., 2021), which impairs insulin action (see myosteatosis below). Resident and blood-derived immune cells affect muscle metabolism and are essential for muscle repair. However, these cells promote muscle inflammation in obesity and type 2 diabetes, which also reduces insulin action (Wu et al., 2017) (see muscle inflammation below).

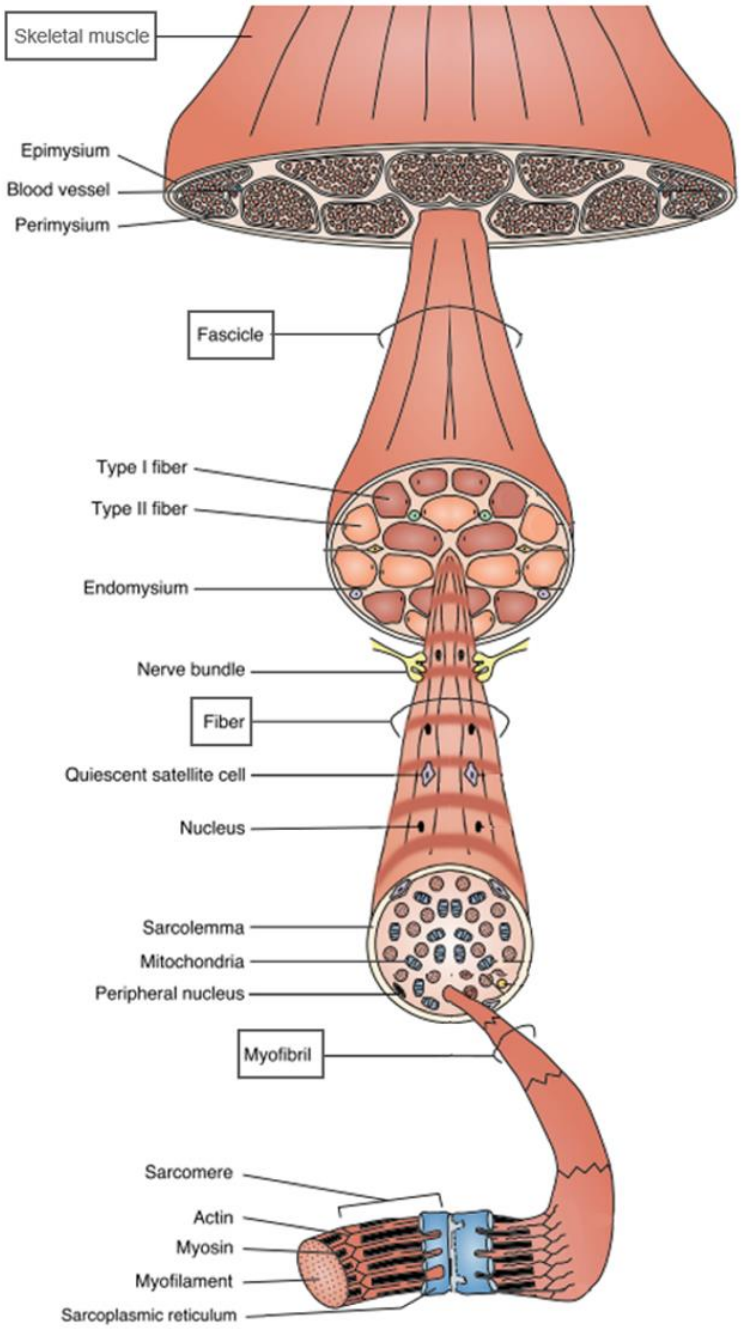


Figure 1. Skeletal muscle structure (adapted from Martin et al., 2021).

1. 2. Skeletal muscle functions

a. Muscle contraction

The main function of skeletal muscle is to generate force by converting biochemical energy into mechanical energy, to produce movement and locomotion, maintain posture, and exert vital functions such as breathing (Tallis et al., 2018, Egan et al., 2013). Force generation is based on excitation-contraction coupling. Briefly, the release of the neurotransmitter acetylcholine at the neuromuscular junction after motor neuron excitation leads to a change in the muscle membrane potential (action potential), which triggers an increase in intracellular calcium from the sarcoplasmic reticulum. By binding to troponin complexes, calcium exposes the tropomyosin active binding sites of the actin filament, leading to cross-bridging with myosin. Then, the actin and myosin filaments slide past each other, resulting in muscle contraction. This mechanism consumes a lot of adenosine triphosphate (ATP). When nerve stimulation ends, calcium is rapidly pumped back into the sarcoplasmic reticulum, resulting in relaxation.

b. Muscle metabolism

Skeletal muscle is dynamic metabolic tissue involved in energy metabolism, nutrient storage, and thermogenesis (Sartori et al., 2021). Specifically, skeletal muscle plays a central role in whole-body glucose homeostasis as the

primary site of glucose uptake and storage as glycogen in response to insulin (Sylow et al., 2021; DeFronzo et al., 1981). At the same time, skeletal muscle is the largest reservoir of proteins and amino acids in the human body (Sartori et al., 2021). Amino acids are released from muscle protein breakdown during fasting and are either recycled into new muscle protein, oxidized for energy, or taken up by other tissues for oxidation, gluconeogenesis (e.g., liver), or to maintain essential protein stores (e.g., heart) (Sartori et al., 2021; Sylow et al., 2021; Egan et al., 2013; Wolfe et al., 2017). Skeletal muscle stores lipids as intramyocellular triglycerides and within adipocytes dispersed between the muscle fibers to serve as energy source during fasting and exercise (Sachs et al., 2019). Finally, muscle can generate heat to maintain the body temperature.

c. Endocrine function

Skeletal muscle was first proposed as an endocrine organ over two decades ago, based on evidence that human skeletal muscle releases interleukin (IL)-6 into the circulation during exercise (Steensberg et al., 2000; Barlow et al., 2018). Since then, numerous bioactive molecules, including proteins, exosomes, metabolites and microRNAs, have been identified as part of the human muscle secretome using computational methods and omics (Bortoluzzi et al., 2006; Hartwig et al., 2014; Le Bihan et al., 2012; Barlow et al., 2018). Specifically, proteins that are released by skeletal muscle are called myokines (Febbraio et al., 2005; Eckardt et al., 2014). More than 600 myokines have been identified in

conditioned media from human muscle cells using mass spectrometry, the most accurate and specific method currently available for secretome analysis (Florin et al., 2020). The expression and secretion of some myokines is regulated by muscle contraction, as observed in human muscle cells and their conditioned media after electrical pulse stimulation, and in human muscle tissue and blood samples after exercise (Raschke et al., 2013; Pourteymour et al., 2017; Catoire et al., 2014). Myokines exert auto, para, or endocrine effects, enabling them to regulate many aspects of metabolism in various tissues, including muscle insulin sensitivity, muscle mass and strength, as well as glucose-stimulated insulin secretion (Figure 2) (Carson, 2017; Eckardt et al., 2014; Barlow et al., 2018; Lee et al., 2019). However, some myokines may exert opposite effects in healthy conditions and metabolic diseases. In addition, the effects of certain myokines are only observable in the context of cellular stress (e.g., inflammation, fasting, exposure to palmitic acid) because their expression and secretion are otherwise low under basal conditions. It is generally accepted that myokines may contribute to the benefits of exercise on whole-body glucose homeostasis, muscle mass, and function by mediating crosstalk between muscles and other organs, including the liver, adipose tissue, heart, brain, and pancreas (Barlow et al., 2018; Leuchtmann et al., 2021).

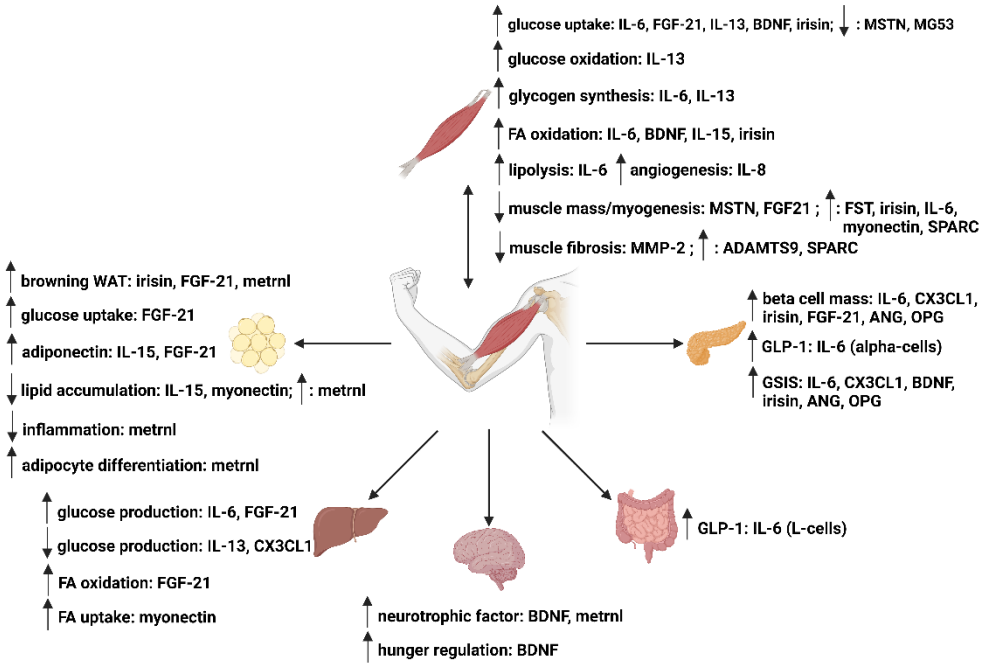


Figure 2. Myokines and their effects on metabolism and muscle mass. Schematic representation (Eckardt et al., 2014, Carson, 2017, Barlow et al., 2018 and Lee et al., 2019). ADAMTS9: ADAM metallopeptidase with thrombospondin type 1 motif 9; ANG: angiogenin; BDNF: brain-derived neurotrophic factor; CX3CL1: fractalkine; FA: fatty acids; FGF21: fibroblast growth factor 21; FST: follistatin; GLP-1: glucagon-like peptide-1; IL: interleukin; metrnl: meteorin-like protein; MG53: mitsugumin (or tripartite motif containing 72, TRIM72); MMP-2: metalloprotease-2; OPG: osteoprotegerin; SPARC: secreted protein acidic and rich in cysteine (osteonectin); WAT: white adipose tissue. Created with Biorender.com.

1.3. Muscle glucose metabolism and its regulation

a. Muscle glucose metabolism

Skeletal muscle plays a central role in whole-body glucose homeostasis in humans as the primary site of insulin-stimulated glucose uptake (≈80%) and glycogen synthesis (Sylow et al., 2021; DeFronzo et al., 2009; Klein et al., 2022).

Binding of insulin to its surface receptors initiates a cascade of molecular events that result in the translocation of an inducible pool of glucose transporters 4 (GLUT4) from intracellular stores to the sarcolemma, rapidly increasing glucose uptake in insulin-sensitive muscle (Figure 3) (Fazakerley et al., 2019; Sylow et al., 2021). Specifically, insulin binds to insulin receptors located at the sarcolemma, triggering their autophosphorylation and the recruitment and phosphorylation of insulin-responsive substrates (IRS, IRS-1), leading to phosphatidylinositol 3-kinase (PI3K) activation. PI3K activation is a bifurcation point that activates two parallel signaling cascades required for GLUT4 translocation, the serine-threonine kinase Akt and the Rho family GTPase Rac1. Downstream of these nodes, signaling leads to the engagement of molecules directly involved in vesicle sorting and mobilization via AS160/TBC1D4 and reorganization of the actin cytoskeleton. Akt-dependent phosphorylation of TBC1D4 eliminates the inhibitory effect imparted by unphosphorylated TBC1D4 on its target Rab GTPases, enabling their action on vesicle traffic functions. GLUT4 translocation is considered the rate-limiting step in insulin-dependent glucose uptake in muscle (Fazakerley et al., 2019). Glucose entering muscle fibers is phosphorylated by hexokinase to glucose-6-phosphate, then mainly oxidized (≈50%) or stored as glycogen (≈35%). The remaining glucose is released as lactate, alanine, and pyruvate by anaerobic glycolysis and used by other tissues as energy sources (Klein et al., 2022; DeFronzo et al., 2009). Glycogen synthase is activated

by an insulin-induced decrease in GS kinase 3 (GSK3) activity. Glycogen synthase is also allosterically activated by glucose-6-phosphate.

Muscle insulin sensitivity can be assessed *in vivo* using several validated techniques. The hyperinsulinemic-euglycemic clamp is the gold standard method for assessing whole-body glucose uptake *in vivo* (i.e., M-value), which is primarily dependent on muscle insulin sensitivity (Bradley et al., 2012). However, the hyperinsulinemic-euglycemic clamp is a complex procedure with limited daily application. The Homeostasis Model Assessment of insulin resistance (HOMA-IR) is commonly used as an acceptable surrogate marker of muscle insulin sensitivity, as HOMA-IR correlates reasonably well with the M-value in various populations (Park et al., 2021). HOMA-IR is easily calculated from fasting plasma insulin and glucose concentrations using the following formula [fasting glucose (mmoL/L)*fasting insulin (mUI/L)/22.5] (Matthews et al., 1985), or a more sophisticated mathematical model (i.e., HOMA-2) (https://www2.dtu.ox.ac.uk/homacalculator).

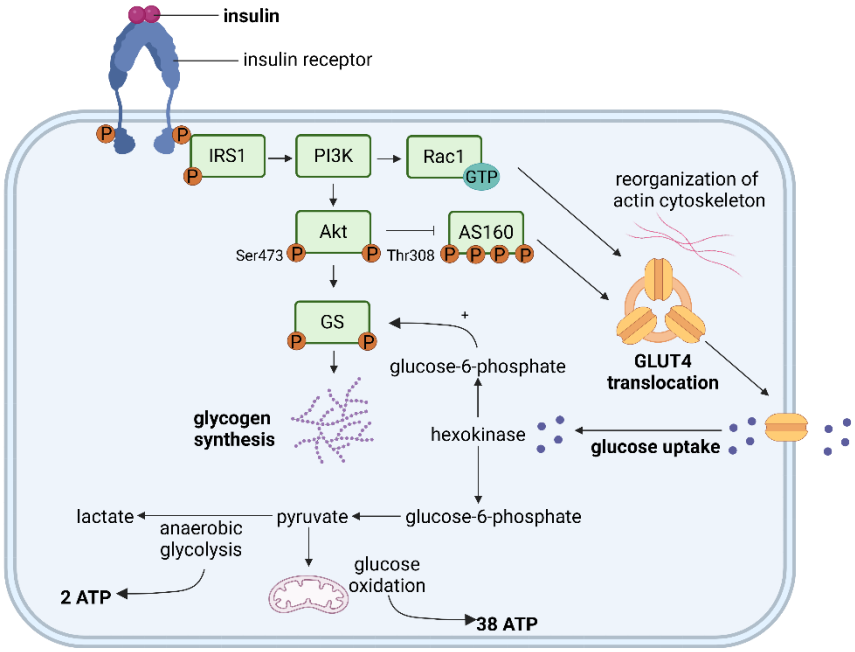


Figure 3. Insulin signaling to glycogen synthesis and fate of glucose entering muscle fibers. Schematic representation (adapted from Sylow et al., 2021). Created with Biorender.com.

b. Role of muscle fiber phenotype

The muscle fiber phenotype is a key determinant of insulin sensitivity and oxidative metabolism in skeletal muscle (Sylow et al., 2021). The metabolic and functional properties of muscle fibers depend on the relative expression of myosin heavy chain isoforms (MHC) and myosin ATPase activity, as well as mitochondrial content (Serrano et al., 2023; Egan et al., 2013; Tallis et al., 2018; Schiaffino et al., 2011). Human muscle fibers are classified as slow oxidative type I (MHC-I, gene: *MYH7*), fast oxidative type IIa (MHC-IIa, gene: *MYH2*), and fast glycolytic type IIx fibers (MHC-IIx, gene: *MYH1*) (Table 1). Type I muscle fibers are the most insulin sensitive due to their

higher protein content for glucose transport and phosphorylation, and glycogen synthesis (e.g. GLUT4, hexokinase, glycogen synthase) (Hood et al., 2019; Sylow et al., 2021; Tallis et al., 2018; Schiaffino et al., 2011; Serrano et al., 2023; Albers et al., 2015, a). In addition, type I muscle fibers contain many mitochondria, allowing them to rely more heavily on the oxidation of glucose and fatty acids to synthesize ATP, a process known as mitochondrial respiration, which is more energy efficient than anaerobic glycolysis (Hood et al., 2019). Functionally, type I muscle fibers are the most resistant to fatigue. They are mainly found in postural or mixed muscles (e.g. rectus abdominis, vastus lateralis) (Albers et al., 2015, a; Egan et al., 2013). At the other end of the physiological continuum, type IIx muscle fibers are glycolytic, rapidly fatigable but capable of producing greater force. They are mainly found in muscles for locomotion and functional tasks (e.g., triceps). Overall, a positive correlation between the proportion of type I muscle fibers in various muscles (e.g., rectus abdominis, vastus lateralis) and whole-body insulin sensitivity has been repeatedly found in humans (Stuart et al., 2013; Albers et al., 2015, a).

Table 1. Muscle fibers in humans: contractile, metabolic, and morphological characteristics

	Type I	Type IIa	Type IIx
General properties			
Myosin heavy-chain isoform	MHC1	MHC2A	MHC2X
Gene	*MYH7*	*MYH2*	*MYH1*
Contractile and metabolic properties	slow twitch high oxidative fatigue resistant	fast twitch oxidative-glycolytic fatigue resistant	fast twitch glycolytic, fast fatigable
Endurance capacity	High	Intermediate	Low
Force production	Weak	Intermediate	Strong
Morphological properties			
Mitochondrial density	High	Intermediate	Low
Distribution in muscle	~50%	~30%	~15%
Metabolic properties			
Oxidative potential	High	Intermediate-high	Low
Glycolytic potential	Low	Intermediate-high	High
Exercise-type dominance	Prolonged low intensity (aerobic/endurance)	Moderate duration, high intensity	Short duration, maximal effort (strength/resistance)

Data from vastus lateralis from untrained men (adapted from Egan et al., 2013)

c. Role of mitochondria and oxidative metabolism

The ability of muscle fibers to oxidize glucose and fatty acids in the mitochondria is essential for adaptation to changes in energy requirements during exercise and fasting (i.e., metabolic flexibility) (Pileggi et al., 2021). Physiologically, muscle fibers store lipids, mainly triglycerides, in the form of lipid droplets for membrane formation and energy production (Kalinkovich et al., 2017; Badin et al., 2013). Insulin stimulates the translocation of fatty acid transporters to the sarcolemma to promote fatty acid uptake and triglyceride storage during postprandial periods (Sylow et al., 2021). Lipolysis of intramyocellular lipids is mainly dependent on adipose triglyceride lipase

(ATGL) and hormone-sensitive lipase (HSL) (Badin et al., 2013). Long-chain fatty acids entering muscle fibers are esterified to long-chain fatty acids-CoA (LCFA-CoA) by acyl-CoA synthetases (ACS) (Figure 4A). LCFA-CoA is incorporated into intramyocellular triglycerides (IMTG) or enters mitochondria via carnitine palmitoyl transferase 1 (CPT1) for beta-oxidation (Figure 4B). On the other hand, glucose entering muscle fibers is broken down by glycolysis to pyruvate which can enter the mitochondria for oxidation (Figure 4A). Mitochondrial pyruvate dehydrogenase (PDH) catalyzes the oxidative decarboxylation of pyruvate to acetyl-CoA. Acetyl-CoA enters the Krebs (or tricarboxylic acid) cycle to produce hydrogen carriers (Figure 4B). Hydrogen carriers from the Krebs cycle and beta-oxidation are used in the electron transport chain (ETC) to synthesize ATP from adenosine diphosphate (ADP). This process, known as mitochondrial respiration, produces 38 ATP per molecule of glucose compared to 2 ATP per molecule glucose for anaerobic glycolysis. In addition, fatty acid oxidation is an energy efficient process that produces ≈ 9 kcal per gram of triglycerides, while glucose oxidation produces ≈ 4 kcal per gram of glycogen (Klein et al., 2022). Intramyocellular triglycerides are therefore an important energy source for muscle fibers, and their accumulation is not harmful if the oxidative capacity of muscle fibers is sufficient. This is the apparent athletes' paradox whose muscles are rich in intramyocellular triglycerides but highly insulin sensitive. Conversely, physical inactivity and obesity are associated with mitochondrial

dysfunction leading to intramyocellular accumulation of toxic bioactive lipids (i.e., diacylglycerol and ceramides) and oxidative stress (Santoro et al., 2023; Klein et al., 2022; Roden et al., 2019).

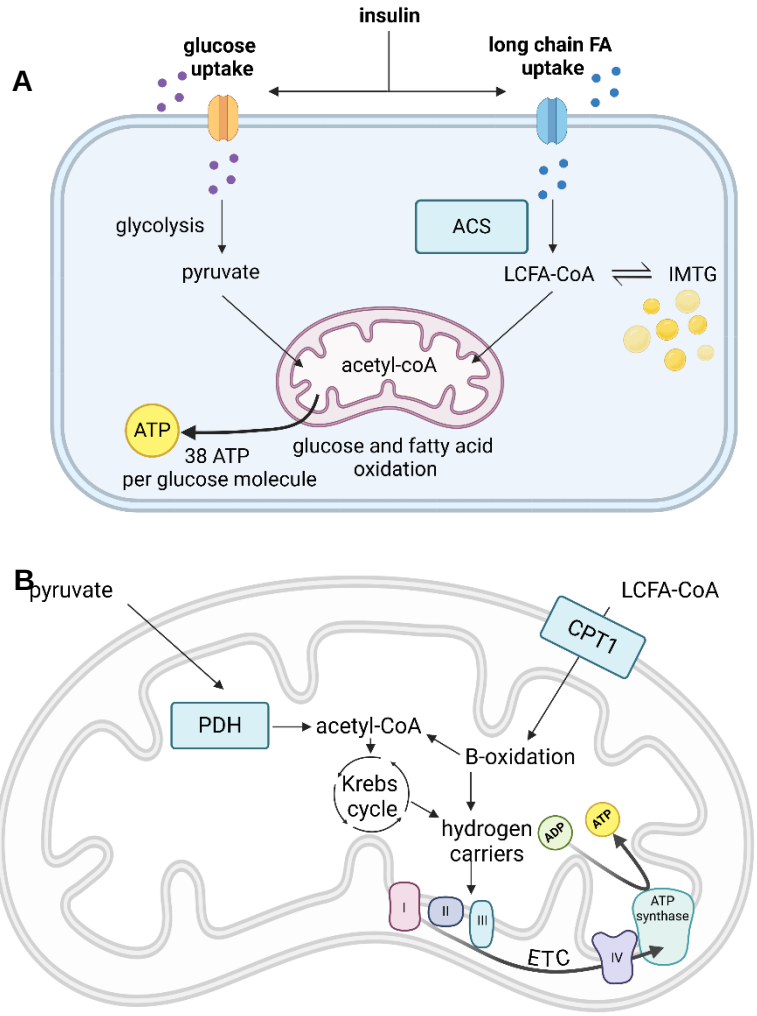


Figure. 4. Glucose and fatty acid oxidation in muscle fibers. (A) Fate of glucose and long-chain fatty acids entering muscle cells (LCFA). (B) Mitochondrial respiration. The oxidative phosphorylation protein complexes (complexes I-IV) of the electron transport chain (ETC) and the ATP synthase are in the inner mitochondrial membrane. IMTG: intramyocellular triglycerides, PDH: pyruvate dehydrogenase, Created with Biorender.com.

d. Role of extracellular matrix and muscle capillaries

The extracellular matrix is a dynamic three-dimensional multifaceted scaffold made of collagens, glycoproteins, elastin, and proteoglycans (Sylow et al., 2021). The extracellular matrix remodels physiologically in response to inflammation and injury, and modulates several physiological processes, ranging from muscle growth and repair to force transmission and muscle contraction (Sylow et al., 2021; Williams et al., 2015). These processes involve transmembrane cell surface receptors called integrins as well as satellite cells (i.e., muscle fiber progenitors), and immune cells for muscle growth and repair, and nerves for muscle contraction (Sylow et al., 2021). In addition, the extracellular matrix is filled with capillaries that provide oxygen, growth factors, insulin, and nutrients delivery to muscle fibers, especially during exercise and postprandial periods (Sylow et al., 2021; Williams et al., 2015; Paavonsalo et al., 2020). Insulin increases muscle capillary perfusion by acting on endothelial insulin receptors, leading to the production of nitric oxide, which increases glucose and insulin delivery to muscle fibers (Sylow et al., 2021). Capillary wall integrity, capillary density and extracellular matrix phenotype (e.g., basement membrane thickness) have been repeatedly reported as determinants of muscle insulin sensitivity in humans (Lillioja et al., 1987; Groen et al., 2014; Akerstrom et al., 2014; Tam et al., 2017; Dantas et al., 2020).

e. Role of muscle contraction and exercise

Hydrolysis of ATP by myosin ATPase provides the immediate energy source for actin-myosin cross-bridge cycling that generates muscle contraction (Egan et al., 2013). 5′-adenosine monophosphate-activated protein kinase (AMPK) activity is central for the metabolic response to muscle contraction (Tallis et al., 2018). AMPK acts as an energy sensor that is activated by energy depletion to facilitate ATP synthesis during exercise (Tallis et al., 2018). In skeletal muscle, acute AMPK activation suppresses glycogen and protein synthesis but promotes lipid oxidation and glucose uptake (Egan et al., 2013). Specifically, acute exercise increases AMPK phosphorylation and enzymatic activity. AMPK phosphorylates AS160/TBC1D4, a molecule that regulates GLUT4 trafficking, at some unique sites, allowing insulin-independent GLUT4 translocation and glucose uptake (Sylow et al., 2021; Egan et al., 2013). The insulin-sensitizing effect of a single bout of acute exercise lasts up to 48 hours in both type I and type II muscle fibers (Sylow et al., 2021). Chronic AMPK activation alters metabolic gene expression and induces mitochondrial biogenesis (Egan et al., 2013). Chronic exercise (i.e., exercise training) therefore results in a longer-lasting increase in insulin sensitivity due to increased GLUT4 and hexokinase expression, muscle capillarization, mitochondrial content and oxidative capacity which optimize glucose uptake, glycogen storage, and substrate utilization (Egan et al., 2013). Globally,

exercise induces short- and long-term metabolic adaptations in muscle that lead to insulin sensitization (Figure 5).

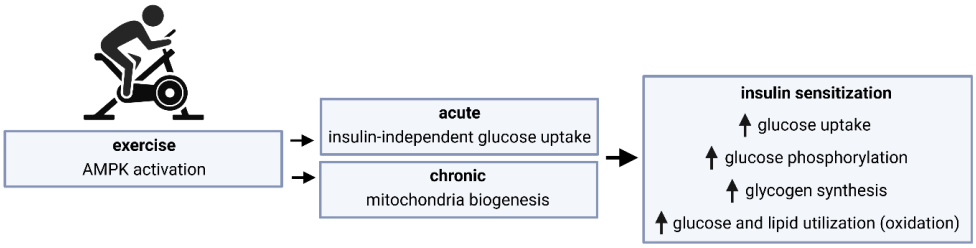


Figure 5. Acute and chronic effects of AMPK activation by exercise on glucose metabolism and insulin sensitivity in skeletal muscle. Schematic representation (Rothschild et al., 2022). Created with Biorender.com.

f. Role of myokines

Data from animal and cell models suggest that several myokines regulate muscle insulin sensitivity, either in a positive or negative manner. Some myokines also regulate glucose-stimulated insulin secretion, suggesting that myokines could be mediators of a muscle-pancreas crosstalk.

1. Brain-derived neurotrophic factor

Brain-derived neurotrophic factor (BDNF) is particularly expressed in the central and peripheral nervous system, where it acts as a potent neuronal survival factor and modulator of synaptic plasticity (Fulgenzi et al., 2020; Uhlén et al., 2015; Uhlén et al., 2019). In humans, 70-80% of circulating BDNF is derived from the brain (Eckardt et al., 2014). Nevertheless, BDNF is also secreted by

human muscle cells (Fulgenzi et al., 2020; Le Bihan et al., 2012; Raschke et al., 2013). Contraction upregulates BDNF mRNA expression in human muscle tissue, and increases its circulating levels (Catoire et al., 2014; Rentería et al., 2022; Matthews et al., 2009; Rodriguez et al., 2018). There are two major isoforms of the mammalian BDNF receptor TrkB, including TrkB.T1, which is expressed in the human pancreas (Fulgenzi et al., 2020).

BDNF has beneficial metabolic effects on muscle insulin sensitivity. Systemic BDNF injections in mouse models of obesity and diabetes improve blood glucose levels by increasing muscle glucose uptake (Tonra et al., 1999; Fulgenzi et al., 2020; Yamanaka et al., 2007). However, BDNF does not have a direct effect on glucose uptake in muscle cells (Eckardt et al., 2014; Tsuchida et al., 2001). In contrast, BDNF increases fatty acid oxidation in muscle by activating AMPK (Eckardt et al., 2014; Matthews et al., 2009). In addition, pancreatic BDNF-TrkB.T1 signaling promotes glucose-stimulated insulin secretion in human islets (Fulgenzi et al., 2020). In fact, muscle-specific BDNF knockout mice exhibit glucose intolerance and blunted insulin secretion, as well as reduced circulating BDNF levels, showing that muscle is a source of circulating BDNF that regulates beta cell function (Fulgenzi et al., 2020). Finally, mice and humans heterozygous for mutations inactivating BDNF or TrkB develop hyperphagic obesity, suggesting a role for BDNF in inhibiting hunger (Fulgenzi et al., 2020).

2. Irisin

Irisin is produced by the cleavage of the membrane protein fibronectin type III domain-containing protein 5 (FNDC5) (Boström et al., 2012). Although irisin is difficult to detect using commercially available ELISA kits, irisin was detected in human plasma samples using mass spectrometry (Jedrychowski et al., 2015). Circulating irisin levels increase in response to acute exercise (Huh et al., 2015; Carson, 2017). However, FNDC5 mRNA is not induced by acute exercise in muscle from healthy people (Catoire et al., 2014). FNDC5 mRNA is higher in human muscle than in liver and adipose tissue, suggesting that muscle could be a significant source of circulating irisin (Huh et al., 2012).

Irisin can exert beneficial effects on muscle insulin sensitivity. Mice fed a high-fat diet are indeed protected against obesity and diabetes by irisin treatment (Boström et al., 2012; Barlow et al., 2018). Similarly, irisin treatment reduces body weight and blood glucose levels in rats with type 2 diabetes (Liu et al., 2017). Muscle cells incubated with irisin exhibit increased glucose uptake and fatty acid oxidation mediated by AMPK, suggesting a direct effect of irisin on muscle (Lee et al., 2015; Carson, 2017; Xin et al., 2016). In addition, irisin treatment improves glucose-stimulated insulin secretion in mice by increasing insulin biosynthesis and beta cell mass (Natalicchio et al., 2017). Human beta cells and islets incubated with irisin are protected from palmitate-induced apoptosis, suggesting that irisin can protect beta cells

from lipotoxicity during obesity (Natalicchio et al., 2017; Barlow et al., 2018). In addition, irisin treatment induces a white-to-brown shift in mouse adipose tissue, which increases whole-body energy expenditure (Boström et al., 2012; Eckardt et al., 2014). Muscle cells incubated with irisin show increased mitochondrial uncoupling, PGC1A expression and mitochondrial fusion genes, resulting in increased oxidative metabolism (Vaughan et al., 2014).

3. Interleukin-6

Muscle is considered the main source of increased circulating IL-6 levels during exercise because contraction upregulates IL-6 expression and secretion by human muscle cells and muscle tissue (Raschke et al., 2013; Catoire et al., 2014; Pourteymour et al., 2017; Hiscock et al., 2004; Steensberg et al., 2000). Type II muscle fibers produce more IL-6 than type I muscle fibers during contraction (Hiscock et al., 2004).

IL-6 release from contracting muscle has been suggested to mediate some of the benefits of exercise on glucose homeostasis (Barlow et al., 2018). Indeed, an acute infusion of IL-6 enhances insulin-stimulated glucose uptake in healthy people, while chronic exposure to IL-6 increases glycogen synthesis and lipid oxidation via AMPK activation in primary human myotubes (Al-Khalili et al., 2006). In addition, acute IL-6 infusion stimulates lipolysis and fatty acid oxidation in muscle from healthy people (van Hall et al., 2003; Wolsk et al., 2010;

Petersen et al., 2005). IL-6 also stimulates insulin release by beta cells through the secretion of glucagon-like peptide-1 from intestinal L-cells and pancreatic alpha cells (Ellingsgaard et al., 2011). These data suggest that IL-6 could mediate a muscle-pancreas crosstalk.

4. Fibroblast growth factor 21

Fibroblast growth factor (FGF) 21, a member of the FGF19 subfamily, is mainly a hepatokine, but also an adipomyokine (Itoh, 2014; Faramia et al., 2020). FGF21 binds to the beta-Klotho complex, an FGF co-receptor that is highly expressed in metabolically active tissues such as the pancreas (Gao et al., 2019). Several observations support the role of FGF21 in glucose homeostasis. FGF21 treatment in both regular chow- and high-fat diet-fed mice improves hepatic and muscle insulin sensitivity, by decreasing hepatocellular and intramyocellular diacylglycerol content (Camporez et al., 2013). Systemic FGF21 administration lowers blood glucose levels in monkeys with diabetes (Gao et al., 2019). In addition, incubation of human myotubes with FGF-21 increases basal and insulin-stimulated glucose uptake, by upregulating GLUT1 expression, suggesting a direct effect of FGF21 on muscle (Mashili et al., 2011). FGF21 also protects beta cells from apoptosis, possibly by reducing glucolipotoxicity (Gao et al., 2019).

5. Myonectin

Myonectin (also known as erythroferrone or CTRP15) is a C1q/TNF-related protein (CTRP) mainly secreted by skeletal muscle (Seldin et al., 2012). Higher myonectin mRNA was found in slow-twitch and oxidative muscles compared to fast-twitch and glycolytic muscles (Seldin et al., 2012). Exercise and nutrient intake (e.g. glucose or lipids) upregulate muscle expression and circulating levels of myonectin in mice, in contrast to fasting and diet-induced obesity (Seldin et al., 2012). These data suggest that myonectin may be involved in muscle oxidative metabolism and that its induction by exercise could promote lipid uptake as a fuel source for liver and possibly for muscle (Seldin et al., 2012). In fact, myonectin increases fatty acid uptake into adipocytes and hepatocytes by increasing the expression of fatty acid transporters, similarly to insulin (Seldin et al., 2012). As a result, myonectin knockout mice have impaired lipid clearance from blood (Little et al., 2019), reduced liver steatosis but increased visceral fat mass and insulin resistance when fed a high-fat diet (Little et al., 2019). These findings suggest a role of myonectin in regulating local and systemic lipid metabolism in obesity. However, myonectin does not lower blood glucose levels (Seldin et al., 2012).

6. Myostatin

Myostatin, also known as growth differentiation factor 8 (GDF-8), is a member of the transforming growth factor (TGF)-beta superfamily that is expressed and secreted primarily by skeletal muscle and to a lesser extent by adipose tissue (Lee, 2021; Hartwig et al, 2014). Myostatin is expressed as a full-length precursor form, processed to latent myostatin by removal of its signal peptide, and subsequently activated by proteolytic cleavage by members of the bone morphogenetic protein-1 family of metalloproteases (Lee, 2021). Mature myostatin is regulated extracellularly by multiple binding proteins, including follistatin (Lee, 2021).

Although myostatin is mainly known as a negative regulator of muscle mass, animal and cell models suggest that myostatin play a pathogenic role in insulin resistance during obesity and type 2 diabetes. Myostatin inhibition indeed protects rodents from obesity and insulin resistance (Cleasby et al., 2014). Specifically, myostatin inhibition increases insulin-dependent and independent glucose uptake and glycogen synthesis in muscle of normal chow-fed mice (Cleasby et al., 2014). This effect is likely mediated by increased GLUT1 and GLUT4 protein levels, and increased muscle mass (Cleasby et al., 2014). Myostatin inhibition also improves muscle glucose uptake in obese high fat-diet-fed mice, by stimulating fatty acid metabolism and mitochondrial function (Eilers et al., 2020). In contrast, myostatin impairs insulin signaling,

decreases AMPK activity, and inhibits GLUT4 expression in muscle cells, thereby impairing muscle glucose uptake (Liu et al., 2018).

7. Other myokines

Leukemia inhibitory factor (LIF) is a cytokine and myokine that belongs to the IL-6 family (Brandt et al., 2015). LIF and its receptor are expressed by skeletal muscle and pancreatic duct cells, to induce the proliferation of progenitor cells (Broholm et al., 2012; De Breuck et al., 2006). Like IL-6, LIF acutely increases muscle glucose uptake in mice (Brandt et al., 2015). In addition, incubation of adult pancreatic exocrine cells with LIF results in their transdifferentiation into functional beta cells, suggesting a beneficial role of LIF in glucose homeostasis (Baeyens et al., 2005).

Follistatin-related protein 1 (FSTL-1) is a small glycoprotein that belongs to the same family as follistatin (Xu et al., 2020). FSTL-1 is secreted by human myotubes in response to contraction and by other tissues, including adipose tissue (Görgens et al., 2013; Xu et al., 2020). FSTL-1 stimulates glucose uptake in muscle cells by inducing GLUT4 protein expression and translocation in an AMPK-dependent manner (Lee et al., 2017).

Apelin is a 36 amino acid peptide and the endogenous ligand of the G protein-coupled receptor APJ receptor. Apelin is secreted by human muscle cells and various tissues including adipose tissue (Besse-Patin et al., 2014; Krist

et al., 2013). Apelin expression increases in response to insulin in adipocytes and to contraction in human muscle cells and tissue (Boucher et al., 2005; Besse-Patin et al., 2014). Muscle apelin expression increases after exercise and is associated with improved whole-body insulin sensitivity (Besse-Patin et al., 2014). In addition, treatment of apelin-null mice or obese insulin-resistant mice with apelin restores glucose tolerance by improving muscle glucose uptake and oxidation (Yue et al., 2010; Dray et al., 2008; Attané et al. 2012).

Fatty acid-binding protein 3 (FABP3) is the major isoform of cytosolic fatty acid binding protein (FABP) expressed in human skeletal muscle and heart (Rosa et al., 2007). FABPs are small proteins that facilitate fatty acid influx across the plasma membrane and transport to the mitochondrial beta-oxidation system (Storch et al., 2023). FABP3-deficient mice have increased plasma fatty acid levels and muscle fatigue during exercise due to impaired muscle fatty acid uptake (Furuhashi et al., 2008). In fact, muscle FABP3 expression increases during exercise and fatty acid exposure due to increased fatty acid demand or availability (Tunstall et al., 2002; Furuhashi et al., 2008). FABP3 is also upregulated in the muscle of obese insulin-resistant mice where it may stimulate fatty acid uptake and oxidation to prevent lipotoxicity (Kusudo et al., 2011). In addition, muscle cells overexpressing FABP3 show enhanced basal and insulin-stimulated glucose uptake (Kusudo et al., 2011).

Other myokines may negatively regulate glucose homeostasis, like myostatin. ADAM metallopeptidase with thrombospondin type 1 motif 9 (ADAMTS9) is a secreted metalloprotease active against proteoglycans (e.g., versican, aggrecan) (Graae et al., 2019). Computational analysis of the human secretome predicts ADAMTS9 as a myokine (Le Bihan et al., 2012; Bortoluzzi et al., 2006). The ADAMTS9 rs4607103 C allele is one of several genetic variants proposed to increase the risk of type 2 diabetes through impaired insulin sensitivity (Graae et al., 2019). This variant is associated with increased expression of ADAMTS9 and decreased insulin sensitivity, impaired insulin signaling, and mitochondrial markers in human muscle.

Tripartite motif containing 72 (TRIM72, also known as mitsugumin or MG53) is a striated muscle-specific E3 ligase that promotes ubiquitin-dependent degradation of the insulin receptor and IRS-1, inducing muscle insulin resistance (Wu et al., 2019; Song et al., 2013). MG53 is detected in conditioned media from human muscle cells (Le Bihan et al., 2012). High glucose and insulin, conditions mimicking obesity and type 2 diabetes, induce MG53 release by muscle (Wu et al., 2019). Similarly, oral administration of glucose increases circulating MG53 in healthy humans (Wu et al., 2019). In addition, systemic administration of recombinant MG53 causes systemic insulin resistance and metabolic syndrome in mice, by blocking the insulin receptor (Wu et al., 2019).

Of note, no reliable ELISA kits are available for the quantification of circulating human MG53.

1.4. Muscle mass and strength and their regulation

a. Muscle mass and its assessment

In addition to being a metabolically active tissue, skeletal muscle is essential for vital functions such as posture, locomotion, and breathing (Sartori et al., 2021). Muscle mass and function are recognized as important determinants of health, survival, and quality of life (Cruz-Jentoft et al., 2019; Donini et al., 2022). Skeletal muscle is the largest tissue in the human body, accounting for 30-50% of body mass in lean people, with approximately 50-60% of skeletal muscle mass in the lower body (Sartori et al., 2021; Janssen et al., 2000, a). Skeletal muscle mass is usually larger in men and young people than in women and older people.

Skeletal muscle mass can be assessed using a variety of validated methods *in vivo*, and reported as total body skeletal muscle mass, appendicular skeletal muscle mass (in limbs), or as muscle cross-sectional area of specific muscle groups (Cruz-Jentoft et al., 2019; Donini et al., 2022). Magnetic resonance imaging and computed tomography scan are considered the gold standards for measuring skeletal muscle mass, and can also assess muscle fat infiltration (i.e., myosteatosis) and fibrosis, which are emerging markers of

muscle quality (Cruz-Jentoft et al., 2019; Donini et al., 2022; Nuijten et al., 2022; Leung, 2019). However, their widespread use is limited by cost and availability. Dual-energy X-ray absorptiometry (DXA) is a reliable alternative, based on tissue absorption of X-rays with dual photon energy. DXA allows the differentiation of three compartments: bone mineral mass, fat mass and lean body mass (i.e., total body mass minus bone and fat mass) (Cruz-Jentoft et al., 2019; Donini et al., 2022). This technique allows a semi-direct assessment of skeletal muscle mass. Its main advantages are good reproducibility, precision and lower radiation dose and cost compared to magnetic resonance imaging and computed tomography. Bioelectrical impedance analysis is another reliable alternative that uses whole-body conductivity to provide an estimate of fat-free mass (i.e., muscles, non-muscle organs, and body fluids) (Nuijten et al., 2022; Donini et al., 2022). In fact, body impedance is inversely proportional to total body water and high-water content tissue mass such as skeletal muscle. Raw resistance and reactance measurements can be used in validated prediction equations to calculate total skeletal muscle mass and appendicular skeletal muscle mass (Janssen et al., 2000, b; Sergi et al., 2015). The advantages of bioelectrical impedance analysis are its low cost and non-invasive nature. However, measurements can be influenced by the hydration status. Skeletal muscle mass can be adjusted for body weight (i.e., percentage value) or square height (i.e. muscle mass index) for comparison between different populations. Notably, lean body mass and fat-free mass

are both considered accurate surrogates for total skeletal muscle mass because skeletal muscle is their main component and changes in skeletal muscle mass assessed by whole-body magnetic resonance imaging mirror those of fat-free mass (Davidson et al., 2018). For these reasons, lean body mass and fat-free mass are most often referred to as "skeletal muscle mass" in the following manuscript.

b. Muscle strength and its assessment

Muscle strength is used as the main measure of muscle function (Donini et al., 2022; Prado et al., 2024). There are three important indicators of muscle strength *in vivo*, including (1) absolute strength capacity of a muscle group, (2) strength adjusted for body weight or fat-free mass (as a surrogate of skeletal muscle mass), and (3) muscle quality, defined as strength adjusted for skeletal muscle mass (Tallis et al., 2018). Muscles undergo length changes during locomotion and functional tasks. Isokinetic measurements (e.g., isokinetic knee extension) are therefore considered the gold standard for evaluating muscle strength (Tallis et al., 2018). However, isometric handgrip strength is considered a reliable and convenient alternative because it is easily measured using a hand dynamometer (Cruz-Jentoft et al., 2019). Muscle strength correlates with skeletal muscle mass and is usually larger in men and young people than in women and older people (Janssen et al., 2000, b).

The muscle fiber phenotype influences muscle growth and force production. Indeed, single fiber studies show that type IIx and IIa muscle fibers have greater power than type I muscle fibers (Widrick et al., 2002). In addition, absolute force is greater in type II fibers because of the greater cross-sectional area of type IIx and IIa fibers than type I fibers, although no differences were found in specific force (force/cross-sectional area) (Malisoux et al., 2006). Finally, type II fibers have a greater capacity for exercise-induced hypertrophy (Widrick et al., 2002). Thus, although type I muscle fibers are more important for oxidative metabolism and muscle insulin sensitivity, type II muscle fibers are important for muscle mass and strength (Albers et al., 2015, a; Egan et al., 2013).

c. Role of insulin

Muscle protein synthesis and muscle protein breakdown are approximately equal throughout the day in healthy people (McGlory et al., 2019; Beals et al., 2019; Sartori et al.; 2021; Egan et al.; 2013). Insulin is a potent anabolic hormone that co-activates mechanistic target of rapamycin complex 1 (mTORC1) together with amino acids during postprandial periods (Figure 6) (Sartori et al., 2021; Beals et al., 2019; Sylow et al., 2021). mTORC1 is a key molecular inducer of muscle protein synthesis and mitochondrial biogenesis, which acts downstream of Akt via its key effectors, p70S6 kinase 1 (p70S6K1) and eIF4E binding protein (4EBP-1) (Sartori et al., 2021; Egan et al., 2013; Sylow et al., 2021). In addition,

molecular events downstream of the insulin receptors, including mTORC1 activation, inhibit the autophagy-lysosome and the ubiquitin-proteasome pathways (Sartori et al., 2021), the two main proteolytic pathways. On one hand, mTORC1 inhibits the autophagy-lysosome pathway. One the other hand, Akt phosphorylates FOXO, a posttranslational modification that excludes FOXO from the nucleus where it would otherwise stimulate protein breakdown by increasing the expression of genes involved in the autophagy-lysosome pathway (e.g., BNIP) and ubiquitin-proteasome pathway. However, removal of old or damaged proteins is equally important for muscle homeostasis. Muscle protein breakdown is primarily dependent on the ubiquitin-proteasome pathway which involves several muscle-specific E3 ubiquitin ligases, most notably muscle atrophy F box (atrogin-1/MAFbx) and muscle RING finger 1 (MuRF1) (Egan et al., 2013; Sartori et al., 2021). These ubiquitin ligases are upregulated during fasting by the transcription factor FOXO (Sartori et al., 2021). Overall, the anabolic action of insulin is critical for maintaining or increasing muscle mass and strength, provided all amino acids are available (Wolfe et al., 2017). Other potent anabolic hormones include insulin-like growth factor I (IGF-I) and testosterone.

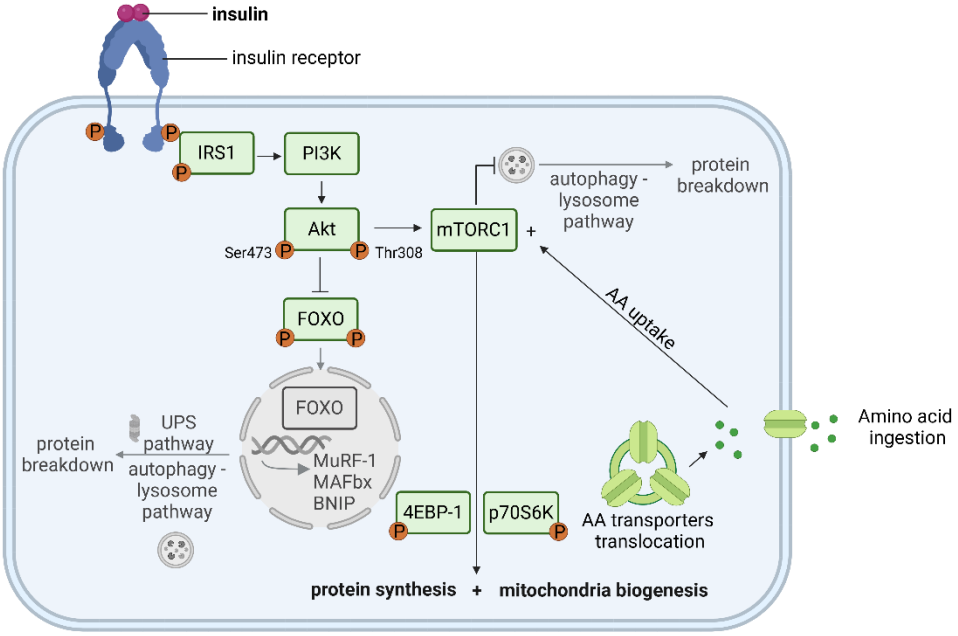


Figure 6. Action of insulin on protein synthesis and breakdown in muscle fibers. Schematic representation (adapted from Sylow et al., 2021). Created with Biorender.com.

d. Role of exercise

Aerobic (or endurance) and resistance (or strength) exercise represent the two extremes of the exercise continuum and elicit specific molecular responses for specific metabolic and functional outcomes (Table 2) (Egan et al., 2013). Aerobic exercise promotes cardiorespiratory capacity and oxidative metabolism via upregulation of peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC1A) (Tallis et al., 2018). PGC1A is a transcription factor that acts as a master regulator of mitochondrial biogenesis. Importantly, PGC1A and AMPK interact in complex signaling pathways that promote type I muscle fiber expression in response to exercise

(Tallis et al., 2018). In short, activation of AMPK lifts the repressive effect of class II histone deacetylases on myocyte enhancer factor 2 (MEF2), a transcription factor activated by the calcium-dependent phosphatase calcineurin. In parallel, calcium signaling lifts the repressive effects of histone deacetylases on MEF2 by activating calmodulin-dependent protein kinase. MEF2 increases the expression of type I fiber-specific genes (e.g., myoglobin, slow troponin isoforms) and PGC1A. Finally, there is a positive feedback loop between MEF2 and PGC1A in which PGC1A induces MEF2 expression. PGC1A expression is positively associated with GLUT4 expression, mitochondrial biogenesis, type I muscle fiber expression, and oxidative metabolism (Tallis et al., 2018). Thus, aerobic exercise promotes a muscle phenotype that maximizes oxidative capacity and insulin sensitivity for improved substrate utilization and fatigue resistance during subsequent exercise (Egan et al., 2013). Resistance exercise, on the other hand, is a powerful anabolic factor that stimulates muscle protein synthesis to increase muscle mass and strength, and maintain basal metabolic rate (Sartori et al., 2021; Egan et al., 2013; Colleluori et al., 2019; Figueiredo et al., 2015; Gil et al., 2021; McGlory et al., 2019). Resistance exercise stimulates amino acid uptake and activates mTORC1 in an insulin-independent manner, likely through a mechanosensory transduction mechanism between muscle fibers and the extracellular matrix (Philp et al, 2011; Egan et al., 2013; Figueiredo et al., 2015). Exercise-regulated genes include, but are not limited to, those encoding key

molecular regulators of muscle protein synthesis, which are primarily involved in repairing muscle damage after exercise (Egan et al., 2013). In the longer term (≈3 weeks), exercise-regulated genes lead to metabolic remodeling, increased muscle protein content, and increased muscle mass and strength. Globally, a combination of aerobic and resistance training is more effective in improving muscle insulin sensitivity and function in healthy people as well as in those with obesity and type 2 diabetes (Egan et al., 2013).

Table 2. Muscle adaptations and health benefits in response to aerobic and resistance exercise

	Aerobic/endurance	**Resistance/strength**
Muscle morphology and performance		
Muscle hypertrophy	↔	↑↑↑
Myofibrillar protein synthesis	↔ ; ↑	↑↑↑
Mitochondrial protein synthesis	↑↑↑	↔ ; ↑
Capillarization	↑↑	↔
Anaerobic capacity	↑	↑↑
Mitochondrial density, oxidative metabolism	↑↑↑	↔ ; ↑
Endurance capacity	↑↑↑	↔ ; ↑
Whole-body and metabolic heath		
Percent body fat	↓↓	↓
Lean body mass	↔	↑↑
Insulin sensitivity	↑↑	↑↑
Inflammatory markers	↓↓	↓
Basal metabolic rate	↑	↑↑
Cardiovascular risk profile	↓↓↓	↓
Ability in activities of daily life	↔ ; ↑	↑↑

Aerobic exercise generally encompasses exercise durations of several minutes up to several hours at various intensities incorporating repetitive, low-resistance exercise such as cycling, running, and swimming. Resistance exercise generally encompasses short-duration activity at high or maximal intensities, and increases the capacity to perform high-intensity, high-resistance exercise such as bodybuilding. ↑: increase; ↓: decrease; ↑↑↑ or ↓↓↓: large effect; ↑↑ or ↓↓: medium effect; ↑ or ↓: small effect increase; ↔ ; ↑: no change or slight change. Adapted from Egan et al., 2013.

e. Role of myokines

Data from animal and cell models suggest that several myokines could regulate muscle mass and strength, either in response to physiological stimuli such as physical exercise, or in response to metabolic factors associated with obesity.

1. Myostatin and its inhibitor, follistatin

Myostatin acts as a direct negative regulator of muscle mass through activin type II receptors and SMAD2-3 transcription factors (Lee, 2021; Lee et al., 2019). Myostatin gene knock-out in mice results in muscle hypertrophy, whereas myostatin overexpression in skeletal muscle results in muscle atrophy (Lee, 2021). On one hand, myostatin activates FOXO-dependent transcription of atrogens (e.g., MURF-1, MAFbx/atrogin-1) which activate muscle protein breakdown and inhibit myogenesis (Lee et al., 2019, Hittel et al., 2009). On the other hand, myostatin inhibits muscle protein synthesis by suppressing Akt-mediated mTORC1 activation (Lee et al., 2019; Sartori et al., 2021; Egan et al., 2013). Therefore, it has been suggested that myostatin could contribute to muscle atrophy in humans (Gonzalez-Cadavid et al., 1998; Yarasheski et al., 2002; Breitbart et al., 2011; Breitbart et al., 2013). In contrast, exercise decreases circulating levels and muscle expression of myostatin in humans, promoting the increase of

muscle mass and strength (Hittel et al., 2010; Hjorth et al., 2016; Catoire et al., 2014; Pourteymour et al., 2017).

Follistatin is a glycoprotein mainly secreted by the liver that is also detected in conditioned media from human myotubes (Ciaraldi et al., 2016). Follistatin inhibits several members of the TGF-beta family, including myostatin (Lee et al., 2010). Muscles of follistatin heterozygous knock-out mice exhibit reduced size and force production, and impaired muscle repair (Lee et al., 2010). In contrast, follistatin overexpression induces muscle hypertrophy through proliferation of satellite cells (i.e., muscle fiber precursors) and inhibition of myostatin and activin A (Gilson et al., 2009).

2. Irisin

Several lines of evidence suggest that irisin promotes muscle hypertrophy (Lee et al., 2019). Irisin expression and secretion increase during myogenic differentiation in human primary myotubes (Huh et al., 2014). In addition, treatment of human primary myotubes with irisin results in muscle cell hypertrophy through downregulation of myostatin and upregulation of IGF-I (Huh et al., 2014). Irisin also induces myogenic differentiation of muscle cells (Reza et al., 2017). *In vivo*, mice injected with irisin exhibit muscle hypertrophy and improved grip strength due to activation of satellite cells and increased muscle protein synthesis (Reza et al., 2017). Finally, intraperitoneal irisin

administration to aged mice mitigates age-related muscle atrophy (Guo et al., 2023). As mentioned above, circulating irisin levels increase in response to exercise, but not muscle *FNDC5* expression (Huh et al., 2015; Carson, 2017).

3. IL-6

The role of IL-6 in the regulation of muscle mass is complex. IL-6 is produced transiently by growing muscle fibers and satellite cells (Serrano et al., 2008). IL-6 gene deletion in mice blunts muscle hypertrophy in response to overload (Serrano et al., 2008). Recovery of muscle mass after hindlimb suspension is also absent in IL-6 knockout mice because of reduced IGF-I expression and Akt/mTOR signaling in muscle (Washington et al., 2011). Thus, IL-6 may play a positive role in muscle growth, through satellite cell activation and muscle protein synthesis stimulation (Serrano et al., 2008). This would be consistent with elevated circulating IL-6 levels during exercise. However, recombinant human IL-6 injected subcutaneously into rats causes muscle wasting (Janssen et al., 2005). In addition, IL-6 overexpression from birth in mice reduces muscle growth during postnatal life, causing muscle atrophy (Pelosi et al., 2021). Similarly, a 3-hour infusion of recombinant human IL-6 in healthy people causes a significant reduction in muscle protein turnover and plasma amino acids (van Hall et al., 2008). These observations suggest that locally produced IL-6 can exert an anabolic effect on muscle, whereas high and chronic circulating IL-6 levels can be detrimental to muscle mass.

4. BDNF

BDNF has long been thought to serve only as a retrograde trophic factor for innervating motor neurons and stabilization of neuromuscular junctions in muscle (Mousavi et al., 2006; Rentería et al., 2022). Mouse and cell models have shown that BDNF is expressed in satellite cells and promotes myogenic differentiation (Mousavi et al., 2006). Muscle-damaging exercise in healthy people is associated with increased muscle BDNF expression and satellite cell proliferation (McKay et al., 2020). These data suggest that BDNF plays an important role in mediating satellite cell response to muscle injury (Lee et al., 2019). However, BDNF overexpression in mouse muscle does not alter muscle mass (Delezie et al., 2019). In line with this, BDNF is associated with muscle strength rather than muscle mass in humans (Tsai et al, 2015).

5. Myonectin

Myonectin may contribute to muscle hypertrophy (Lee et al., 2019). Indeed, disruption of myonectin exacerbates muscle atrophy and mitochondrial dysfunction related to age, sciatic denervation and dexamethasone in mice

(Ozaki et al., 2023). In contrast, myonectin treatment attenuates or suppresses muscle atrophy in these mouse models via activation of AMPK/PGC1A signaling (Ozaki et al., 2023). Slow-twitch muscle fibers exhibit higher myonectin

expression than fast-twitch muscle fibers, suggesting that myonectin may control mitochondrial biogenesis and muscle function (Seldin et al., 2012).

6. FGF21

FGF21 expression is very low in healthy muscle (Oost et al., 2019). However, cellular stressors including fasting, endoplasmic reticulum stress, mitochondrial myopathies, and metabolic disorders induce FGF-21 release from muscle (Oost et al., 2019; Vandanmagsar et al., 2016). Muscle-specific FGF21 knockout mice have similar muscle fiber size than control mice, indicating that FGF21 does not control muscle mass under basal conditions (Oost et al., 2019). In contrast, FGF21 knockout mice are protected against starvation-induced muscle atrophy, whereas FGF21 overexpression in muscle induces muscle atrophy by activating autophagy (Oost et al., 2019). Similarly, systemic FGF21 treatment in female mice induces muscle atrophy, possibly through glucocorticoid signaling (Larson et al., 2024). In contrast, skeletal muscle-specific Akt1 transgenic mice which are characterized by muscle fiber hypertrophy exhibit increased FGF-21 muscle expression and circulating levels (Izumiya et al., 2008). Taken together, these data suggest that muscle is a source of circulating FGF21 and that FGF21 induces muscle atrophy in the context of cellular stress.

7. Secreted protein acidic and rich in cysteine

Secreted protein acidic and rich in cysteine (SPARC or osteonectin) is a glycoprotein that regulates cell-extracellular matrix interactions and exerts profibrotic effects in various tissues, including muscle (Wu et al., 2011; Lee et al., 2014). SPARC is expressed in satellite cells and stimulates myogenesis (Jørgensen et al., 2009; Cho et al., 2000; Nakamura et al., 2012). As a result, SPARC knockout mice have decreased muscle mass and impaired force recovery when isolated muscle is subjected to an *in vitro* fatigue stimulation protocol (Bradshaw et al., 2003; Nakamura et al., 2012; Jørgensen et al., 2017). In fact, reduced SPARC expression in mouse muscle upregulates atrogens, increases TGF-beta signaling, and decreases myofiber size, suggesting that SPARC deficiency leads to muscle atrophy (Nakamura et al., 2012). SPARC treatment in young rats was found to be effective in promoting myogenesis (Nakamura et al., 2012). These data suggest that SPARC plays a role in the regulation of satellite cell function and protects against muscle atrophy. Paradoxically, SPARC is expressed in myotubes, myofibers, and satellite cells in several inherited and idiopathic muscle wasting diseases (Jørgensen et al., 2009). In fact, transient increase in SPARC expression occurs during muscle regeneration and correlates with the expression of myogenic muscle factors (e.g., MyoD, Myf5, Myf6, and Myogenin) (Petersson et al., 2013). Therefore,

increased SPARC expression in myopathies can be interpreted as a mechanism aimed at muscle repair.

2. Impact of obesity on muscle insulin sensitivity and muscle mass and strength

2.1. Impact on muscle insulin sensitivity

a. Muscle insulin resistance

Muscle insulin resistance is a rather selective and strong impairment of insulin-stimulated glucose uptake and glycogen synthesis (Figure 7) (Sylow et al., 2021; Klein et al., 2022; Roden et al., 2019; Fazakerley et al., 2019; DeFronzo et al., 2009). Although GLUT4 translocation is decreased in response to insulin, GLUT4 protein expression is unchanged or only moderately reduced in muscle of people with obesity (Kalinkovich et al., 2017; Albers et al., 2015, a; Gaster et al., 2001). In fact, proximal defects in the insulin signaling cascade such as defective phosphorylation and/or reduced protein content have been repeatedly found at the level of IRS-1, and PI3K but inconsistently at the level of Akt in human muscle (Sylow et al., 2021; Roden et al., 2019; Fazakerley et al., 2019). Distal signaling defects including defective phosphorylation of AS160/TBC1D4, reduced activation of Rac1, and dysfunctional reorganization of the actin cytoskeleton have also been reported (Sylow et al., 2021; Fazakerley et al., 2019). Compared to glucose-tolerant people with obesity,

those with type 2 diabetes have lower insulin receptor, GLUT4, and hexokinase content in muscle, and altered phosphorylation of Akt and AS160/TBC1D4 in response to insulin (Albers et al., 2015, a; Gaster et al., 2001). Given the multifactorial and polygenic nature of obesity, it is unlikely that a single molecular defect causes muscle insulin resistance (Sylow et al., 2021; Perseghin et al., 1996). Whatever the underlying molecular mechanisms, muscle insulin resistance is considered the primary defect leading to type 2 diabetes (Sylow et al., 2021; Roden et al., 2019; DeFronzo et al., 2009).

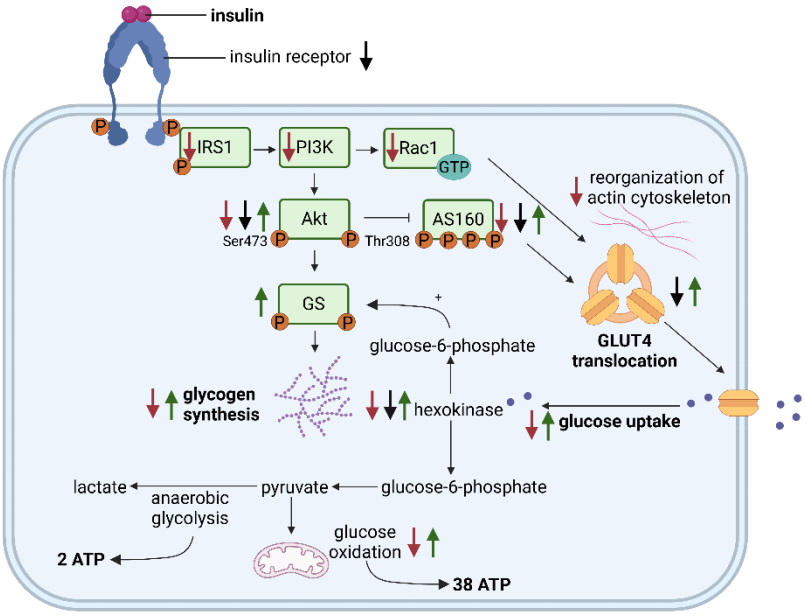


Figure. 7. Impacts of obesity and bariatric surgery on insulin action on glucose metabolism in muscle. Schematic representation. *Red arrows*: people with obesity versus healthy people; *black arrows*: people with obesity with type 2 diabetes versus glucose-tolerant people with obesity; *green arrows*: people with obesity or myotubes from people with obesity after bariatric surgery. ↑: increase; ↓: decrease. Created with Biorender.com.

b. Slow-to-fast muscle fiber transition

Muscle from people with obesity with or without type 2 diabetes is characterized by a lower proportion of type I fibers and, at the same time, a higher proportion of type IIx fibers compared to lean people (Damer et al., 2022; Tallis et al., 2018; Tanner et al., 2002; Serrano et al., 2023; Albers et al., 2015, a). BMI and percentage fat mass are positively associated with type IIx fiber proportion, and negatively associated with type I fiber proportion, independent of gender and muscle group (Damer et al., 2022; Tanner et al., 2002). Primary myotubes from people with obesity also show a lower proportion of type I MHC than myotubes from lean people (Bollinger et al., 2015). This "slow-to-fast" shift in muscle fiber-type distribution impairs muscle glucose uptake and oxidative metabolism (Tanner et al., 2002; Serrano et al., 2023; Egan et al., 2013; Tallis et al., 2018; Albers et al., 2015, a). However, it remains uncertain whether this muscle fiber-type distribution is the cause or consequence of obesity. On the one hand, the heritability of muscle fiber-type distribution has been estimated at 40-50% (Tanner et al., 2002; Serrano et al., 2023). A higher proportion of type IIx fibers has been found in insulin-resistant first-degree relatives of people with type 2 diabetes, suggesting a genetic component in muscle fiber-type distribution associated with metabolic diseases (Tanner et al., 2002). In addition, lean people with a higher proportion of type IIa fibers experience greater fat mass gain during chronic overfeeding (Sun et al., 2002), suggesting a causal relationship between muscle fiber-type distribution and obesity (Serrano et al.,

2023; Tanner et al., 2002). On the other hand, human data suggest that a lower proportion of type I muscle fibers results from exposure to metabolic and environmental factors associated with obesity (Serrano et al., 2023; Tanner et al., 2002; Tallis et al., 2018). Specifically, insulin regulates muscle gene expression (Rome et al., 2003) and hyperinsulinemia during a 3-hours clamp rapidly increases type IIx MHC mRNA in muscle of healthy people (Houmard et al., 1999), while a 9-day high-fat diet decreases synthesis rate of MHC-I isoform to a greater extent than that of MHC-II isoform (Camera et al., 2017; Serrano et al., 2023). In addition, physical inactivity reduces type I muscle fiber expression (Tallis et al., 2018). In fact, the two hypotheses are not mutually exclusive: changes in muscle fiber-type distribution can be the cause and consequence of obesity. Inherited factors involved in muscle fiber-type distribution likely act synergistically with metabolic and environmental factors associated with obesity to produce a “slow-to-fast” shift. In turn, decreased oxidative metabolism promotes detrimental intramyocellular lipid accumulation, oxidative stress, and insulin resistance in muscle from people with obesity (Figure 8) (Tanner et al., 2002; Serrano et al., 2023; Egan et al., 2013; Tallis et al., 2018; Albers et al., 2015, a).

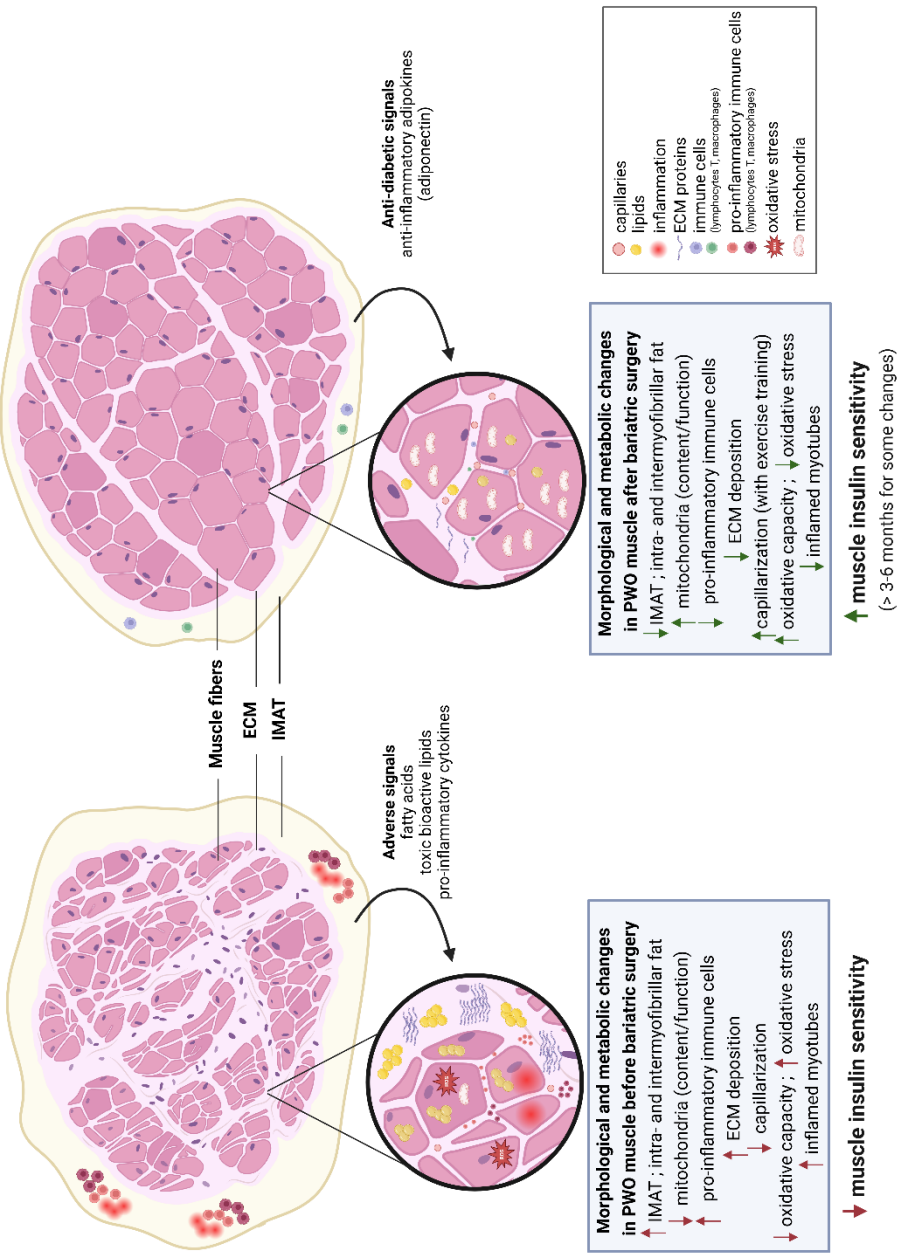


Figure. 8. Summary of morphological and metabolic changes in muscle of people with obesity before and after bariatric surgery. Schematic representation. PWO: people with obesity; IMAT: intermuscular adipose tissue; ECM: extracellular matrix. Created with Biorender.com.

c. Mitochondrial dysfunction

Mitochondrial dysfunction, characterized by decreased mitochondrial content and enzyme activity, has been repeatedly reported in the muscle of people with obesity and type 2 diabetes (Pileggi et al., 2021; Gancheva et al., 2019; Coen et al., 2013; Campbell et al., 2016; Barberio et al., 2021; Gastaldi et al., 2007). Compared to glucose-tolerant people with obesity, those with type 2 diabetes show exacerbated mitochondrial dysfunction, as evidenced by a coordinated downregulation of genes involved in oxidative metabolic pathway (Barberio et al., 2021). In addition, the interconnected mitochondrial network is smaller in muscle from people with obesity compared to that of healthy people, probably due to an imbalance between mitochondrial fusion/biogenesis and fission (Pileggi et al., 2021). The expression of PGC1A and mitochondrial fusion proteins (e.g., mitofusin-2) is lower in muscle from people with obesity, while cellular and mouse models suggest increased expression of mitochondrial fission proteins (e.g., mitochondrial fission 1 protein) in response to metabolic factors (fatty acids, high glucose, and high fat-diet) (Bach et al., 2003; Gancheva et al., 2019; Patti et al., 2003; Colberg et al., 1995; Pileggi et al., 2021). A single meal rich in saturated fat induces PGC1A expression in muscle of healthy people, whereas a three-day high-fat diet or prolonged exposure to fatty acid using lipid infusion decreases PGC1A expression (Boyle et al., 2011; Pileggi et al., 2021). Metabolic inflexibility in muscle from people with obesity (i.e., inability for muscle to acutely shift mitochondrial fuel selection between lipids or glucose

during fasting or postprandial periods) is evidenced by the absence of PGC1A induction in response to acute exposure to saturated fat, as opposed to healthy people (Boyle et al., 2011; Pileggi et al., 2021). Thus, chronic overfeeding and excess fatty acids likely contribute to mitochondrial dysfunction, lower proportion of type I muscle fibers, and ultimately lower oxidative metabolism in muscle of people with obesity. Carnitine palmitoyltransferase-1 (CPT1) is a target of PGC1A and the main regulator of long-chain fatty acid transport across the mitochondrial membrane (Gastaldi et al., 2007). A defect in CPT1 activity has been reported in muscle from people with obesity, promoting detrimental accumulation of intramyocellular lipids (Kim et al., 2000). Finally, inefficient beta-oxidation results in cytosolic backup of acylcarnitine derivatives which promotes diacylglycerol accumulation and stimulates the production of oxygen reactive species, leading to stress pathways activation (e.g., IKK/NF-κB pathway) (Roden et al., 2019; Kalinkovich et al., 2017). Mitochondrial dysfunction is thus considered a key driver of muscle insulin resistance in people with obesity (Figure 8).

d. Myosteatosis

Reduced oxidative metabolism in muscle fibers, coupled with increased fatty acid availability from adipose tissue and diet, contributes to ectopic fat accumulation in muscle from people with obesity, called myosteatosis (Henin et al., 2023). Myosteatosis develops as both intermuscular adipose tissue

(IMAT, outside muscle fascia) and intramuscular fat (inside muscle fascia). Intramuscular fat accumulates as extramyocellular (or interfibrillar fat, in the muscle interstitium) and intramyocellular lipids (inside muscle fibers) (Henin et al. 2023). The expansion of IMAT is positively correlated with percentage fat mass and visceral adipose tissue content (Boettcher et al., 2009). IMAT and visceral adipose tissue share common features including a negative correlation with insulin sensitivity, increased expression of proinflammatory cytokines, and increased basal lipolytic rate compared to subcutaneous adipose tissue (Boettcher et al., 2009; Sachs et al., 2019). Their secretome elicits insulin resistance in primary human myotubes with equal potency, presumably via sustained release of proinflammatory cytokines and fatty acids that trigger inflammation in muscle fibers (Figure 9) (Sachs et al., 2019; Santoro et al., 2023; Klein et al., 2022; Mingrone et al., 2002). Lipid infusion in healthy people increases both plasma fatty acid and intramyocellular lipid levels which impairs insulin-stimulated glucose uptake in muscle (Klein et al., 2022; Santoro et al., 2023). Therefore, increased basal lipolytic activity of IMAT directly increases fatty acid influx towards adjacent muscle fibers exceeding their oxidative capacity and leading to detrimental intramyocellular lipid accumulation (Santoro et al., 2023; Klein et al., 2022; Sachs et al., 2019). Incomplete fatty acid oxidation and accumulation of toxic fatty acid intermediates has been demonstrated in myotubes from people with obesity (Aguer et al., 2015; Kovalik et al., 2011). In muscle tissue, altered expression of muscle lipases (i.e.,

increased ATGL and decreased HSL expression) promotes intramyocellular lipid accumulation, particularly diacylglycerol (Roden et al., 2019; Badin et al., 2013; Kase et al., 2015; Sachs et al., 2019). Notably, intramyocellular lipids are more abundant in people with obesity with type 2 diabetes than those without (Camastra et al., 2017). In humans, the association between muscle fat infiltration and insulin resistance is rather to be caused by intramyocellular rather than extramyocellular lipids, as measured by proton spectroscopy (Virkamäki et al., 2001). In fact, diacylglycerol and ceramides impair insulin signaling at the level of IRS1 and Akt, respectively, through novel protein kinase C (PKC) isoforms activation (Badin et al., 2013; Roden et al., 2019). Finally, intramyocellular lipid accumulation and impaired insulin signaling blunt the expression of mitochondrial genes, including PGC1A, further decreasing muscle oxidative metabolism (Barberio et al., 2021). Collectively, these defects link myosteatosis to lipotoxicity and muscle inflammation which are both major drivers of muscle insulin resistance in people with obesity (Figure 8).

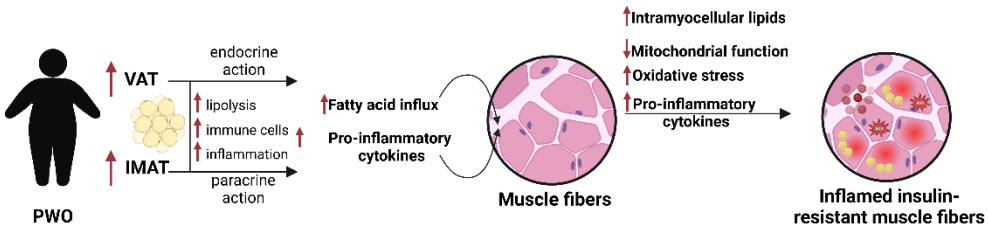


Figure 9. Lipotoxicity and inflammation as major drivers of muscle insulin resistance in people with obesity (PWO). Schematic representation. FA: fatty acids; VAT: visceral adipose tissue; IMAT: intermuscular adipose tissue; DAG: diacylglycerol. ↑: increase; ↓: decrease; *red arrows*: PWO versus healthy people. Created with Biorender.com.

e. Muscle inflammation

Muscle from people with obesity is infiltrated by a greater number of immune cells than that of lean people (Varma et al., 2009; Fink et al., 2013; Sachs et al., 2019). Specifically, pro-inflammatory macrophages (CD11c+, CD68+, M1 phenotype) and T cells (CD4+, CD8+) accumulate in IMAT as observed in VAT (Wu et al. 2017; Varma et al., 2009; Fink et al.; 2013; Kalinkovich et al., 2017). Although the signals that trigger muscle inflammation are unknown, pro-inflammatory cytokines and fatty acids released by ectopic muscle fat probably exert paracrine effects on muscle fibers (Figure 9) (Wu et al., 2017; Kalinkovich et al., 2017). Fatty acids, particularly long-chain saturated fatty acids (e.g. palmitic acid), induce inflammation in human cells (Abildgaard et al., 2014.; Rauen et al., 2021). Similarly, primary human myotubes exposed to macrophages (Varma et al., 2009) or pro-inflammatory cytokines (Bouzakri et al., 2011) show increased expression and secretion of pro-inflammatory myokines (e.g., MCP-1/CCL2, TNF-a), suggesting muscle fiber inflammation (Kalinkovich et al., 2017). In addition, pro-inflammatory cytokines secreted by adipose tissue (e.g., IL-6) stimulate lipolysis, thereby increasing fatty acid influx into muscle fibers and promoting lipotoxicity (Santoro et al. 2023; Klein et al., 2022; Sachs et al., 2019). Importantly, inflamed myotubes are insulin resistant, as evidenced by impaired insulin signaling and insulin-stimulated glucose uptake (Varma et al., 2009; Bouzakri et al., 2011; Austin et al., 2008; Mäkinen et al., 2017;

Bouzakri et al., 2007; Austin et al., 2008). Proinflammatory cytokine infusion (e.g., TNF-a) causes muscle insulin resistance in healthy people (Plomgaard et al., 2005). These data establish a link between muscle fiber inflammation and muscle insulin resistance. Activation of stress pathways (e.g., IKK/NF-κB pathway) presumably links obesity- or nutrient-induced inflammation to muscle insulin resistance (Austin et al., 2008; Bandyopadhyay et al., 2005; Bouzakri et al., 2003; Mashili et al., 2013; Wu et al., 2017). Finally, pro-inflammatory factors released from immune cells and inflamed muscle fibers skew resident immune cells towards proinflammatory phenotypes, attract monocytes from blood, and promote muscle inflammation and insulin resistance in a feedforward fashion (Figure 9) (Sylow et al. 2021; Wu et al., 2017).

f. Muscle fibrosis

Chronic overfeeding and excess lipids trigger a pathological extracellular matrix remodeling in human muscle, eventually leading to muscle fibrosis (Figure 8) (Musale et al., 2023; Williams et al., 2015; Richardson et al., 2005; Tam et al., 2014). Rapid weight gain and lipid infusion in healthy people leads to increased expression of various extracellular matrix genes, including collagen, fibronectin-1 and lumican (Richardson et al., 2005; Tam et al., 2014). The extracellular matrix in muscle from people with obesity is remodeled, ticker, and characterized by increased collagen I and III deposition (Musale et al., 2023; Berria et al. 2006; Dantas et al. 2020). Murine models of obesity suggest that

muscle inflammation, and the subsequent increase in TGF-beta signaling, the canonical pathway of fibrosis, are predominant mechanisms leading to extracellular matrix remodeling (Williams et al., 2015). In healthy people, rapid weight gain upregulates TGF-beta signaling in muscle (Musale et al., 2023). TGF-beta activates SMAD 2-3, which form a complex with SMAD4 followed by nuclear translocation and regulation of expression of target genes (Musale et al., 2023). An increase in phosphorylated SMAD2-3 has been observed in muscle from people with obesity (Watts, 2013). Furthermore, studies in humans and rodents suggest that extracellular matrix remodeling contributes to muscle insulin resistance (Musale et al., 2023; Richardson et al., 2005; Tam et al., 2017). Obesity in rodents is associated with delayed insulin delivery to muscle fibers, supporting the idea that the remodeled extracellular matrix increases diffusion distance and impedes insulin delivery to muscle fibers (Williams et al., 2020; Sylow et al., 2021). Data in rodents and humans also suggest that obesity disrupts the angiogenic response, leading to morphological and functional alterations in capillary networks (Paavonsalo et al., 2020; Gavin et al., 2005). Decreased capillary density and perfusion, thicker capillary walls and impaired insulin-stimulated vasodilation have been described in muscle from people with obesity (Dantas et al., 2020; Sylow et al., 2021; Gavin et al., 2005). Finally, mouse and cell models suggest that excessive extracellular matrix deposition may promote muscle insulin resistance by activating specific

integrin receptors interfering with insulin action and muscle capillarity (Williams et al., 2015; Musale et al., 2023).

g. Changes in myokines in relation with muscle insulin sensitivity

Myokine secretion from muscle cells of people with obesity and type 2 diabetes is altered and characterized by increased release of interleukins (ILs), including IL-6, IL-8, and IL-15, and pro-inflammatory cytokines, such as tumor necrosis factor alpha (TNF-a) and monocyte chemotactic protein-1 (MCP-1) (Ciaraldi et al., 2016; Hittel et al., 2009). In addition, increased release of follistatin and myostatin, which exert opposing effects on muscle mass, has also been reported (Ciaraldi et al., 2016; Hittel et al., 2009). Altered myokine secretion may be an intrinsic property of muscle in people with obesity and type 2 diabetes, due to genetic or epigenetic factors (Batista et al., 2020). Alternatively, altered myokine secretion may result from exposure to obesity-related metabolic factors such as lipotoxicity and inflammation, which impair muscle insulin sensitivity (Bouzakri et al., 2011; Yoon et al., 2011; Deshmukh et al., 2015). In addition, insulin itself may play a role in myokine secretion. Indeed, insulin directly regulates myokine secretion by muscle cells (Mizgier et al., 2017) and indirectly by increasing the expression of type IIx muscle fibers in muscle tissue (Houmard et al., 1999; Damer et al., 2022). Therefore, hyperinsulinemic states such as obesity and type 2 may alter myokine secretion, especially since some myokines appear to be fiber-specific (e.g., myonectin, IL-6,

angiogenin, osteoprotegerin) (Rutti et al., 2018; Hiscock et al., 2004). On the other hand, some myokines secreted by insulin-resistant and inflamed myotubes negatively affect glucose-stimulated insulin secretion, beta cell survival, insulin action, and muscle growth and metabolism (Bouzakri et al., 2011; Ciaraldi et al., 2016; Hittel et al., 2009; Varma et al., 2009). In contrast, increased expression and secretion of other myokines, such as fractalkine and chitinase-3-like protein 1, by human myotubes exposed to pro-inflammatory cytokines may represent an auto-protective mechanism against inflammation (Eckardt et al., 2014; Görgens et al., 2014). Understanding the beneficial and detrimental effects of myokines on glucose homeostasis, muscle mass and function may provide novel therapeutic targets and biomarkers for obesity, type 2 diabetes, and sarcopenia (Table 3).

1. BDNF

Circulating BDNF levels are decreased in people with type 2 diabetes independently of obesity and associated with impaired glucose homeostasis (Eckardt et al., 2014; Krabbe et al., 2007). Based on its biological properties, reduced BNDF levels may contribute to beta cell dysfunction, reduced muscle oxidative capacity, and altered eating behavior (see above).

2. Myostatin

Circulating myostatin levels are usually higher in people with obesity and type 2 diabetes than in healthy people (Hittel et al., 2009; Amor et al., 2019; Wang et al., 2012). Higher myostatin expression in muscle has also been reported in people with obesity and type 2 diabetes (Hittel et al., 2009; Brandt et al., 2012). In addition, there is a positive correlation between circulating myostatin levels or muscle myostatin expression and HOMA-IR in people with obesity and type 2 diabetes (Amor et al., 2019; Wang et al., 2012; Hittel et al., 2009). Myotubes from insulin-resistant women exhibit increased myostatin secretion, suggesting that muscle is a source of increased circulating myostatin levels in people with obesity and type 2 diabetes (Hittel et al., 2009). Therefore, increased myostatin may play a pathogenic role in insulin resistance during obesity and type 2 diabetes.

3. Irisin

Some studies have reported lower circulating irisin levels in people with obesity and type 2 diabetes than in healthy people (Liu et al., 2013; Choi et al., 2013; Yan et al., 2014; Demirpence et al., 2016; Moreno-Navarrete et al., 2013). Circulating irisin levels are inversely correlated with BMI and insulin resistance in healthy people and people with obesity (Demirpence et al., 2016; Liu et al., 2013; Choi et al., 2013; Yan et al., 2014). In contrast, several studies have reported higher

circulating irisin levels in people with obesity (Pardo et al., 2014; Crujeiras et al., 2014) and increased irisin secretion by insulin-resistant human myotubes (Natalicchio et al., 2017). Consistent with these latter observations, FNDC5 mRNA expression in muscle is positively correlated with BMI (Huh et al., 2012). In addition, human visceral and subcutaneous adipose tissue secrete irisin and may contribute to higher circulating irisin levels in people with obesity (Pardo et al., 2014; Crujeiras et al., 2014). Different methods of irisin detection may account for at least part of the inconsistencies between studies. However, decreased irisin may be associated with decreased oxidative metabolism and insulin sensitivity in muscle.

4. Interleukin-6

Circulating IL-6 levels are chronically elevated in people with obesity and type 2 diabetes (Roytblat et al., 2000; Gancheva et al., 2019; Eckardt et al., 2014). Adipose tissue, rather than muscle, is considered the primary source of chronically elevated circulating IL-6 and others pro-inflammatory factors (e.g., TNF-a) levels in obesity (Klein et al., 2022; Santoro et al., 2023). However, human myotubes exposed to TNF-a show increased IL-6 secretion, suggesting that muscle fiber may also contribute to increased circulating IL-6 levels in people with obesity and type 2 diabetes (Bouzakri et al., 2011). On the other hand, high-fat diet increases circulating IL-6 levels in mice, while IL-6 blockade improves insulin sensitivity by enhancing glucose uptake in

muscle (Yamaguchi et al., 2015). In humans, subcutaneous administration of recombinant IL-6 induces dose-dependent increase in fasting blood glucose in healthy people, probably by stimulating glucagon release and/or by inducing peripheral insulin resistance (Tsigos et al., 1997), whereas IL-6 blockade improves glycated hemoglobin A1c (HbA1c) in people with type 2 diabetes (Genovese et al, 2020). Thus, chronically elevated circulating IL-6 levels may contribute to impaired glucose homeostasis in obesity. In addition, skeletal muscle response to IL-6 is blunted in people with type 2 diabetes. Indeed, acute IL-6 infusion/treatment does not increase glucose uptake in people with type 2 diabetes and myotubes from people with type 2 diabetes, conversely to healthy people (Harder-Lauridsen et al., 2014; Jiang et al., 2013). In contrast, acute IL-6 infusion stimulates lipolysis and fatty acid oxidation in muscle from people with type 2, as observed in healthy people (Petersen et al., 2005). These data suggest that IL-6 may be effective in preventing type 2 diabetes by increasing muscle glucose uptake, but ineffective in stimulating glucose uptake in people with type 2 diabetes.

5. Fibroblast growth factor 21

Elevated circulating FGF21 levels have been reported in people with prediabetes and type 2 diabetes, which may be a consequence of metabolic imbalance and/or FGF21 resistance (Gao et al., 2019; Fisher et al., 2010; Mashili et al., 2011). Circulating FGF21 levels correlate with fasting insulin, HOMA-IR,

and BMI in people with type 2 diabetes but not in healthy people (Mashili et al., 2011). However, muscle FGF21 mRNA expression is similar in people with type 2 diabetes and healthy people (Mashili et al., 2011), suggesting that increased circulating FGF-21 levels do not originate from muscle.

6. Meteorin-like protein

Data on circulating Metrnl levels in people with obesity and type 2 diabetes are conflicting (Schmid et al., 2021). Some studies have reported higher circulating Metrnl levels in people with obesity and type 2 diabetes (Alkhairi et al., 2019), while other studies have found lower levels negatively correlated with HbA1c (Schmid et al., 2021; Pellitero et al., 2018).

7. Myonectin

Conflicting results have been reported on circulating myonectin levels in people with obesity. Several studies have found lower circulating myonectin levels in people with obesity compared to healthy people and a negative association between myonectin and indicators of metabolic risk such as BMI, visceral fat content, and insulin resistance (Li et al, 2020; Petro et al, 2023). In contrast, other studies have found higher circulating myonectin levels in people with obesity and type 2 diabetes, and a positive association between myonectin, fat mass, and insulin resistance (Mi et al., 2019). Exercise training

increases circulating myonectin levels in people with obesity (Pourranjbar et al., 2018).

8. SPARC and MMP-2

Circulating SPARC levels and its muscle mRNA are higher in people with obesity and type 2 diabetes than in lean people (Wu et al., 2011). Similarly, circulating MMP-2 levels (72 kDa type IV collagenase), a key metalloproteinase involved in the removal of excess extracellular matrix (Lee et al., 2014), are higher in people with obesity than in lean people (Sajoux et al., 2019). These data suggest that SPARC and MMP2 may play opposing roles in the extracellular matrix remodeling during obesity, thereby regulating muscle insulin sensitivity.

9. Apelin

Circulating apelin levels are higher in people with obesity and type 2 diabetes and inversely associated with insulin sensitivity, suggesting either apelin resistance or increased secretion by adipose tissue (Boucher et al., 2005; Krist et al., 2013; Soriguer et al., 2009; Heinonen et al., 2005). In fact, muscle apelin expression is similar between healthy people and people with type 2 diabetes. However, adipose tissue apelin expression is increased in people with type 2 diabetes, suggesting that adipose tissue is responsible for

increased apelin circulating levels during type 2 diabetes (Dray et al., 2010; Krist et al., 2013).

10. Other myokines

Circulating FSTL-1 levels are higher in people with type 2 diabetes than in healthy people, and positively associated with BMI and HOMA-IR, suggesting either FSLT-1 resistance or increased secretion by adipose tissue (Xu et al., 2020). On the other hand, circulating FSTL-1 levels increase in response to exercise in healthy people. Together these observations suggest that acutely and chronically elevated FSTL-1 may result from physiological (exercise) or pathological (type 2 diabetes) stimuli, as with other myokines (e.g., IL-6) (Xu et al., 2020). In fact, FSTL-1 is induced at both gene and circulating level in various inflammatory conditions to promote IL-1β secretion by immune cells (Chaly et al., 2014; Xu et al., 2020). Conversely, FSTL-1 secretion by primary human myotubes is increased in response to proinflammatory cytokines, suggesting that muscle may contribute to increased circulating FSTL-1 levels in people with obesity and type 2 diabetes, both conditions being associated with chronic low-grade inflammation (Gorgens et al., 2013; Xu et al., 2020).

Table 3. Changes in circulating levels and muscle expression of myokines involved in the regulation of glucose homeostasis and muscle mass or function

	Obesity		Surgery	
Myokines	**Circulating levels**	**Muscle mRNA**	**Circulating levels**	**Muscle mRNA**
BDNF	↓	-	↓	NR
Irisin	↑↓	↑	↑↓↔	↓
IL-6	↑	↑	↓↔	NR
FGF21	↑	↔	↑	NR
Myonectin	↑↓	↑	↑	NR
Meteorin-like protein	↑↓	NR	↑↓	NR
Myostatin	↑	↑	↓	↓
FSTL1	↑	NR	↓	NR
Apelin	↑	↔	↓	NR
FST	↑	↑↓	↑↓	NR
SPARC	↑	↑	↓	NR
Fractalkine	↑↓	↑↓	NR	NR

BDNF: Brain Derived Neurotrophic Factor; IL: interleukin; FGF21: fibroblast growth factor 21; FSTL1: Follistatin-related protein 1; NR: not reported; SPARC: secreted protein acidic and rich in cysteine (osteonectin)

2.2. Impact on muscle mass and strength

a. Anabolic resistance

Anabolic resistance refers to the blunted response of muscle protein synthesis to anabolic stimuli, including amino acids, insulin, and exercise (McGlory et al., 2019). The muscle protein synthesis response to protein-rich food or amino acid infusion is weaker in people with obesity than in healthy

people, and the stimulation of myofibrillar and mitochondrial protein synthesis rates is reduced (Paulussen et al., 2021; Stephens et al., 2015). Overall, muscle protein synthesis in response to anabolic stimuli is inversely related to body fat mass (Paulussen et al., 2021; Stephens et al., 2015). At the molecular level, people with obesity have increased basal and phosphorylation levels of anabolic signaling proteins (e.g., mTORC1) in muscle, but impaired response to insulin, in particular insulin-induced Akt phosphorylation (Paulussen et al., 2021; Stephens et al., 2015; Williamson et al., 2015). Myosteatosis is thought to be an important driver of anabolic resistance in people with obesity as accumulation of intramyocellular lipids impairs mTORC1 activity and insulin-stimulated amino acid uptake in muscle fibers (Beals et al., 2019; Paulussen et al., 2021; Tallis et al., 2018). Altered muscle protein breakdown has also been demonstrated in human primary myotubes from people with obesity (Bollinger et al., 2015). Specifically, flux through the autophagy-lysosome pathway is decreased in myotubes from people with obesity, which may contribute to mitochondrial dysfunction, possibly by slowing the degradation of damaged or dysfunctional mitochondria (Bollinger et al., 2015). On the other hand, muscle inflammation promotes muscle protein breakdown through upregulation of muscle-specific E3 ubiquitin ligases, atrogin-1/MAFbx and MuRF1 (Varma et al, 2009). Overall, postprandial muscle protein synthesis and muscle protein turnover are impaired in obesity and likely affect muscle metabolism and function. Anabolic resistance likely limits positive

adaptations to the overload stimulus, which may promote sarcopenic obesity (see below) (Donini et al., 2022; Tomlinson et al., 2016; Villareal et al., 2004).

b. Muscle mass

There are limitations to the assessment of skeletal muscle mass in people with obesity. Magnetic resonance imaging has been validated in healthy people through post-mortem studies that included people with obesity but with a limited BMI range (≤ 31 kg/m²) (Sizoo et al., 2021; Donini et al., 2022; Nuijten et al., 2022). The ESPEN/EASO consensus statement insists on the use of computed tomography scan to measure selected muscle areas, particularly at the level of the third lumbar vertebra, because of its strong association with whole-body skeletal muscle mass (Donini et al., 2022). However, weight (≈ 150-200 kg) and radial height (≈ 60 cm) limits may preclude the use of magnetic resonance imaging or computed tomography scan in people with severe obesity (Sizoo et al., 2021). DXA can accurately predict lean body mass and appendicular lean mass in people with obesity (Sizoo et al., 2021). However, some people do not fit within the DXA field of view, but half-body analysis has been shown to be comparable to full-body analysis (Sizoo et al., 2021). Bioelectrical impedance analysis has been validated against magnetic resonance imaging in healthy people but not in people with obesity (Sizoo et al., 2021). Compared to DXA, bioelectrical impedance analysis tends to overestimate fat-free mass in people with obesity, with errors increasing with

BMI (Sizoo et al., 2021; Coppini et al., 2005; Brunani et al., 2021; Donini et al., 2022). In fact, fat-free mass estimation using bioelectrical impedance analysis is based on a constant hydration assumption of 73%, which is violated by hyperhydration in people with obesity (Sizoo et al., 2021). Nevertheless, the use of DXA and bioelectrical impedance analysis to assess skeletal muscle mass in people with obesity as a second-line alternatives to magnetic resonance imaging or computed tomography scan is supported by the ESPEN/EASO consensus statement (Donini et al., 2022).

People with obesity typically have an absolute skeletal muscle mass comparable to or greater than that of lean people (Donini et al., 2022; Tomlinson et al., 2016; Villareal et al., 2004), which is expected given the strong positive correlation between muscle and fat mass (Baker et al., 2020). In addition, a higher overall body mass increases the workload on muscles in daily activities, exerting a training effect leading to increased skeletal muscle mass particularly in lower limbs (Donini et al., 2022; Tomlinson et al., 2016; Villareal et al., 2004). Finally, the larger muscles themselves add to the already higher body mass, further increasing the strength needed to overcome the body inertia (Tallis et al., 2018). Although absolute skeletal muscle mass is most often greater in people with obesity, skeletal muscle mass adjusted to body weight is lower (Figure 10), leading to the concept of sarcopenic obesity (see below) (Donini et al., 2022).

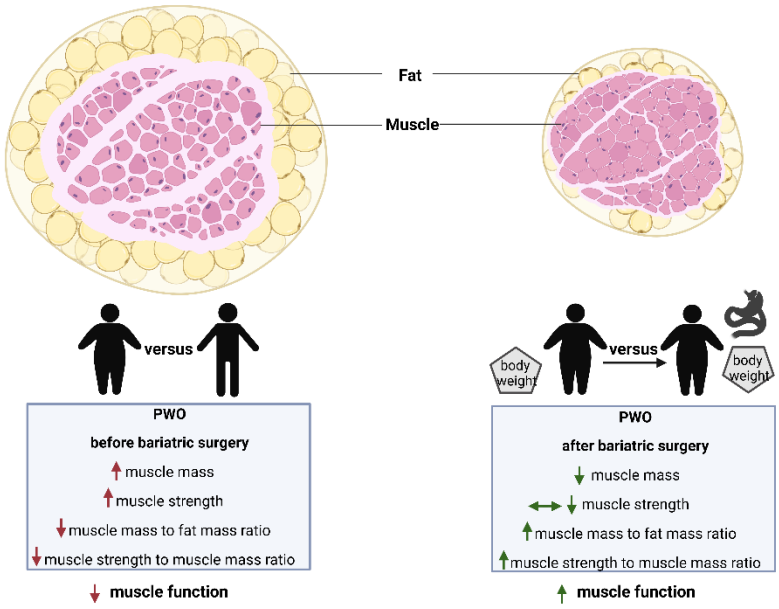


Figure. 10. Muscle mass and strength in people with obesity before and after bariatric surgery. Schematic representation. PWO: people with obesity. ↑: increase; ↓: decrease; ↔: no change; *red arrows*: PWO versus healthy people; *green arrows*: effect of bariatric surgery. Created with Biorender.com.

c. Muscle strength

People with obesity have greater muscle strength than lean people (Figure 10), as expected from the correlation between muscle mass and strength (Tomlinson et al., 2016; Hulens et al., 2001). In particular, the antigravity muscles of the knee extensors, back extensors, and oblique abdominals are stronger in people with obesity than in lean people, in contrast to the upper body muscles (Tomlinson et al., 2016; Hulens et al., 2001; Lafortuna et al., 2005). Increased body mass simulates a resistance training stimulus placed on weight-bearing muscles during standing and locomotion, increasing their ability to produce

absolute strength (Tallis et al., 2018; Tomlinson et al., 2016). However, muscle strength (e.g., maximum knee extensor strength) normalized to body mass or skeletal muscle mass (i.e., muscle quality) is lower in people with obesity compared to lean people (Tomlinson et al., 2016; Hulens et al., 2001; Maffiuletti et al., 2008). This suggests that muscle quality and functional performance are impaired in people with obesity which contributes to sarcopenic obesity (see below), even though skeletal muscle mass is usually increased (Figure 10) (Tallis et al., 2018; Cruz-Jentoft et al., 2019). In addition, muscle strength and quality, particularly in the lower limbs, are generally lower in people with type 2 diabetes than in those without, suggesting that diabetes and possibly diabetes-related peripheral neuropathy exacerbates muscle dysfunction (Andersen et al., 2004; Park et al.,2006; Hilton et al., 2008; Tomlinson et al., 2016).

The mechanisms by which obesity affects contractile function are unclear (Serrano et al., 2023). In mouse models of obesity, isolated muscle preparations showed reduced muscle quality (i.e., force per unit muscle area), particularly in muscles containing lower proportions of type I muscle fibers (e.g., extensor digitorum longus) (Tallis et al., 2018). The slow-to-fast muscle fiber-type shift observed in muscle from people with obesity likely affects contractile function in humans (Tallis et al., 2018). The activity of AMPK is inhibited by chronic overfeeding, hyperinsulinemia, and intramyocellular diacylglycerol accumulation (Tallis et al., 2018). AMPK inhibition enhances the inhibitory effect

of histone deacetylases on MEF2 and PGC1A expression which reduces type I muscle fiber expression and muscle oxidative capacity, further promoting intramyocellular lipid accumulation and muscle insulin resistance (Tallis et al., 2018). At the same time, calcium signaling is altered by oxidative stress resulting from intramyocellular lipid accumulation, muscle inflammation, and mitochondrial dysfunction (Tallis et al., 2018). Contractile dysfunction in people with obesity also involves reduced myogenesis, changes in neuromuscular recruitment, and alterations at the neuromuscular junction (Tallis et al., 2018). Muscle contractile dysfunction contributes to muscle fatigue, reduced muscle quality, and functional impairment well before old age, which creates a vicious cycle of excess fat, decreased energy expenditure, and further weight gain promoting sarcopenic obesity (Donini et al., 2022).

d. Sarcopenic obesity

Sarcopenic obesity was first described nearly thirty years ago. Its current definition considers the coexistence of excess fat and sarcopenia, defined as low skeletal muscle mass and function, with muscle strength being the predominant measure of muscle function (Donini et al., 2022; Prado et, 2024). The prevalence of sarcopenic obesity varies widely among studies, from less than 20% to more than 40%, depending on the population studied and the diagnostic criteria used (Prado et al., 2024; Baker et al., 2020). Sarcopenic obesity

is primarily described in the elderly, as aging is naturally associated with a progressive decline in muscle mass and function (Prado et al., 2024). In addition, older people are more prone to metabolic disorders and intercurrent diseases that can exacerbate skeletal muscle mass loss (Prado et al., 2024). However, the disturbances in glucose, lipid, and protein metabolism, as well as the expansion and inflammation of adipose tissue, and myosteatosis observed with aging are quite like those seen in people with obesity (Prado et al., 2024; Tallis et al., 2018). Thus, obesity-related changes in muscle metabolism and function exacerbate the aging process well before old age, resulting in inadequate muscle adaptation during weight gain (Figure 11) (Tallis et al., 2018). Sarcopenic obesity is associated with an increased risk of disability, poor quality of life, and mortality compared to obesity or sarcopenia alone (Benz et al, 2024; Kalinkovich et al., 2017; Prado et al., 2024).

The diagnosis of sarcopenic obesity requires the assessment of muscle function as a first step and skeletal muscle mass as a second step. The currently recommended cut-offs for low muscle strength and mass in people with obesity according to the ESPEN/EASO consensus statement are summarized in Table 4. Specifically, the diagnosis of sarcopenic obesity requires low skeletal muscle mass adjusted to body weight, as a relative reduction in skeletal muscle mass may have significant functional consequences even in the absence of an absolute reduction in skeletal

muscle mass (Donini et al., 2022). The ESPEN/EASO consensus statement recommends the use of appendicular lean mass/body weight or skeletal muscle mass/body weight (Table 4). However, further research is needed to define "adequate/low" relative muscle mass and strength in people with obesity (Prado et al., 2024; Donini et al., 2022). Sarcopenic obesity is a distinct phenotype of obesity that is gaining importance as obesity and sarcopenia can reinforce each other by reducing activity levels and energy expenditure, which further increases fat mass (Figure 9) (Donini et al., 2022; Tallis et al., 2018; Maffiuletti et al., 2007.; Paulussen et al., 2021; McGlory et al., 2019).

Table 4. Diagnosis of sarcopenic obesity (adults, Caucasians)

Muscle function/strength			
Parameter	Cut-offs	Sample characteristics	Reference
HGS (kg)	< 16 kg in females < 27 kg in males	N= 49964 Females and males ≥ 5 Years; Caucasians	(Dodds et al., 2014)
Muscle mass			
Parameter	Cut-offs	Sample characteristics	Reference
SMM/BW (% BW) Based on BIA or DXA	Class I sarcopenia: 22.1-27.6% in females 31.5-37% in males Class II sarcopenia: <31.5% in males <22.1% in females	N=6414 Females and males 18-39 years Mixed ethnicity	(Janssen et al., 2002)
ALM/BW (% BW) Based on DXA	< 19.4% in females < 25.7% in males	N=4984 Females and males ≥ 60 years; Mixed ethnicity	(Batsis et al., 2013)

ALM: appendicular lean mass; BIA: bioelectrical impedance analyses; BW: body weight; DXA: dual-energy X-ray absorptiometry; HGS: handgrip strength; SMM: skeletal muscle mass. Adapted from Donini et al., 2022.

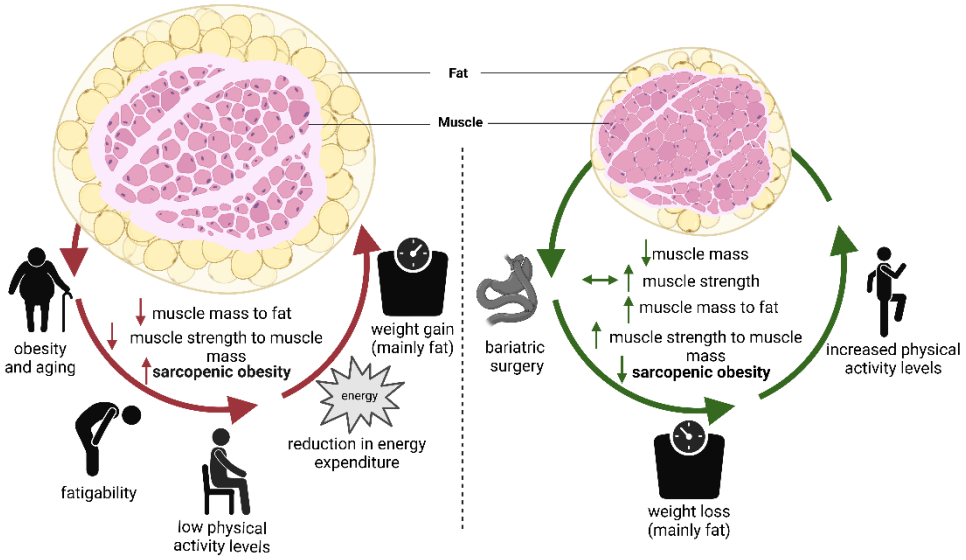


Figure. 11. Sarcopenic obesity before and after bariatric surgery. Schematic representation. ↑: increase or improvement; ↓: decrease; *red arrows*: people with obesity versus healthy people; *green arrows*: effect of bariatric surgery. Created with Biorender.com.

e. Changes in myokines in relation with muscle mass

Changes in myokines involved in the regulation of muscle mass in people with obesity compared to healthy people are summarized in table 3 (see above).

1. Myostatin

People with obesity and type 2 diabetes have usually higher circulating levels and muscle expression of myostatin than healthy people (see above). In addition, people with obesity and type 2 diabetes have increased levels and phosphorylation of SMAD2-3 transcription factors, which alters myogenesis

(Tallis et al., 2018). In contrast, the inhibition of the activin type II receptor with bimagrumab results in fat mass loss, increased lean mass, and improved insulin sensitivity in people with obesity and type 2 diabetes (Garito et al., 2018; Heymsfield et al., 2021). These data suggest that, in addition to insulin resistance, increased myostatin signaling may contribute to sarcopenic obesity (Prado et al., 2024; Park et al., 2006; Park et al., 2009). However, a positive correlation between circulating myostatin levels and muscle mass has been shown in various conditions, using immunoassays detecting active myostatin (Alexopoulos et al., 2021; Arrieta et al., 2019; Zhou et al., 2021; Yamada et al., 2016; Delanaye et al., 2019; Burch et al., 2017; Echeverria et al., 2021; Bergen et al., 2015). These data suggest that myostatin may not play a pathogenic role in muscle wasting in these conditions.

2. Follistatin

Circulating FST levels are usually higher in people with insulin resistance and type 2 diabetes than in healthy people (Hansen et al., 2013; Sylow et al., 2020). Follistatin and myostatin secretion are increased by primary myotubes from people with obesity and type 2 diabetes, suggesting that muscle may contribute to increased circulating follistatin and myostatin levels (Ciaraldi et al., 2016). In addition, circulating follistatin levels are negatively associated with muscle strength, mass, and function, suggesting that follistatin may be used as a biomarker of sarcopenia (Fife et al., 2018; Du et al., 2021). In fact,

increased follistatin at the circulating and muscle level could be interpreted as a compensatory mechanism to counteract increased myostatin in conditions such as obesity.

3. Irisin

Circulating irisin levels are positively correlated with muscle mass adjusted for body weight in postmenopausal women, and inversely associated with sarcopenia (Park et al., 2019). In addition, irisin expression decreases with age in human muscle (Guo et al., 2023). These data suggest that irisin may positively regulate muscle mass in humans. Therefore, decreased levels in people with obesity could promote sarcopenic obesity.

4. IL-6

Circulating IL-6 levels are usually elevated in people with obesity and type 2 diabetes but are much lower than in people with cancer and muscular dystrophy (Pelosi et al., 2021; Belizário et al., 2016). Human studies have suggested a possible association between age-related decline in muscle mass and strength and elevated circulating IL-6 levels (Pelosi et al., 2021). Indeed, older people with sarcopenia have higher IL-6 levels than those without, and IL-6 levels are associated with sarcopenia (Rong et al., 2018). In addition, higher circulating IL-6 levels in older people are associated with impaired muscle response to exercise training, and impaired physical performance (Grosicki et

al., 2020). Therefore, chronically elevated IL-6 could be an important contributor to a decline in muscle strength, quality, and function in people with obesity and type 2 diabetes (Grosicki et al., 2020). The effect of IL-6 on muscle mass and function may differ under physiological and pathological conditions that affect IL-6 signaling. Briefly, classical IL-6 signaling involves the binding of IL-6 to the membrane-bound IL-6 receptor alpha-subunit and glycoprotein 130 (gp130) signal-transducing subunit. In contrast, IL-6 trans-signaling, which has emerged as the predominant pathway by which IL-6 promotes disease pathogenesis, involve the binding of complexes of IL-6 and the soluble form of IL-6 receptor to membrane-bound gp130 (Rose-John et al., 2023).

5. BDNF

Circulating BDNF levels increase after exercise training in people with obesity and type 2 diabetes and correlate positively with muscle strength in lower limbs (Tsai et al, 2015). In older people, those with frailty (i.e., muscle weakness, slow walking speed, and low physical activity) have lower circulating BDNF levels than those without, and lower BDNF levels are associated with frailty regardless of age (Roh et al., 2022). Circulating BDNF levels are also correlated with exercise capacity and muscle strength, but not with muscle mass in people with heart failure (Nakano et al., 2020). Thus,

decreased BDNF levels in people with obesity may promote sarcopenic obesity.

6. FGF21

Older people with sarcopenia have higher circulating FGF21 levels than those without (Jung et al. Bone., 2021). In addition, FGF21 levels were found to be associated with an increased likelihood of sarcopenia, low muscle mass, and low muscle strength, independent of gender, age, and BMI (Jung et al. Bone., 2021; Roh et al., 2021). However, a meta-analysis found no difference in circulating FGF21 levels between people with and without sarcopenia, and no strong correlation between the onset of sarcopenia and circulating FGF21 levels (Liu et al., 2023). Nevertheless, these data suggest that increased FGF-21 levels in people with obesity may be associated with sarcopenic obesity.

7. SPARC

People with sarcopenia have higher circulating SPARC levels than those without (Kwak et al., 2018). This suggests that SPARC can serve as a potential biomarker for sarcopenia. Higher circulating SPARC levels and its muscle mRNA in people with obesity and type 2 diabetes may therefore be associated with sarcopenic obesity (Wu et al., 2011).

3. Impact of bariatric surgery on muscle insulin sensitivity and muscle mass and strength

3.1. Impact on muscle insulin sensitivity

a. Improved muscle insulin sensitivity

Bariatric surgery improves insulin sensitivity whether assessed by hyperinsulinemic-euglycemic clamp or HOMA-IR (Yoshino et al., 2020; Bradley et al. 2012). In fact, bariatric surgery improves sequentially systemic and tissue-specific insulin sensitivity (Figure 12) (Gancheva et al., 2019; Gastaldi et al., 2007). Systemic insulin sensitivity improves within days of bariatric surgery, allowing 30-100% of people with type 2 diabetes to discontinue all glucose-lowering drugs while maintaining euglycemia (Bradley et al., 2012; Schauer et al., 2003; Klein et al., 2023). Specifically, hepatic insulin sensitivity is particularly sensitive to acute caloric restriction, as evidenced by the marked reduction in hepatic fat content and glucose production early after bariatric surgery (Bradley et al., 2012). In contrast, muscle insulin sensitivity is not improved or only to a minor extent early after bariatric surgery (Camastra et al., 2011; de Weijer et al., 2013; Gancheva et al., 2019; Gastaldelli et al., 2016; Guidone et al., 2006). Improved muscle insulin sensitivity becomes apparent 10 to 12 weeks after bariatric surgery, when significant weight loss of > 15-20% has occurred (Yoshino et al., 2020; Madsbad et al., 2014; Bojsen-Møller et al., 2014; Gancheva et al., 2019; Gastaldi et al., 2007).

Further weight loss only slightly increases muscle insulin sensitivity (Bradley et al., 2012). Roux-en-Y gastric bypass and sleeve gastrectomy induce similar changes in muscle insulin sensitivity over time (Gancheva et al., 2019). Partial and complete reversal of muscle insulin resistance have been reported after bariatric surgery (Dantas et al., 2020; Barberio et al., 2021; Camastra et al., 2011; Gancheva et al., 2019). The length of postoperative follow-up and the combination of bariatric surgery with exercise training in some studies may explain, at least in part, different results.

Bariatric surgery induces extensive changes in insulin signaling improving muscle insulin sensitivity (Figure 7) (Dantas et al., 2020). Improved glucose uptake and oxidation, glycogen synthesis and insulin signaling have been reported in primary human myotubes as early as 4 weeks after bariatric surgery (Hinkley et al., 2017; Severino et al., 2016; Albers et al., 2015, b; Nascimento et al., 2015). In this model, no change was observed in total Akt, AS160/TBC1D4 or GLUT4 protein content, while hexokinase was slightly increased after bariatric surgery (Hinkley et al., 2017). In muscle tissue, increased protein expression of GLUT4, phosphorylated levels of AS160/TBC1D4, and insulin-induced changes in phosphorylation of Akt and glycogen synthase activity have been reported after bariatric surgery in people with and without type 2 diabetes at baseline (Greco et al., 2002; Mingrone et al., 2002; Albers et al., 2015, b).

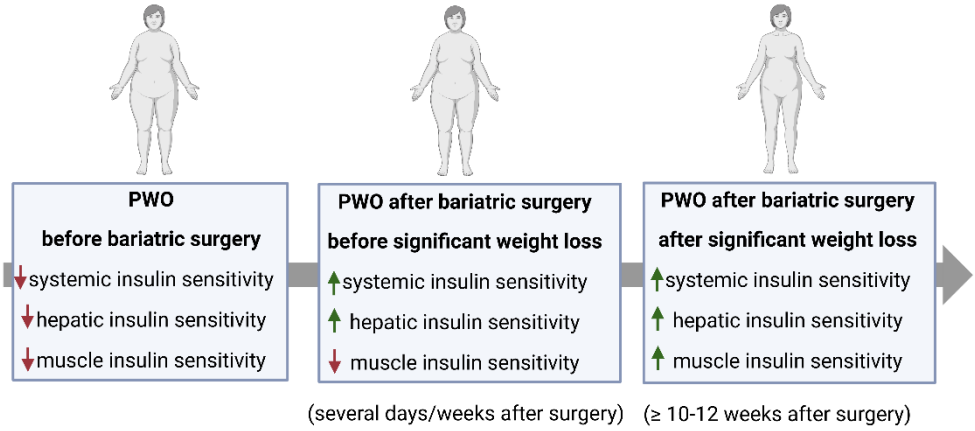


Figure. 12. Changes in insulin sensitivity in people with obesity before and after bariatric surgery. Schematic representation. (A) Muscle insulin resistance in PWO is characterized by a reduction in glucose uptake, phosphorylation, and PWO: people with obesity. ↑: increase; ↓: decrease. Created with Biorender.com.

b. Changes in fiber-type distribution

To the best of our knowledge, no studies have performed longitudinal assessment of muscle fiber-type distribution in people with obesity undergoing bariatric surgery, especially using immunochemistry, although it has been shown that bariatric surgery improves muscle oxidative capacity (Gancheva et al., 2019; Vijgen et al., 2013; Mingrone et al., 2005; Kugler et al., 2020). It should be noted that muscle metabolic function could improve without fiber-type transition, as observed after exercise training (Tallis et al., 2018). In contrast, it has been shown that a greater proportion of type I muscle fibers in rectus abdominis at baseline was associated with greater weight loss after bariatric surgery (Tanner et al., 2002).

c. Improved mitochondrial function

Bariatric surgery exerts variable effects on mitochondria over time, and conflicting results between studies may result from different timepoints (Gancheva et al., 2019). Vijgen *et al.* showed that mitochondrial dysfunction completely recovered one year after bariatric surgery using high-resolution respirometry (Vijgen et al., 2013). Similarly, Barberio *et al.* reported normalization of expression of genes involved in mitochondrial function and aerobic metabolism one year after bariatric surgery in women with and without type 2 diabetes at baseline (Barberio et al., 2021). Other studies reported improved muscle mitochondrial biogenesis and function at earlier (~3 months) and later (~6-12 months) timepoints after bariatric surgery (Figure 8), via upregulation of PGC1A and mitochondrial fusion proteins (Gastaldi et al., 2007; Gancheva et al., 2019). Upregulation of PGC1A protein and reduced mitochondrial fission have also been reported in primary myotubes of people with obesity after bariatric surgery (Hinkley et al., 2017; Kugler et al., 2020). In contrast, unbiased analysis of the muscle proteome and transcriptome at an early timepoint (~3 months) after bariatric surgery showed no significant enrichment in oxidative phosphorylation, and Krebs cycle proteins, and even decreased transcription of genes involved in both pathways (Campbell et al., 2016). On the one hand, decreased mitochondrial content and function early after bariatric surgery could result from the dramatic reduction in caloric and

protein intake (Bertoni et al., 2021), which induces an energy economy phenomenon essentially due to low lipid oxidation and the breakdown of muscle proteins and organelles (Bobbioni-Harsch et al., 2000). On the other hand, decreased mitochondrial function could result from increased adipose tissue lipolysis and transient lipotoxicity early after bariatric surgery (Gancheva et al., 2019). Finally, exercise training improves oxidative phosphorylation and muscle insulin sensitivity beyond bariatric surgery alone (Dantas et al., 2020). Thus, although it is generally expected that muscle mitochondrial function and biogenesis improve after bariatric surgery, early time points may be characterized by decreased oxidative metabolism.

d. Improved myosteatosis

Bariatric surgery reduces total and visceral adipose tissue contents, as well as ectopic fat in liver and pancreas, all of which are expected to improve glucose homeostasis (Sandoval et al., 2023; Petrov et al., 2022; Toro-Ramos et al. 2015). However, few studies have investigated the effects of bariatric surgery on myosteatosis. Using whole-body magnetic resonance imaging, Toro-Ramos *et al.* showed significant reductions in IMAT one year after bariatric surgery, so that IMAT content reached that of lean people (Toro-Ramos et al. 2015). Another study showed that extramyocellular lipids, but not intramyocellular lipids, were reduced at 6 and 12 months after bariatric surgery using magnetic resonance imaging and magnetic resonance

spectroscopy (Johansson et al. 2008). Several studies have however shown that intramyocellular lipids and perivascular and interfibrillar fat depots were all reduced after bariatric surgery, using quantitative immunochemistry (Camastra et al., 2017; Greco et al., 2002). These findings were similar in people with or without type 2 diabetes at baseline and observed despite most people remaining in the obesity range after bariatric surgery (Greco et al., 2002). Muscle insulin sensitivity is inversely related to intramyocellular lipid content after bariatric surgery (Greco et al., 2002). Decreased expression of the sterol regulatory element-binding protein 1c (SREBP1c), the main lipogenic transcription factor in human muscle (i.e., vastus lateralis) after bariatric surgery, suggests that lipogenesis is decreased (Mingrone et al., 2003). In conclusion, bariatric surgery causes substantial and robust loss in total and regional fat depots, including IMAT, and intramyocellular lipids (Figure 8) (Camastra et al., 2017). Intramyocellular lipid withdrawal seems to be a critical factor in improving muscle insulin sensitivity after bariatric surgery.

e. Improved muscle inflammation

Few studies have specifically investigated the effects of bariatric surgery on muscle inflammation. Nevertheless, some general findings indicate that bariatric surgery decreases both circulating and muscle inflammatory markers. Tamboli *et al.* showed a general downregulation of inflammatory pathways in muscle after bariatric surgery, an effect that was amplified in a

subgroup of individuals that also underwent omentectomy (Tamboli et al., 2011). Some genes downregulated after bariatric surgery, with or without omentectomy, encoded for myokines such as IL-6 and monocyte chemoattractant protein-1 (MCP-1/CCL2) (Tamboli et al., 2011). Both myokines are upregulated in conditioned media from insulin-resistant myotubes (Bouzakri et al., 2011) and considered to exert pro-inflammatory effects during obesity (Eckardt et al., 2014). Most of the inflammatory genes that were downregulated only in the omentectomy subgroup were associated with macrophage activation (e.g., CXCL2) (Tamboli et al., 2011). Similarly, Yan K. *et al.* have shown that inflammation-related signaling pathways are significantly altered after bariatric surgery, using integrative bioinformatics analysis (Yan et al., 2022). Specifically, the IL6-JAK-STAT3 and IL2-STAT5 signaling pathways are downregulated in muscle after bariatric surgery. IL6-JAK-STAT3 signaling is a classic inflammatory pathway that is activated in muscle from people with obesity (Mashili et al., 2013; Wu et al., 2017), while IL2-JAK-STAT5 signaling controls the homeostasis of pro- and anti-inflammatory T-cells (Yan et al., 2022). Elevated IL-2 expression correlates with adipose tissue insulin resistance during obesity (Yan et al. 2022). Mingrone *et al.* also reported marked decrease in TNF-a mRNA levels in muscle after bariatric surgery, which was associated with decreased intramyocellular lipid content and improved muscle insulin sensitivity (Mingrone et al., 2002). In addition, circulating levels of pro-inflammatory factors such as TNF-a, IL-1β, IL-6, IL-8, and MCP-

1 are generally decreased at early and later timepoints after bariatric surgery (Villarreal-Calderon et al., 2021; Tamboli et al., 2011; Gancheva et al., 2019). All these factors are expressed and secreted by insulin-resistant human myotubes (Bouzakri et al., 2011). However, the potential contribution of muscle to changes in inflammatory markers at the circulating level is unknown. Most changes observed after bariatric surgery are generally attributed to reduced adipose tissue inflammation (Sandoval et al., 2023.; Madsbad et al., 2014).

f. Healthier extracellular matrix phenotype

Bariatric surgery promotes extracellular matrix degradation and collagen digestion via changes in metalloproteinases expression in muscle (Dantas et al., 2020). Reversal of the prominent basement membrane thickness, decreased collagen I and III deposition, and increased capillary density have been observed in people with obesity undergoing bariatric surgery combined with exercise training (Figure 8) (Dantas et al., 2020). Exercise training potentiates the effects of bariatric surgery on muscle insulin sensitivity and extracellular matrix remodeling, including further reduction of collagen I and III deposition compared to bariatric surgery alone as well as specific effects on extracellular matrix-related genes (e.g. vimentin) (Dantas et al., 2020). In addition, exercise training combined with bariatric surgery decreases TGF-beta signaling, myostatin expression, SMAD 2-3 phosphorylation but increases follistatin expression, compared to bariatric surgery alone (Dantas

et al., 2020). All these factors are expected to improve muscle fibrosis and counteract muscle mass loss. Finally, bariatric surgery increases the expression of angiogenesis markers, including vascular endothelial growth factor (VEGF) (Gil et al., 2021). However, greater muscle fiber capillarization after bariatric surgery was observed in exercised people only (Gil et al., 2021).

g. Changes in myokines in relation with muscle insulin sensitivity

Improved glucose homeostasis after bariatric surgery is characterized by improved systemic, liver, and muscle insulin sensitivity as well as recovery of beta cell morphology, cell mass, and glucose-stimulated insulin secretion (Liu et al., 2023). These processes contribute to the remission of type 2 diabetes after bariatric surgery. The recovery of beta cells is promoted by reduced islet inflammation and glucotoxicity, inducing beta cell apoptosis and dedifferentiation (Liu et al., 2023). In addition, the increased secretion of certain gastro-intestinal hormones such as glucagon-like-peptide-1 is thought to promote islet function restoration. Finally, as insulin resistance is largely alleviated after bariatric surgery, the workload of beta cells (i.e., compensation) decreases (Liu et al., 2023; Weir et al., 2004). Improvement of homeostasis model assessment of beta-cell function (HOMA-B) has been reported after bariatric surgery. Myokines may be involved in both improved insulin sensitivity, especially at the muscle level, and improved beta-cell function after bariatric surgery (Table 3).

1. BDNF

Several studies show decreased circulating BDNF levels after bariatric surgery (Merhi et al., 2009; Muñoz-Rodríguez et al., 2018). However, none of these studies have linked changes in BDNF to changes in glucose homeostasis after bariatric surgery.

2. Myostatin

Kumar *et al.* have shown that circulating myostatin levels decrease by 22% six months after Roux-en-Y gastric bypass in adolescent females (Kumar et al., 2019). Milan *et al.* observed a 60% reduction in quadriceps myostatin mRNA in people with obesity and normal glucose tolerance at baseline 18 months after bariatric surgery (i.e. biliopancreatic diversion) (Milan et al., 2004). However, no association was found between changes in myostatin and changes in HOMA-IR (Kumar et al., 2019).

3. Irisin

Changes in circulating irisin levels after bariatric surgery show no discernible pattern. Kumar *et al.* have reported increased circulating irisin levels in adolescent females 6 months after bariatric surgery (Kumar et al., 2019). However, no association was found with changes in HOMA-IR. In other studies, circulating irisin levels remained unchanged or even decreased from

one month to one year after bariatric surgery in people with or without type 2 diabetes at baseline (Demirpence et al., 2016; Lee et al., 2019; Sajoux et al., 2019; Huh et al., 2012). Decreased *FNDC5* expression in skeletal muscle has been reported 6 months after bariatric surgery (Huh et al., 2012). The inconsistent results of circulating irisin levels after bariatric surgery preclude meaningful speculation about its role in this context in humans.

4. Interleukin-6

Kumar *et al.* have reported reduced circulating IL-6 levels in adolescent females 6 months after bariatric surgery (Kumar et al., 2019). However, no association was found with HOMA-IR. Similarly, Villarreal-Calderon *et al.* have found reduced circulating IL-6 levels 6 months after bariatric surgery, while metabolic improvement was evident as early as 3 months (Villarreal-Calderon et al., 2021). In contrast, Sajoux *et al.* have reported unchanged circulating IL-6 levels after bariatric surgery (Sajoux et al., 2019).

5. Fibroblast growth factor 21

A steep increase in circulating FGF21 levels has been observed within weeks of bariatric surgery (Crujeiras et al., 2017; Gomez-Ambrosi et al., 2017; Lips et al., 2014). Increased FGF21 in muscle after bariatric surgery would be expected to improve muscle insulin sensitivity, particularly through intramyocellular lipid depletion. However, no association was found between changes in

circulating FGF21 levels and HOMA-IR (Crujeiras et al., 2017). People with obesity and type 2 diabetes who had undergone bariatric surgery 12 years earlier exhibited similar circulating FGF21 levels to those who had not undergone bariatric surgery, despite a lower BMI (Butler et al., 2023).

6. Meteorin-like protein

The data on circulating Metrnl levels after bariatric surgery are also conflicting. Pellitero *et al.* have found increased circulating Metrnl levels 12 months after bariatric surgery, which inversely associated with HOMA-IR, suggesting a potential insulin-sensitizing effect of Metrnl (Pellitero et al., 2018; Li et al., 2015). In contrast, Schmid *et al.* have reported decreased circulating Metrnl levels within days after bariatric surgery, followed by a progressive return to baseline (Schmid et al., 2021). Decreased circulating Metrnl levels after bariatric surgery have been also observed in rats while Metrnl expression was increased in muscle and white adipose tissue (Jamal et al., 2020). In addition, hepatic Metrnl expression is reduced in humans after bariatric surgery, suggesting that tissues other than muscle may contribute to changes in circulating Metrnl levels after bariatric surgery (Grander et al., 2022).

7. Myonectin

Li *et al.* have shown increased circulating myonectin levels 6 months after bariatric surgery (Li et al., 2020). Although circulating myonectin levels were

negatively associated with HOMA-IR at baseline, no association with HOMA-IR was searched after bariatric surgery. In contrast, Butler *et al.* have shown that people who had undergone bariatric surgery had similar circulating myonectin levels to those who had not undergone bariatric surgery, even though BMI was lower after bariatric surgery (Butler et al., 2023). However, these data were obtained 12 years after bariatric surgery.

8. SPARC

Lee *et al.* have shown decreased circulating SPARC and MMP-2 levels after bariatric surgery (Lee et al., 2014). Changes in circulating SPARC levels, but not MMP2, were significantly correlated with changes in HOMA-IR, suggesting that reduced SPARC is associated with improved insulin sensitivity (Lee et al., 2014). In contrast, Sajoux *et al.* have shown that circulating MMP2 levels increase after bariatric surgery, which may contribute to a healthier extra-cellular matrix phenotype in muscle (Sajoux et al., 2019). Notably, resistance exercise also increases circulating MMP2 levels in humans (Sajoux et al., 2019). These data suggest that SPARC and MMP2 may play opposing roles in the extracellular matrix remodeling during obesity and after bariatric surgery, thereby regulating muscle insulin sensitivity.

9. Apelin

Circulating apelin levels decrease after bariatric surgery and decreased apelin levels are associated with improved insulin sensitivity (Krist et al., 2013; Soriguer et al., 2009; Heinonen et al., 2005). Apelin expression in adipose tissue is decreased after bariatric surgery, which likely impacts circulating apelin levels and may be associated with improved glucose homeostasis after bariatric surgery (Krist et al., 2013).

10. Other myokines

Circulating FSTL-1 levels decrease after bariatric surgery (Santos et al., 2024). However, no association has been reported with glucose homeostasis.

11. Myokines and beta cell function

Fractalkine, also known as C-X3-C motif chemokine ligand 1 (CX3CL1), is the only member of the CX3C chemokine family (Rutti et al., 2014). Fractalkine is initially synthesized in a plasma membrane-bound form, and its soluble form is released by enzymatic cleavage (Rutti et al., 2014). Fractalkine is the specific ligand for a G protein-coupled receptor called CX3CR1, which mediates its effects on chemotaxis, cell adhesion, and increased cell survival during inflammation (Rutti et al., 2014). Fractalkine is secreted by human muscle cells, although it has a low tissue specificity (Raschke et al., 2013; Bouzakri

et al., 2011; Uhlén et al., 2015; Uhlén et al., 2019). Contraction upregulates fractalkine expression in human muscle cells and tissue, and increases its circulating levels (Raschke et al., 2013; Catoire et al., 2014; Pourteymour et al., 2017).

Fractalkine may have beneficial metabolic effects, particularly on beta cells. Indeed, CX3CR1 knockout mice exhibit impaired glucose-stimulated insulin secretion, which is also observed in islets isolated from these mice (Lee et al., 2013). In contrast, CX3CR1 knockout mice treated with fractalkine show improved glucose tolerance and glucose-stimulated insulin secretion, while fractalkine also potentiates glucose-stimulated insulin secretion in mouse and human islets (Lee et al., 2013). Similarly, chronic administration of a long-acting form of fractalkine improves glucose tolerance in obese rodents, as evidenced by increased glucose-stimulated insulin secretion and hepatic insulin sensitivity (Riopel et al., 2018). Furthermore, fractalkine protects rodent beta cells from apoptosis and protects glucose-stimulated insulin secretion against pro-inflammatory cytokines (Lee et al., 2013; Rutti et al., 2014). Fractalkine expression in islets is reduced in mice fed a high-fat diet and obese mice, suggesting that reduced fractalkine signaling may contribute to beta dysfunction during obesity and type 2 diabetes (Lee et al., 2013). Some studies have reported higher circulating fractalkine levels in people with obesity and type 2 diabetes, while others have not (Shah et al., 2011; Eckardt et al., 2014). In

addition, data on fractalkine expression in muscle from people with obesity and type 2 diabetes are conflicting (Eckardt et al., 2014).

In conclusion, bariatric surgery is associated with changes in circulating levels and muscle expression of several myokines regulating glucose homeostasis (Table 3). However, for most of them, no association was found between changes in circulating levels or muscle expression and changes in glucose homeostasis after bariatric surgery. Thus, the potential contribution of myokines to improved glucose homeostasis after bariatric surgery remains poorly understood.

3.2. Impact on muscle mass and strength

a. Changes in muscle protein turnover

Current data suggest that muscle protein synthesis is reduced after bariatric surgery due to the early acute caloric and protein restriction (Katsanos et al., 2016). Dietary protein intake is reduced to about 30g/day early after bariatric surgery, as part of an overall restriction of food intake (i.e., 500-800 kcal/day) (Nuijten et al., 2022). Reasons for protein restriction after bariatric surgery include the difficulty of consuming solid protein-based foods (e.g., meat) due to surgical restriction (Bertoni et al., 2021; Moize et al., 2003) and changes in gastrointestinal hormones that promote early satiety (e.g., increased post-prandial glucagon-like-peptide-1 secretion), whereas proteins are the most

satiating macronutrients (Bertoni et al., 2021). This makes it difficult to achieve the recommended protein intake after bariatric surgery of at least 60g/day or 1.1g/kg ideal body weight (Nuijten et al., 2022; Tabesh et al., 2019; Bertoni et al., 2021). In addition, 20-40g of proteins per meal are needed for an optimal response of muscle protein synthesis (Nuijten et al., 2022). In contrast, malabsorption is unlikely to be a determining factor in protein restriction, as it is minimal after Roux-en-Y gastric bypass and not observed after sleeve gastrectomy (Katsanos et al., 2016; Madsbad et al., 2014; Nguyen et al., 2017). Periods of muscle unloading lead to muscle mass loss, which is likely to occur after bariatric surgery given that clinical guidelines discourage exercise (other than walking and activities of daily living) and prohibit weightlifting for up to six weeks after bariatric surgery (Nuijten et al., 2022; Tabesh et al., 2019). At the molecular level, mTORC1 basal and insulin-stimulated phosphorylation (Magkos et al., 2013), and mTORC1 pathway-related proteins seem unaffected by bariatric surgery (Gil et al., 2021).

On the other hand, several factors may increase muscle protein breakdown early after bariatric surgery. Initial surgical stress may transiently increase muscle protein breakdown through the effects of inflammation and catabolic hormones (Bertoni et al., 2021; Gore et al., 1993). In addition, caloric restriction is a powerful physiological inducer of autophagy, which aims to release amino acids from muscle proteins (Chung et al., 2019; Wolfe et al., 2017). Caloric

restriction also induces epigenetic chromatin remodeling that enables FOXO to stimulate Atrogin-1/MAFbx and MuRF1 expression (Chung et al., 2019; Sartori et al., 2021; Gil et al., 2021). However, several lines of evidence suggest that muscle protein breakdown is decreased following bariatric surgery. Campbell *et al*. showed that proteins of the ubiquitin-proteasome pathway were enriched among downregulated proteins 3 months after bariatric surgery, whereas ribosome proteins were enriched among the upregulated proteins, suggesting reduced muscle protein breakdown and an attempt to increase muscle protein synthesis (Campbell et al., 2016). Gil *et al*. reported that the expression of autophagy genes and MuRF-1 mRNA decreased 9 months after bariatric surgery (Gil et al., 2021). Similarly, Tamboli *et al*. reported that breakdown of myofibrillar proteins was reduced 6 months after bariatric surgery (Tamboli et al., 2010). However, the effect could be transient, as muscle protein breakdown returned to baseline at 12 months, coinciding with the attenuation of skeletal muscle mass loss (Tamboli et al., 2010). Milan et al. reported that overall protein degradation was similar to baseline when measured 18 months after bariatric surgery, when skeletal muscle mass had already stabilized (Milan et al., 2004). Overall, these data can be interpreted as an attempt to preserve muscle mass after bariatric surgery by reducing muscle protein breakdown and overall muscle protein turnover. However, protein intake following bariatric surgery does not cover protein requirements

for most individuals (Guillet et al., 2020; Nuijten et al., 2022; Katsanos et al., 2016), ultimately leading to skeletal muscle mass loss.

b. Changes in muscle mass

Loss of skeletal muscle mass is one of the most notable but unintended changes occurring after bariatric surgery (Davidson et al., 2018). The amount of skeletal muscle mass loss after bariatric surgery is directly related to the amount of weight loss (Chaston et al., 2007; Nuijten et al., 2022). Weight loss occurs primarily in the first year after bariatric surgery, averaging 30-35% of baseline body weight for Roux-en-Y gastric bypass and 20-30% for sleeve gastrectomy (Maciejewski et al., 2016). Maximum weight loss is expected ≈1.5-2 years after bariatric surgery, then some weight regain may be observed during long-term follow-up (Noria et al., 2023; Madsbad et al., 2014; Davidson et al., 2018). As a result, the most favorable body composition (i.e., lowest body weight and fat mass) is expected to occur ≈12-18 months after bariatric surgery, with an average loss of fat mass of ≈30-50% and skeletal muscle mass of ≈10-15% of their baseline values (Nuijten et al., 2020; Davidson et al., 2018). Specifically, skeletal muscle mass loss begins as early as the first month after bariatric surgery, continues at a high rate for up to 6 months, and then declines slightly until reaching a plateau ≈12-18 months after bariatric surgery (Maïmoun et al., 2019; Nuijten et al., 2020; Sherf-Dagan et al, 2019; Davidson et al., 2018). In contrast, skeletal muscle mass adjusted to body weight increases

during the first year after bariatric surgery because of the predominant loss of fat mass (Figure 10) (Davidson et al., 2018; Nuijten et al., 2020; Sherf-Dagan et al., 2019). Overall, the contribution of skeletal muscle to weight loss after bariatric surgery averages 15-25%, depending on individual factors such as age and gender, the amount of weight loss, the surgical procedure, and the method of assessment (Davidson et al., 2018; Chaston et al., 2007). Skeletal muscle mass loss after Roux-en-Y gastric bypass is either greater, similar, or less than that observed after sleeve gastrectomy (Davidson et al., 2018; Martínez et al., 2022; Kim et al., 2019; Otto et al., 2016; Nuijten et al., 2020). Skeletal muscle mass loss after bariatric surgery occurs in both the trunk and limbs (i.e. arms and legs) (Tamboli et al., 2010).

c. Changes in muscle strength

A reduction in muscle strength would be expected following a reduction in skeletal muscle mass after bariatric surgery, as the two are positively correlated. In a meta-analysis of 10 studies, the pooled analysis of handgrip strength showed no significant change after bariatric surgery, despite a significant decrease in skeletal muscle mass (Jung et al., 2023). Only one of the included studies reported a reduction in handgrip strength (Jung et al., 2023). However, two studies reported improved handgrip strength-to-BMI and handgrip strength-to-skeletal muscle mass ratios, which is clinically relevant because higher handgrip strength-to-BMI ratio is inversely associated with

the risk of metabolic syndrome, and the association is stronger than with absolute handgrip strength (Jung et al., 2023). Weight-bearing muscles such as the back, pelvis and lower limbs reveal different results. A meta-analysis of three studies showed decreased absolute muscle strength in the lower limbs after bariatric surgery (Herring et al., 2016). However, a recent study showed increased muscle strength-to-body weight ratio in the lower limbs after bariatric surgery although absolute muscle strength was reduced (Gadducci et al., 2024). Thus, changes in muscle strength are different from changes in skeletal muscle mass, but in both cases the relationship to body and fat mass is improved (Figure 10) (Jung et al., 2023). Of note, most studies have been conducted at 6 or 12 months after bariatric surgery, when muscle mass and strength are most likely to be affected (Jung et al., 2023). Finally, a meta-analysis showed that objectively measured and self-reported exercise is greater after bariatric surgery, which may help improve the muscle phenotype and function (Herring et al., 2016; Dantas et al., 2020). Physical function, as measured by validated techniques such as the 6-minute walk distance, the short physical performance battery, or the timed up-and-go test, improves as early as 3 months after bariatric surgery (Steele et al., 2015; Herring et al., 2016). Functional capacity is more strongly related to muscle strength than to skeletal muscle mass, and low muscle strength is significantly associated with impaired physical function and adverse outcomes (Jung et al., 2023; Cruz-Jentoft et al., 2019). The improved muscle strength-to-body weight and muscle

strength-to-skeletal muscle mass ratios after bariatric surgery may counteract the negative effects of reduced skeletal muscle mass and improve daily activities and mobility after bariatric surgery, therefore opposing sarcopenic obesity (see below).

To our knowledge, few studies have investigated the key molecular mechanisms involved in muscle contractile function after bariatric surgery. Type II muscle fibers are more prone to atrophy that type I muscle fibers, which potentially affect muscle mass and strength following bariatric surgery (Wang et al., 2013). Dantas *et al.* showed that bariatric surgery increases AMPK phosphorylation and AMPK total content in muscle and this effect is enhanced by exercise training (Dantas et al., 2020). Caloric restriction, decreased insulin levels, and reduced muscle inflammation and myosteatosis after bariatric surgery are all expected to restore AMPK activity and calcium signaling (Tallis et al., 2018). PGC1A expression and mitochondrial function improve at later timepoints after bariatric surgery. Improved muscle quality and physical performance after bariatric surgery suggest underlying beneficial molecular adaptations.

d. Changes in sarcopenic obesity

The loss of muscle mass and strength after bariatric surgery raises concerns about the onset of sarcopenia or sarcopenic obesity in people who do not

lose enough weight to be classified as overweight or normal weight (Nuijten et al., 2022; Molero et al., 2022). However, studies evaluating the prevalence of sarcopenia and sarcopenic obesity after bariatric surgery are scarce. One case series showed that the prevalence of sarcopenia, defined as low skeletal muscle mass (i.e., skeletal muscle mass index <38.5 cm^2/m^2 for women and <52.4 cm^2/m^2 for men based on computed tomography scan), increased from 8% at baseline to 30% after bariatric surgery (Voican et al., 2018). This finding is consistent with skeletal muscle mass loss after bariatric surgery, but muscle strength was not assessed. Another study reported that low skeletal muscle mass, defined as a skeletal muscle mass index (kg/m^2) >-1 standard deviation from the reference group, was present in 20% of people at baseline and 25% 5 years after bariatric surgery (Molero et al., 2022). These data suggest that low skeletal muscle mass is not uncommon in people after bariatric surgery, but skeletal muscle mass was not adjusted for body weight. In these studies, older age, male sex, lower baseline BMI or skeletal muscle mass, and sleeve gastrectomy were found to be independent predictors of sarcopenia or low skeletal muscle mass after bariatric surgery (Molero et al., 2022; Voican et al., 2018). There are no guidelines for defining excessive or harmful skeletal muscle mass loss after bariatric surgery (Chaston et al., 2007). However, preservation of muscle strength, increased muscle strength-to-body weight and muscle strength-to-skeletal muscle

mass ratios, as well as improved muscle function after bariatric surgery do not support an increased risk of sarcopenia (Figure 11).

e. Changes in myokines in relation with muscle mass and strength

Changes in myokines involved in muscle mass regulation after bariatric surgery are shown in Table 3.

1. Myostatin

Decreased myostatin levels (Kumar et al., 2018) and mRNA in muscle have been reported after bariatric surgery (Milan et al., 2004). Milan *et al.* have found a positive correlation between decreased myostatin mRNA and decreased muscle mass 18 months after bariatric surgery (Milan et al., 2004). On the one hand, these data suggest that decreased myostatin expression is responsible for decreased circulating myostatin levels and that circulating myostatin does not play a major pathogenic role in muscle wasting after bariatric surgery. On the other hand, these data suggest that circulating myostatin levels may be used as a biomarker of muscle mass in people with obesity undergoing bariatric surgery. No study to date has investigated whether myostatin is a biomarker of muscle mass in people with obesity undergoing bariatric surgery.

2. Follistatin

The effects of bariatric surgery on circulating follistatin levels are inconsistent. Some studies have reported increased levels (Han et al., 2019; Pham et al., 2021), while others have reported decreased or unchanged levels (Tao et al., 2018; Perakakis et al., 2019; Pham et al., 2021) after bariatric surgery. Changes in follistatin after bariatric surgery could regulate muscle mass, but current data are insufficient to support this hypothesis.

3. Irisin

Although circulating irisin levels do not follow a clear pattern after bariatric surgery, irisin expression in muscle is decreased (see above) (Huh et al., 2012). Based on the documented anabolic effect of irisin on muscle cells, decreased irisin expression in muscle after bariatric surgery could contribute to muscle wasting after bariatric surgery.

4. IL-6

Bariatric surgery is associated with decreased circulating IL-6 levels (see above) which likely reflects reduced adipose tissue inflammation. Data on IL-6 expression in muscle after bariatric surgery is lacking, and associations with muscle mass after bariatric surgery have not been reported.

5. BDNF

Decreased circulating BDNF levels after bariatric surgery could reflect or contribute to muscle mass loss. However, data on BDNF mRNA in muscle after bariatric are lacking, and associations with muscle mass after bariatric surgery have not been reported.

6. Myonectin

The observed increase in circulating myonectin levels after bariatric surgery (see above) may contribute to attenuate the decline in muscle mass and mitochondrial function observed at early timepoints after bariatric surgery. However, associations with muscle mass after bariatric surgery have not been reported.

7. FGF21

The steep increase in circulating FGF21 levels early after bariatric surgery followed by a slight decrease over time (see above), may be related to initial muscle wasting in response to caloric restriction, which tends to decrease over time. However, associations with muscle mass after bariatric surgery have not been reported.

8. SPARC

Decreased circulating SPARC levels have been reported after bariatric surgery (see above). Although SPARC may regulate muscle mass, no study has assessed the relationship between changes in SPARC and muscle mass after bariatric surgery.

In conclusion, bariatric surgery is associated with changes in circulating levels and muscle expression of myokines that may be involved in the regulation of muscle mass and strength in humans (Table 3). However, the potential contribution of changes in these myokines and changes in muscle mass after bariatric surgery has not been investigated. Furthermore, some myokines may be biomarkers of sarcopenia. However, the relationship between these myokines and sarcopenic obesity has not been investigated.

Based on this literature review, we are preparing two narrative reviews:

Orioli L, Thissen JP. Impacts of obesity and bariatric surgery on muscle mass and insulin sensitivity: a narrative review *(in preparation).*

Orioli L, Thissen JP. Potential contribution of myokines to changes in glucose homeostasis and muscle mass after bariatric surgery: a narrative review *(in preparation).*

II. AIMS OF THE WORK

This work aims to investigate the potential contribution of myokines to changes in glucose homeostasis and muscle mass after bariatric surgery.

Our hypothesis are as follows:

1) Bariatric surgery alters the expression of myokines in muscle samples and myotubes.

2) These changes may translate into changes of the circulating level.

3) Changes in myokines expression and circulating levels are associated with changes in glucose homeostasis induced by bariatric surgery.

4) Changes in myokines expression and circulating levels are associated with changes in body composition, especially skeletal muscle mass, induced by bariatric surgery.

The specific objectives of this work are as follows:

1) to identify myokines susceptible to improve glucose homeostasis after bariatric surgery on muscle samples.

2) to identify myokines susceptible to improve glucose homeostasis after bariatric surgery using a primary human skeletal muscle cell culture model.

3) to identify myokines susceptible to improve glucose homeostasis after bariatric surgery using multiplex immunoassay on plasma samples.

4) to determine whether myostatin is a biomarker of muscle mass in people with obesity undergoing bariatric surgery

III. MATERIAL AND METHODS

1. Study design

The MYDIASECRET study is a prospective, longitudinal, and interventional study led from January 2018 through October 2020 (NCT03341793). The study included 62 Caucasians participants scheduled for bariatric surgery aged 19-69 years. Eligibility criteria for bariatric surgery consisted of a 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 (American Diabetes Association, 2021). Glycated hemoglobin A1c (HbA1c) at inclusion was ≤7.5% (≤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. Participants were evaluated before (T0) and 3 months (T3) after bariatric surgery, which consisted of either a sleeve gastrectomy or a Roux-en-Y gastric bypass. Anthropometric parameters included height, weight, BMI, waist, and hip circumferences. Body composition was determined using bioelectrical impedance analysis (Tanita, BC-418 MA), which provided an estimate of fat mass and fat-free mass. In addition, muscle mass was assessed using bioelectrical impedance analysis (BIA101 device,

Akern®, Pisa, Italy). Whole body resistance and reactance were measured with the subject in a supine position, with arms and legs slightly separated. Surface electrodes were placed on the right side of the body on the dorsal surface of the hand and foot. Fat mass was estimated using Bodygram® software and proprietary manufacturer algorithms (Akern®, Pisa, Italy). Skeletal muscle mass was calculated using Janssen's equation which is based on magnetic resonance imaging as reference method (Janssen et al., 2000, b). Appendicular skeletal muscle mass was calculated using Sergi's equation which is based on DXA as reference method (Sergi et al., 2015). Fat mass, skeletal muscle mass, and appendicular skeletal muscle mass were expressed as absolute values (kg), percentage values (percentage of body weight, %BW), and indexes (I, kg/m²). Longitudinal changes from baseline (T0) were expressed as absolute changes (kg or kg/m²) or percentage changes (percentage change from T0, %T0). Handgrip strength was assessed using a Jamar hand-held dynamometer at T0 and T3. Three measures were made on the nondominant side in a time interval of 30 seconds. The highest value (kg) was used. Changes in muscle strength were expressed as described above. 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) (Levy et al., 1998). 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 (Camara et al., 2014). 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 either immediately frozen in liquid nitrogen for RNA analysis or immediately transferred to cold phosphate-buffered saline (PBS) for muscle cell culture. 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.

Control individuals were identified from a cohort of healthy men (HbA1c <6.5%) recruited at the Institute of NeuroScience (UCLouvain, Belgium) (De Groote et al., 2021). Eighteen healthy male individuals without obesity (BMI <30kg/m²) from this cohort were included in a control group. Fasting insulin and fasting glucose from control individuals were used to compute HOMA-IR using to HOMA2 calculator, as described above. RNA from muscle samples (vastus lateralis) taken using a Bergstrom needle was kindly provided by Pr. L. Deldicque (Institute of NeuroScience, UCLouvain, Belgium).

2. Identification of myokines susceptible to improve glucose homeostasis after bariatric surgery on muscle samples

2.1. RT-qPCR

Muscle samples for RNA analysis were taken in 39 participants at T0 and T3. 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 (Gueugneau et al., 2018). Relative gene expression after bariatric surgery was calculated using the comparative Ct method and normalized by the expression of the housekeeping gene GAPDH. In addition, muscle RNA from control individuals were used to compare the expression of myosin heavy chains (*MYH1*, *MYH2*, *MYH7*) and *PPARGC1A* (encoding PGC1A*)* with the study group, using RT-qPCR. Primers used for amplification during RT-qPCR were tested to avoid primer dimers, self-priming formation, or any unspecific amplification (Supplementary Table 1).

2.2. Targeted study

A literature search was performed on PubMed to identify myokines known to regulate glucose homeostasis. These myokines included brain-derived neurotrophic factor (BDNF), fractalkine (CX3CL1), angiogenin (ANG), osteoprotegerin (TNFRSF11B), a disintegrin and metalloproteinase with thrombospondin motifs 9 (ADAMTS9), irisin (FNDC5), myostatin (MSTN), apelin (APLN), interleukin-6 (IL6), tripartite motif-containing protein 72 (TRIM72), and secreted frizzled-related protein 4 (SFRP4). Relative expression levels of these myokines after surgery were determined using RT-qPCR in muscle samples.

2.3. Untargeted study

High-throughput RNA-sequencing (RNA-Seq) was performed on muscle biopsies from 12 participants. Total RNA was extracted from muscle samples as described above. 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 per sample. Bioinformatic methods are detailed in Supplementary Methods. Differential expression of the transcripts and genes was evaluated with the Bioconductor R package DESeq2 (v.1.30.0) for R (v.4.0.4) using a paired design. P values were corrected for multiple testing using Benjamini–Hochberg false discovery rate (FDR) and set at ≤ 0.10. Genes that were upregulated (≥1.3-fold) or downregulated (≤0.7-fold) were searched among all regulated genes after surgery. Those encoding putative myokines were identified using predicted signal peptide as described elsewhere (Massart et al., 2020), in combination with annotations from UniProtKB (secreted/extracellular space, https://www.uniprot.org/).

GSEA was performed using the R package clusterProfiler (Wu et al., 2021; Yu et al., 2012) (Supplementary Figure 1). GSEA aims to determine whether genes belonging to a particular pathway are clustered at the top or bottom positions of the ranked gene list. The normalized enrichment score (NES) reflects the degree of overrepresentation at the top or bottom positions of the ranked gene list. GSEA was performed on all genes. Genes were sorted in descending order by log2(FC). Gene sets from Gene Ontology (http://geneontology.org/), KEGG https://www.genome.jp/kegg/kegg.html), and Reactome (https://reactome.org/) were used. FDR was set at ≤ 0.10. RNA-Seq and downstream statistical analysis were performed by the Inserm U1283/CNRS UMR 8199 LIGAN-PM Genomics platform, Lille, France.

2.4. Circulating levels of selected myokines

Blood samples were taken in standardized conditions as described above, then 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.

2.5. Associations between changes in myokines and changes in glucose homeostasis

In the targeted study, a linear regression analysis was performed to search for associations between changes in glucose homeostasis (HOMA test) and changes in myokine expression (fold change). For regression analyses, independent variables were first tested in univariate analysis. All candidate independent variables with $R^2 \geq 0.10$ and $p<0.10$ 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. Results are expressed as adjusted estimates (95% confidence interval [CI]).

In the untargeted study, an exploratory linear regression analysis was first performed to search for associations between changes in glucose homeostasis (HOMA test) and changes in putative myokine expression (transcriptions per million). Myokines associated with glucose homeostasis ($R^2 \geq 10\%$, $p<0.10$) were quantified in RT-qPCR. Linear regression analyses

were then performed to search for associations between changes in glucose homeostasis and changes in myokine expression, as described previously.

2.6. Associations between changes in myokines and changes in body composition

A linear regression analysis was also performed to search for associations between changes in myokine expression (fold change) and changes in body composition (i.e., fat mass and fat-free mass) after bariatric surgery, as described above.

3. Identification of myokines susceptible to improve glucose homeostasis after bariatric surgery using a primary human skeletal muscle cell culture model

3.1. Primary human skeletal muscle cell culture

a. Muscle tissue dissociation and cell culture before isolation of satellite cells

Muscle samples were carefully dissected, minced, and washed twice with cold PBS to remove blood, fat, and connective tissue under a laminar flow hood. Muscle tissue was dissociated by three consecutive treatments of 15 minutes each in 0.05% Tryspine/EDTA at 37°C in an orbital shaker set at 150 rpm. Dissociated cells were filtered through a 40µm strainer, centrifuged at 150g for 5 minutes at 37°C, and suspended in growth medium. Growth

medium consisted of 50% Dulbecco's modified Eagle's medium (DMEM) with GlutamaxTM and 50% Ham's F12 nutrient mix supplemented with 2% fetal bovine serum (FBS), 2% UltroserG (Pall BioSepra, France), and 1% Antibiotic-Antimycotic 100x (10,000 units/mL penicillin, 10,000 µg/mL streptomycin, and 25 µg/mL amphotericin B). Cells were seeded in a 35 mm dish (PrimariaTM, Corning®, US) and incubated at 37°C in an incubator containing 5% CO_2. The culture medium was changed every 2 to 3 days. Cells were grown to 60-70% confluence before satellite cell isolation. Cell culture materials were purchased from GibcoTM, Fischer Scientific SAS (France), unless otherwise noted.

b. Isolation of satellite cells and differentiation

CD56 (Neural cell adhesion molecule 1, NCAM1) and CD82 (CD82 antigen, CD82) are both myogenic markers that allow selection of a highly myogenic cell population using fluorescence-activated cell sorting (FACS) (Pakula et al., 2019). Magnetic activated cell sorting (MACS) is also commonly used to select satellite cells using CD56 as a surface marker, with the advantage that MACS is readily available (Agley et al., 2015). Contamination of muscle cells by other cell types, especially fibroblasts, represents a potential bias in subsequent analyses. Therefore, both sorting techniques were applied in parallel to several muscle samples to determine which selected the most myogenic cell population in our study. Prior to FACS, cells were suspended

in medium containing 1% BSA and 1mM EDTA and then incubated with monoclonal antibodies against CD56, CD82, CD90, CD45 and CD31 (Biolegend, US) for 10 minutes at room temperature. CD90, CD45 and CD31 antibodies were used to eliminate fibroblasts, white blood cells, and endothelial cells, respectively (Pakula et al., 2019). CD56+CD82+ cells were selected using a FACSAria III cytometer (BD Biosciences, US). Prior to MACS, cells were suspended in medium containing 1% BSA and 1mM EDTA and then incubated with CD56 microbeads (Miltenyi Biotec, Germany) for 15 minutes at 4°C. Positive selection of CD56+ cells was performed on MS columns (Miltenyi Biotec, Germany). After cell sorting, CD56+CD82+ and CD56+ cells were seeded in 35 mm dishes (Primaria, Corning®, US) at a density of at least 30,000 cells/dish. These cells were grown to 60-70% confluence at passage 2 to prevent spontaneous fusion, which would have resulted in variable mixtures of myoblasts and myotubes. Subcultured cells were frozen in FBS with 10% dimethyl sulfoxide (DMSO) and stored in liquid nitrogen. For subsequent experiments, cells were thawed, seeded into 35 mm dishes (PrimariaTM, Corning®, US) or slide flasks (NuncTM Lab-TekTM flash on slide, ThermoFisher Scientific, US) at a density of at least 30,000 cells/dish, and grown to near confluence. Confluent cells were differentiated in DMEM containing GlutamaxTM supplemented with 2% horse serum, 1% FBS and 1% anti-anti 100x until fusion. Muscle cells collected at T0 and T3

from the same participant were thawed and cultured simultaneously to limit bias in culture conditions.

c. Immunostaining

Differentiated cells in slide flasks (#170920, Thermo Scientific) were washed twice with PBS, and then fixed in 4.0% formaldehyde (#9713.5000, VWR) in PBS for 20 minutes at room temperature. Cells were then permeabilized with 0.2% Triton X-100 (#93426, Sigma-Aldrich) in PBS for 15 minutes at room temperature, blocked with 2% goat serum (#S2000-100, Biowest), and incubated with a primary antibody overnight at 4°C. Cells were incubated with either MF20 antibody (1:20, #MF20, DSHB, US) to stain myosin heavy chains (MHC) in muscle cells or TE7 antibody (1:50, #CBL271, Merck, Germany) to stain fibroblasts (Agley et al., 2015). Cells were then washed with PBS, incubated with Alexa Fluor 488-conjugated goat anti-mouse IgG secondary antibody (1:400, ThermoFisher Scientific, US) for 1 hour, and mounted in Vectashield Antifade Mounting Medium with DAPI (Vector Laboratories, US). Cells were observed by fluorescence microscopy (Axioimager Z1, Zeiss, Germany). Myotubes were identified as MF20+ multinucleated cells (≥ 3 nuclei), whereas fibroblasts were identified as TE7+ mononucleated cells (Agley et al., 2015).

d. Assessment of myogenicity

Differentiation potential was determined by (1) calculation of the fusion index as the ratio of nuclei within myotubes to the total number of nuclei using Visiopharm® software (Visiopharm A/S, Denmark), and (2) expression of genes encoding myogenic markers (*Pax7*, *MyoD1*, *MYOG*, *Myf6*) and MHC (*MYH1*) using RT-qPCR. These markers are sequentially expressed during the muscle fiber differentiation process (Iberite et al. 2022). CD56+CD82+ cells were compared to CD56+ cells (considered as control cells).

3.2. RT-qPCR

Total RNA from differentiated cells was harvested using RNAprotect Cell Reagent (Qiagen, Germany) and isolated using the RNeasy Micro Kit with on-column DNase (Qiagen, Germany) according to the manufacturer's instructions. Genomic contamination was minimal (< 1.0% of total RNA) as assessed by QubitTM dsDNA HS Assay Kit, Qubit™ RNA BR Assay Kit (Invitrogen, USA). The isolated RNA was quantified using a Nanodrop spectrophotometer (#ND-1000, Isogen) and stored at -80°C. One microgram of RNA was reverse transcribed and Sybr Green® RT-qPCR was performed as previously reported (Gueugneau et al., 2018). Relative mRNA levels were calculated using the comparative Ct method and normalized to the expression of the housekeeping gene GAPDH.

3.3. Targeted study

RT-qPCR was performed on RNA from CD56+CD82+ myotubes collected at T0 and T3 to quantify changes in myokine expression. Based on our previous review of the literature and study in muscle samples (see above), candidate myokines included brain-derived neurotrophic factor (*BDNF*), xylosyltransferase 1 (*XYLT1*), leucine-rich repeat-containing G protein-coupled receptor 5 (*LGR5*), serine protease inhibitor Kazal type 5 (*SPINK5*), fractalkine (*CX3CL1*), angiogenin (*ANG*), osteoprotegerin (*TNFRSF11B*), A disintegrin and metalloproteinase with thrombospondin motifs 9 (*ADAMTS9*), irisin (*FNDC5*), interleukin-6 (*IL-6*), myostatin (*MSTN*), Apelin (*APLN*), tripartite motif-containing protein 72 (*TRIM72*) and secreted frizzled-related protein 4 (*SFRP4*). Primers used for amplification during RT-qPCR were tested to avoid primer dimers, self-priming, or unspecific amplification (Supplementary Table 1).

3.4. Untargeted study

High-throughput RNA-Seq was performed on RNA from CD56+CD82+ myotubes collected at T0 and T3 to identify novel bariatric surgery-regulated myokines and metabolic pathways susceptible to improve glucose homeostasis after bariatric surgery, as described above. Bioinformatic methods are detailed in Supplementary Methods. RNA expression was

measured at the gene and transcript levels. Differential expression of the genes and transcripts was evaluated with the R package DESeq2 (v. 4.2.0) using a paired design (Love et al., 2014), as described above. RT-qPCR was performed as described above on RNA from CD56+CD82+ myotubes obtained at T0 and T3 to quantify changes in the expression of selected myokines identified by RNA-Seq. Primers used for amplification during RT-qPCR were tested to avoid primer dimers, self-priming, or unspecific amplification (Supplementary Table 1).

4. Identification of myokines susceptible to improve glucose homeostasis after bariatric surgery using multiplex immunoassays on plasma samples

4.1. Assessment of glucose homeostasis (see study design)

4.2. Assessment of muscle mass (see study design)

4.3. Measurement of plasma myokine levels

Blood samples (EDTA) were collected under standardized conditions, as described above. Multiplex immunoassays were performed on plasma samples using a Luminex bead-based multiplex system (HMYOMAG-56K, Millipore Corporation, USA), which is a sensitive and reproducible method for measuring myokine levels (Weigert et al., 2021). The assays included apelin (APLN), erythropoietin (EPO), SPARC (also known as osteonectin), leukemia inhibitory factor (LIF), interleukin-15 (IL-15), fatty acid-binding protein 3 (FABP3), irisin, follistatin-related protein 1 (FSTL-1), oncostatin M, interleukin-6 (IL-6), fibroblast growth factor 21 (FGF-21), and musclin (also known as osteocrin). Plasma samples were diluted according to the manufacturer's instructions and standard curves were generated for each myokine from the standards provided with the assay. All samples and standards were analyzed in duplicate using a Luminex 200 instrument (Luminex, R&D Systems, Inc, USA) and XPonent 3.1 software (Millipore

Corporation, USA). Levels were determined for each myokine relative to a 5-point regression standard curve and expressed in pg/mL. Multiplex immunoassays were performed by ImmunXperts (Charleroi, Belgium). Changes from baseline (T0) were expressed as absolute changes (pg/mL) or percentage changes (percentage change from T0, %T0).

4.4. Associations between changes in myokines and changes in glucose homeostasis

Associations between changes in myokines and changes in glucose homeostasis were explored using univariate and multivariate linear regression analysis, as described above. Multivariate analysis was adjusted for age and sex (model 1), age, sex, and percentage change in SMM (%T0) (model 2), age, sex, and change in ASMM (%T0) (model 3). Results are expressed as estimates with 95% CI.

4.5. Associations between changes in myokines and changes in muscle mass

Associations between changes in myokines and changes in muscle mass were explored using univariate and multivariate linear regression analysis, as described above. Multivariate analysis was adjusted for age and sex (model 1). Results are expressed as estimates with 95% CI.

5. Myostatin as a biomarker of muscle mass and strength in people with obesity undergoing bariatric surgery

5.1. Assessment of muscle mass (see study design)

5.2. Assessment of muscle strength (see study design)

5.3. Definition of sarcopenic obesity

Sarcopenic obesity was considered in individuals with obesity (BMI ≥ 30 kg/m² and/or percentage fat mass ≥ 25% in men and 35% in women) and low muscle mass. Low muscle mass was defined as skeletal muscle mass adjusted for body weight (SMM/BW, %) <37.7% in men and <26.7% in women (Donini et al., 2022). Class I and class II sarcopenic obesity were defined as skeletal muscle mass/body weight ratio between 31.5 and 37.7% in men and <27.6% in women, and <31.5% in men and <22.1% in women, respectively (Donini et al., 2022).

5.4. Measurement of plasma myostatin levels (see circulating levels of selected myokines)

5.5. Associations between myostatin, muscle mass, and muscle strength

Correlations between myostatin, muscle mass and muscle strength were estimated using Spearman's Rank correlation coefficient. Associations

between myostatin, muscle mass, and muscle strength were assessed using uni- and multivariate linear regression. Multivariate analysis was adjusted for age and sex (Model 1), and for age, sex, and BMI (Model 2). Results were expressed as estimates with 95%CI.

5.6. Associations between myostatin and sarcopenic obesity

Associations between myostatin and sarcopenic obesity were assessed using uni- and multivariate logistic regression. Multivariate analysis was adjusted using Model 1 and Model 2, unless there was a collinearity issue. Results were expressed as odds ratio (OR) with 95%CI. Receiver operating characteristic (ROC) curve analysis was performed to identify an optimal myostatin cutoff level for the prediction of sarcopenic obesity.

6. Statistical analysis

Continuous variables are reported as medians with 95% confidence interval (95%CI) or interquartile ranges (IQR). Discrete variables are reported as numbers with frequencies. Longitudinal differences were assessed using the non-parametric Wilcoxon signed rank test for continuous variables and using the McNemar test for discrete variables. Cross-sectional differences with the control group were assessed using the Mann-Whitney U test. For RT-qPCR analysis, longitudinal differences were calculated on delta Ct and cross-sectional differences on fold-changes. Correlations and associations were

searched as described above. The sample size was not prespecified given the exploratory nature of the study. Statistical significance was set at $p < 0.05$, unless otherwise stated. Statistical analyses were performed using SPSS Statistics software (IBM SPSS Statistics, Version 27.0. Armonk, NY, USA) and SAS software (version 9.4; SAS Institute Inc, Cary, NC, USA). Graphs were made with GraphPad Prism (version 8.0.0 for Windows, GraphPad Software, San Diego, California USA, www.graphpad.com).

7. Supplementary material

7.1. Supplementary methods - Targeted study on muscle samples:

Literature search

At the time of our study, over 300 proteins had been identified as proteins secreted by muscle cells using a computational approach (Bortoluzzi et al., 2006) and over 600 proteins had been detected in conditioned culture media of human or murine muscle cells using untargeted proteomic analysis (i.e. mass spectrometry) (Florin et al., 2020). We have performed a literature search on Pubmed using keywords that included “myokine” in combination with either “glucose homeostasis”, “insulin resistance” or “insulin secretion” (as free language not as Mesh terms). The results were cross-referenced with data from descriptive studies of the human muscle secretome when it was unclear whether the protein had previously been reported or predicted in culture

media conditioned by human muscle cells (Bortoluzzi et al., 2006; Le Bihan et al., 2012; Raschke et al., 2013; Hartwig et al., 2014). Only papers published in English were considered. Recent and mechanistic studies were given priority. This search has identified Brain-Derived Neurotrophic Factor (*BDNF*), Fractalkine (*CX3CL1*), Angiogenin (*ANG*), Osteoprotegerin (*TNFRSF11B*), Irisin (*FNDC5*), Myostatin (*MSTN*), Tripartite motif-containing protein 72 (*TRIM72*), apelin (*APLN*), interleukin-6 (*IL6*), A disintegrin and metalloproteinase with thrombospondin motifs 9 (*ADAMTS9*), and Secreted frizzled-related protein 4 (*SFRP4*) as myokines involved in the regulation of glucose homeostasis. These myokines were then searched in the Human Protein Atlas for muscle cells/tissue expression and known functions (Uhlén et al., 2015). Of note, most of the identified myokines are not specific of skeletal muscle, the contribution of each tissue to circulating levels is most often unknown, and the mode of action of these myokines (auto-, para- and/or endocrine) cannot be systematically determined.

7.2. Supplementary methods - Untargeted study on muscle samples:

RNA-Sequencing

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 x 1 minute, at 6m/second, with 5 minutes rest on ice between rounds. Total RNA was isolated according to the manufacturer instructions. This protocol ensured high yields of RNA despite small-sized biopsies (± 0.4 µg/mg of muscle tissue) together with minimal contamination with genomic DNA (< 1.1% of total RNA), assessed using Qubit™ dsDNA HS Assay Kit, Qubit™ RNA BR Assay Kit (Invitrogen, USA). Purity was assessed using Nanodrop (A260/A280 > 1.8, A260/A230 > 1.8).

Bioinformatics

Data demultiplexing was performed using the bcl2fastq software (Illumina, v.2.19.1). Quality controls were used by FastQC (Babraham Institute, v.0.11.8). The alignment and counting of the transcripts were made using RSEM software (v.1.3.0) in association with the STAR aligner (v.2.5.2b) to the hg38 reference genome as well as to the gtf file containing the Ensemble transcripts in version 89. RNA-Seq and downstream statistical analysis were performed by the Inserm U1283/CNRS UMR 8199 LIGAN-PM Genomics platform, Lille, France.

7.3. Supplementary methods - Untargeted study on myotubes:

Bioinformatics

RNA-Seq data (i.e., RSEM files) were imported using the R package tximport. Constant-, low, and non-expressed genes or transcripts were excluded from the analysis (i.e., gene- or transcript-wise variance and

median should be greater than 0, and the mean should be greater than 1). Principal component analysis was performed to detect possible outliers, defined as the Euclidean distance from the cohort centroid greater than 3 times the interquartile range above the 75th percentile.

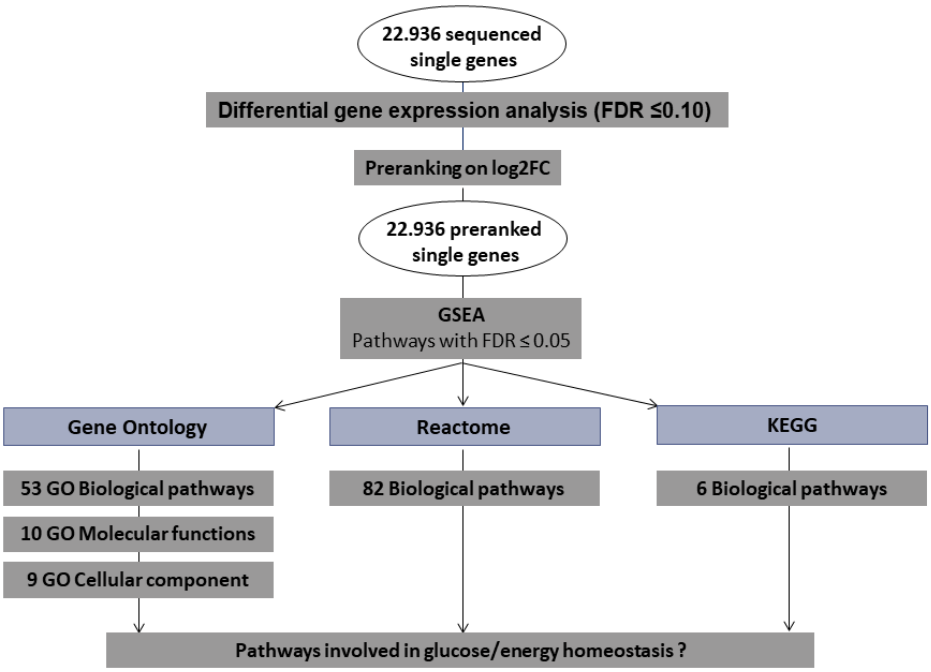
7.4. Supplementary Tables

Supplementary Table 1. List of primers sequences used for RT-qPCR analysis

Gene symbol	Forward (5'-3')	Reverse (5'-3')
Targeted studies on muscle samples and myotubes		
CX3CL1	CACCACGGTGTGACGAAATG	ATCTGCTTCTCGAAGGTGCC
TNFRSF11B	TGGCACCAAAGTAAACGCAG	TGCTCGAAGGTGAGGTTAGC
IL6	CGGGAACGAAAGAGAAGCTCTAT	AGGCGCTTGTGGAGAAGGA
ADAMTS9	AGCCTCTATCTATAAAGACCCAAGT	TCCACCTGGACTGTTCTTCG
BDNF	CCTTGACCCTGCAGAATGGC	CCACCTTGTCCTCGGATGTT
ANG	CATCATGAGGAGACGGGG	TCCAAGTGGACAGGTAAGCC
TRIM72	GACCCGCTGAGCATCTACTG	AGCCACACTCTTCTCCTTGC
MSTN	CTCCACTCCGGGAACTGATT	TGGGTTTTCCATCCACTTGC
FNDC5	GATCCAGCCATCAAGGACAT	TTGTCCAAGCTAGCATTTCTGA
SFRP4	CGAACTCAAGTCCCGCTCAT	ACTGTTCTCCGCTGTTCCTG
APLN	AGCATGAATCTGCGGCTCTG	GGAATTTCCTCCGACCTCCC
Untargeted study on muscle samples		
KDR	GTAACCCGGAGTGACCAAGG	AACCAAGGTACTTCGCAGGG
XYLT1	CGCTCATCACCCTGGAGACT	TTTTGAGTCCTGGGTGCGAA
LGR5	ATGGACACCTCCCGGCT	GGGAGCAGCTGACTGATGTT
SPINK5	CCTCTGTTGCCAGGATGAGTA	ACTGAAAGAGTCTGGGCTGTC
ADGRB2	CCTGCTCAGCTCAGCCTC	TCTGGCACTTCACCACATCC
CD6	AGAAGTTTTCATGCTGCCCAT	TGCTGGACCTCCTCATCTGT
LIPG	ACACCTACACGCGTTCCTTC	TCGGCTTGTCCTGATTCACC
DLK1	CACGGACTCTGTGGAGAACC	GCAGTCCTTTCCCGAGTACC
PF4	TTGCTGCTCCTGCCACTTG	GTGGCTATCAGTTGGGCAGT
FCRL1	TGTGCTCCACTCTGTGAACC	CTTCTTTCCACATGGCAGCG
ELFN2	CCTTTCGCCTCCCTACAAGG	ATGGAACCACTGGACGACAC
Myosin heavy chains and PGC1A		
MYH1	TCCACTTTAAGGTCGCATCTCT	GTTCTGGGCTTCAATTCGCTC
MYH2	AGCCCTTGGAATGAGGCTGA	GCTCCGCCACAAAGACAGAT

MYH7	GAGCAAGCCAACACCAACCT	TGTGGCAAAGCTACTCCTCCATT
PPARGC1A	AAAGGATGCGCTCTCGTTCA	TCTACTGCCTGGAGACCTTGATC
Myogenic markers		
Pax7	GTGAGTTCGATTAGCCGCGT	GTTCCGACTCCACATCCGAG
MyoD1	CGACGGCATGATGGACTACA	GGCAGTCTAGGCTCGACAC
MYOG	GCCATCCAGTACATCGAGCG	ATCTGTAGGGTCAGCCGTGA
Myf6	CTTGAGGGTGCGGATTTCCT	AAGCGCAGGCTCAGTTACTT
Untargeted study on myotubes		
ADGRG6	CCACCCACTGTCACCACTAA	ATTCTGCCACCTTGCTCTGT
BACE1	CCCGGGAGACCGACGAA	CAGCTGCCTCTGGTAGTAGC
BRINP2	GCTGACTTCATGGAGCGGTA	CCTTCCAAGGAGGCGAATGT
BTC	AGGTGTGAGAGAGTTGACTTGTT	AGGTAACTTCATAGCCTTTTAAGCA
BTD	TGTAAAGGGAGAATGGCGCA	TGAGAGCCAGAGGGTTCAGA
CD24	GACATGGGCAGAGCAATGGT	GACCACGAAGAGACTGGCTG
CDHR2	ATCCAGGTGCAGAGGGAGAT	CCAGGATCCGGAAGAGATGC
COL18A1	CTCCTGGACGTGCTCGC	AAGAGGCTGGGGAAGTGGTA
CTSB	CCACAGTGTCCCACCATCAA	GGATGCAGATCCGGTCAGAG
FCGR1A	CTGCTCCTTTGGGTTCCAGT	CACTGTGTAGAGCTGCTCCC
FGF18	GAGGAGAACGTGGACTTCCG	CTTGCCCTTGATCCGGACTT
GDE1	GGCAGGCAGCTAAGAATGGA	CACTCTGCAACAGCTTCCCT
IGLV5-52	TGGCCTGGACTCTTCTCCTT	CAGCATGCAGGTGAGTCTGA
ILR11A	AGTTCCGTTTGCAGTACCGT	TGATCACCTCCTCCAGTCCA
MEGF11	AACTTAGACACCTCAGCCGC	GGCGTAAGGGTTTTCACTGC
POMC	CAGGACCTCACCACGGAAAG	GAAGTGGCCCATGACGTACT
THSD8	AGTACTTAGGGCAGCAGGGC	TTCTCCGGGTCCATGAAAACC
TIMP2	TCTGTGACTTCATCGTGCCC	TGGTGCCCGTTGATGTTCTT
WNT4	CTCGTCTTCGCCGTCTTCTCA	TGGATCAGGCCCTTGAGTTTC
Housekeeping gene		
GAPDH	CGCTGAGTACGTCGTGGAGTC	GCAGGAGGCATTGCTGATGA

7.5. Supplementary figures



Supplementary Figure 1. Identification of enriched pathways after bariatric surgery.

IV. RESULTS

1. Identification of myokines susceptible to improve glucose homeostasis after bariatric surgery on muscle samples

1.1. Published manuscript

This work has been published as: Orioli L, Canouil M, Sawadogo K, Ning L, Deldicque L, Lause P, de Barsy M, Froguel P, Loumaye A, Deswysen Y, Navez B, Bonnefond A, Thissen JP. Identification of myokines susceptible to improve glucose homeostasis after bariatric surgery. Eur J Endocrinol. 2023 Sep 1;189(3):409-421. doi: 10.1093/ejendo/lvad122.

1.2. 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*=0.006) while BDNF circulating levels were decreased (−12%, *p*=0.001). Upregulated BDNF expression was associated with decreased HOMA of insulin resistance (HOMA-IR) (adjusted estimate [95%CI]: −0.51 [−0.88 to −0.13], *p*=0.010). 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*=0.046). In the untargeted study, upregulated putative myokines included *XYLT1* (xylosyltransferase 1) (+64%, *p*<0.001), LGR5 (leucine-rich repeat-containing G protein-coupled receptor 5) (+57%, *p* <0.001), and SPINK5 (serine protease inhibitor Kazal type 5) (+46%, *p*<0.001). Upregulated LGR5 was associated with decreased HOMA-IR (adjusted estimate [95% CI] = −0.50 [−0.86 to −0.13], *p*=0.009). 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*=0.009 and 16.5 [0.87-32.19], *p*=0.039).

Conclusions: Improved glucose homeostasis following bariatric surgery is associated with changes in myokines expression and circulating levels.

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.

1.3. Significance of the data

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. Specifically, upregulation of *BDNF*, *XYLT1*, *SPINK5*, and *LGR5* is associated with improved insulin sensitivity. In addition, our data show that changes in body composition seem to drive most of the impact of bariatric surgery, particularly on myostatin at both muscle and circulating levels (Orioli et al, 2023). These results suggest that several myokines could contribute to improved glucose homeostasis and reflect changes in body composition following bariatric surgery.

1.4. Supplementary data

Importance and Objective: Muscle from people with obesity is generally characterized by a lower proportion of type I fibers and, at the same time, a higher proportion of type IIx fibers compared to lean people. These changes are thought to alter muscle insulin sensitivity in people with obesity. In this

additional study, we aimed to compare glucose homeostasis as well as muscle phenotype in our patients with obesity and healthy lean controls.

Methods: The expression of myosin heavy chains (*MYH1*, *MYH2*, *MYH7*) and *PPARGC1A* mRNA in muscle were compared between patients with obesity before and 3 months after bariatric surgery and healthy subjects without obesity (control group, n=18, HbA1c <6.5%, BMI<30kg/m²). Fasting insulin and fasting glucose from control individuals were used to compute HOMA-IR using the HOMA2 calculator. In addition, we assessed the impact of bariatric surgery on the expression of myosin heavy chains and *PPARGC1A* mRNA muscle samples from individuals with obesity.

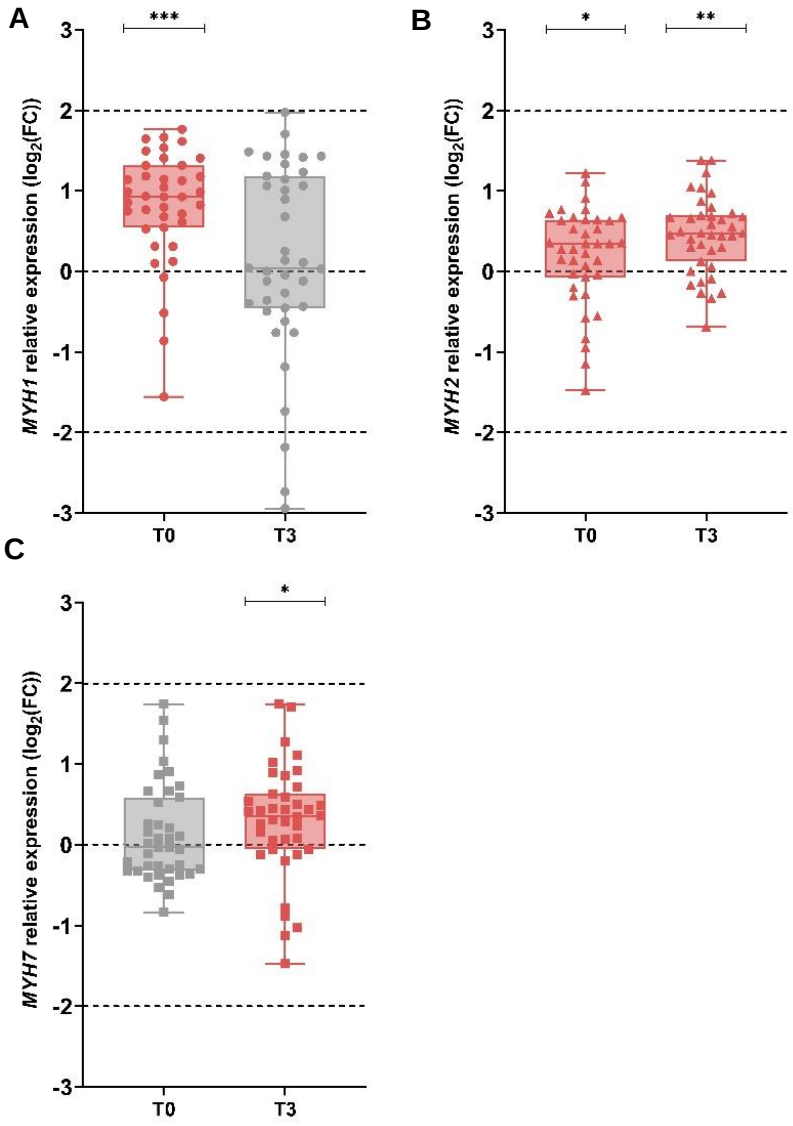
Results: The characteristics of the study and control groups are summarized in Supplementary Table 1. As expected, the study group had higher HOMA-IR than the control group, before and after bariatric surgery (respectively, $p<0.001$ and $p=0.002$). Before bariatric surgery, RT-qPCR showed higher expression of *MYH1* (+90%, $p<0.0001$) and *MYH2* (+27%, $p=0.032$) in the study group compared to the control group, whereas *MYH7* expression was similar in both groups ($p>0.05$) (Supplementary Figure 1). After bariatric surgery, the study group had higher expression of *MYH2* (+39%, $p=0.001$) and *MYH7* (+28%, $p=0.034$) expression than the control group, whereas *MYH1* was similar in both groups ($p>0.05$) (Supplementary Figure 1). *PPARGC1A* expression was similar in both groups throughout the study ($p>0.05$, not shown). In the study group, RT-qPCR showed decreased

expression of *MYH1* (-38%, *p*=0.002) and increased expression of *MYH2* (+21%, *p*=0.022) after bariatric surgery (Supplementary Figure 2). The expression of *MYH7* (+16%, *p*=0.074) and *PPARGC1A (+6%, p=0.087)* was unchanged after bariatric surgery.

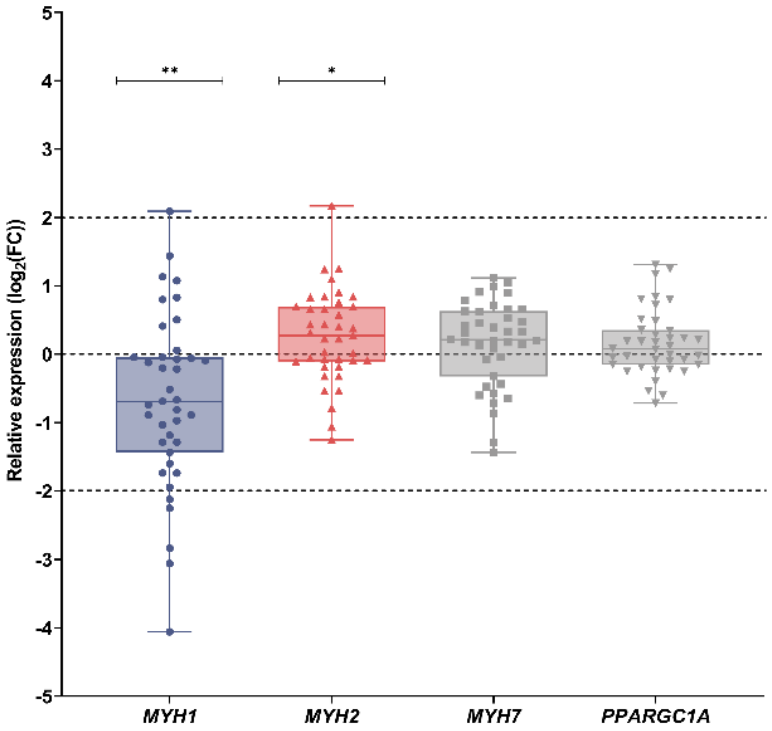
Supplementary Table 1. Characteristics of the control and study groups

Variables	Control group (n=18)	Study group-T0 (n=62)	Study group-T3 (n=62)
Age (years)	32 [25-56]	44 [34-54]	-
Sex (females/males)	0/18 (0/100)	36/26 (58/42)	-
Body weight (kg)	76.6 [71.2-84.5]	124.8 [114.2-136.4]	103.4 [91.7-110.9]
BMI (kg/m²)	24.9 [24.0-26.3]	43 [41-44]	35 [32-38]
HbA1c (%)	5.2 [5.0-5.4]	5.7 [5.4-6.2]	5.4 [5.1-5.6]
HOMA-IR	0.8 [0.6-1.9]	3.2 [2.4-3.8]	1.5 [1.1-2.0]

Data expressed as medians [IQR]. Abbreviations: BMI: body mass index; HbA1c: glycated hemoglobin A1c; HOMA-IR, homeostatic model assessment of insulin resistance. T0 and T3 refer to before and 3 months after bariatric surgery.



Supplementary Figure 1. Changes in the expression of genes encoding myosin heavy chains and PGC-1A in the study group compared to control group. (A) *MYH1* expression, (B) *MYH2* expression, (C) *MYH7* expression. n = 39 in the study group, n = 18 in the control group, results presented as median $\log_2$(FC) [min, max]. Gene expression in control individuals arbitrary set at 0. Mann-Whitney U test performed on ΔCT to assess relative mRNA expression. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Red and gray indicate significant and non-significant differences, respectively.



Supplementary Figure 2. Changes in the expression of genes encoding myosin heavy chains and PGC-1A after bariatric surgery. Study group, n = 39, results presented as median log_2(FC) [min, max]. Pre-operative expression (control value) arbitrary set at 0. Wilcoxon signed rank test performed on ΔCT to assess relative mRNA expression between pre- and post-surgery visits. * $p < 0.05$, ** $p < 0.01$.

Discussion: Our study first shows that the expression of type 2x muscle fibers (*MYH1*) and type 2a muscle fibers (*MYH2*) is higher in insulin-resistant people with obesity compared to control individuals. Our data are consistent with the literature showing an increased proportion of type 2 muscle fibers, particularly type 2x muscle fibers, in people with obesity compared to lean people (Damer et al., 2022; Tallis et al., 2018; Tanner et al., 2002; Serrano et al., 2023).

This “slow-to-fast” shift in muscle fiber-type distribution is thought to contribute to impaired glucose uptake and oxidative metabolism in skeletal muscle of people with obesity (Tanner et al., 2002; Serrano et al., 2023; Egan et al., 2013; Tallis et al., 2018; Albers et al., 2015, a).

Interestingly, our study also shows that bariatric surgery changes the expression of genes encoding myosin heavy chains. Specifically, the expression of type 2x muscle fibers (*MYH1*) is decreased after bariatric surgery, whereas the expression of type 2a muscle fibers (*MYH2*) is increased. These results suggest that the muscle phenotype may evolve towards a more insulin-sensitive and oxidative nature after bariatric surgery, which could contribute to improved glucose homeostasis. In addition, some myokines are fiber-specific (Rutti et al., 2018; Hiscock et al., 2004). It is therefore possible that the changes in myokine expression and circulating levels observed in our study are influenced by changes in muscle phenotype.

In contrast, the expression of *PPARGC1A* which encodes PGC1A was unchanged after bariatric surgery in our study, as reported by others (Campbell et al., 2016). PGC1A expression is positively associated with GLUT4 expression, mitochondrial biogenesis, type I muscle fiber expression, and oxidative metabolism (Tallis et al., 2018). It is generally expected that bariatric surgery improves muscle mitochondrial function and promotes mitochondrial biogenesis through upregulation of PGC-1A (Dantas et al., 2020; Barberio et al.,

2021; Gastaldi et al., 2007). However, we have shown previously that some mitochondrial pathways are downregulated after bariatric surgery, as reported by others (Orioli L et al., 2023; Campbell et al., 2016). Our findings are therefore consistent with the literature suggesting that decreased mitochondrial function may be observed early after bariatric surgery, because of an energy economy phenomenon essentially due to low lipid oxidation and transient lipotoxicity due to increased lipolysis of adipose tissue (Bertoni et al., 2021; Bobbioni-Harsch et al., 2000).

This study has limitations. Firstly, the control group included only men, unlike our study group. Secondly, some control individuals had a BMI consistent with being overweight (25.0-29.9 kg/m²) which may result in higher or lower than normal gene expression. Thirdly, muscle samples and muscle RNA from control individuals were obtained using different techniques to ours (De Groote et al., 2021). These factors may influence measurements made on muscle. Fourthly, RT-qPCR provides relative changes in the genes encoding myosin heavy chains, which may give different results compared to the quantification of muscle fiber types by immunochemistry.

In conclusion, bariatric surgery changes the expression of myosin heavy chains associated with type IIA and IIX muscle fibers, which could contribute to a more insulin-sensitive and more oxidative muscle phenotype associated with improved glucose homeostasis after bariatric surgery.

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Original Research

Identification of myokines susceptible to improve glucose homeostasis after 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 ≤7.5% (≤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]

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 ≤.10. Genes that were upregulated (≥1.3-fold) or downregulated (≤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 ≥ 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.

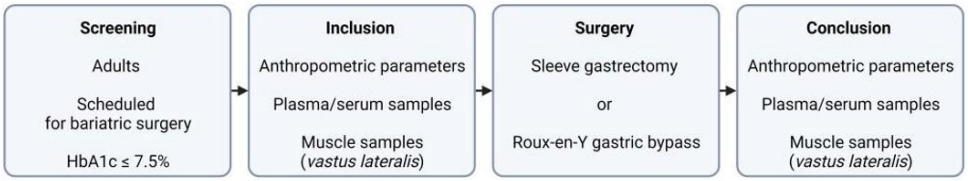

Figure 1. Overview of the study design. Sixty-two patients were evaluated before and 3 months after surgery (i.e., sleeve gastrectomy/Roux-en-Y gastric bypass). Plasma and serum samples were taken to quantify circulating levels of myokines. Muscle biopsies were taken from vastus lateralis for RNA analysis.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

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.

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 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 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 alsoassociated 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 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. 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 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 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 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 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 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 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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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.

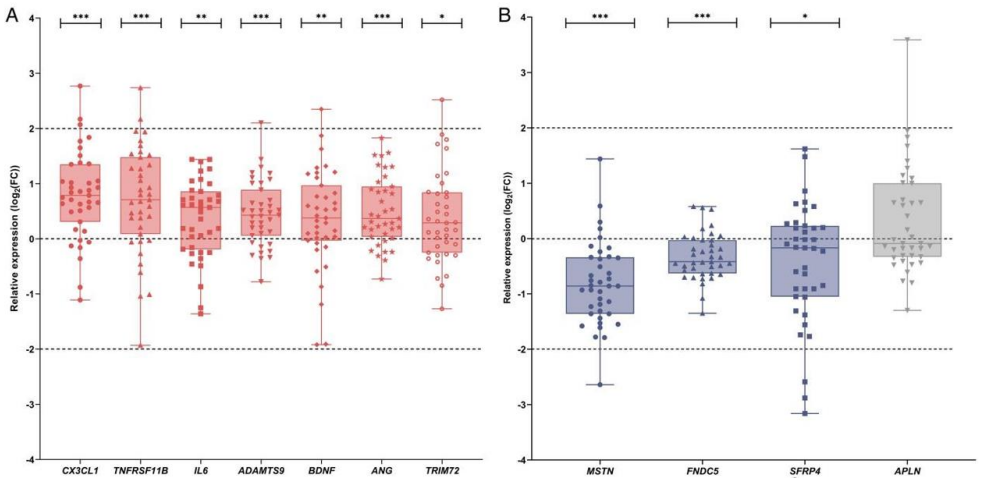


Figure 2. Targeted study: changes in the expression of myokines by RT-qPCR (A) Upregulated genes and (B) downregulated and unchanged genes. *n* = 39, results were presented as median log_2(FC) (min, max). Preoperative expression (control value) arbitrary set at 0. Wilcoxon signed-rank test performed 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.

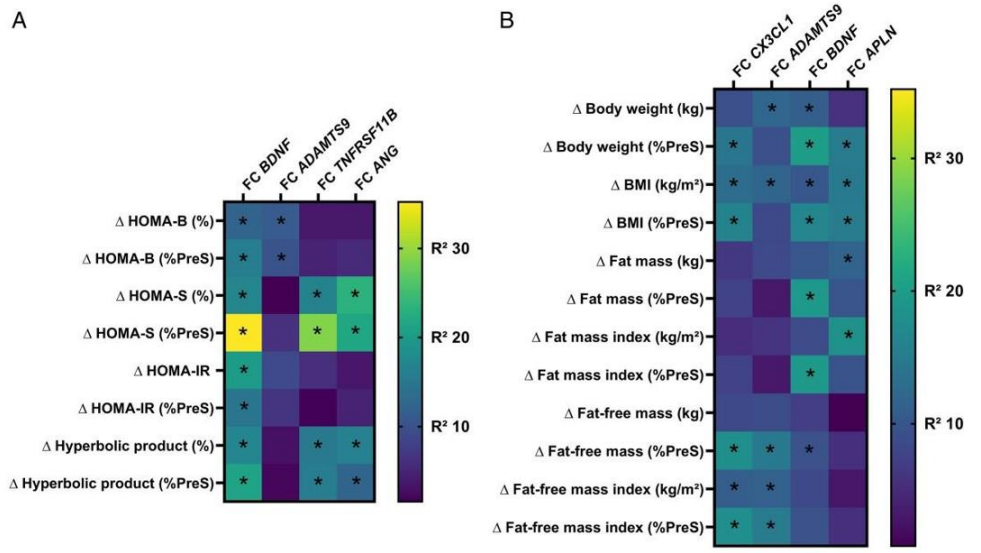


Figure 3. Associations between myokines expression, changes in glucose homeostasis, and changes in body composition after bariatric surgery. (A) Univariate linear regression between changes in myokines expression (fold change) and changes in glucose homeostasis (HOMA test). (B) Univariate linear regression 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.

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 assoc	iated 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.

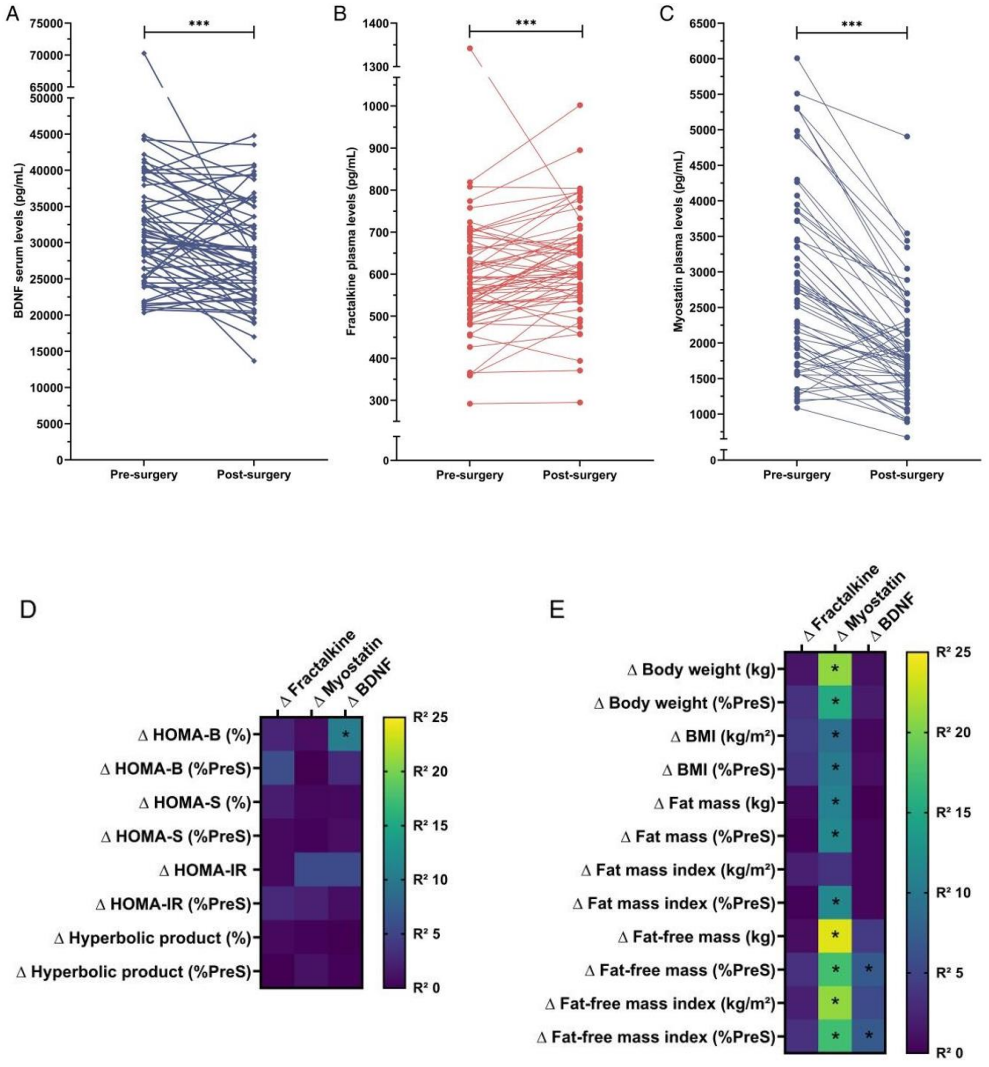
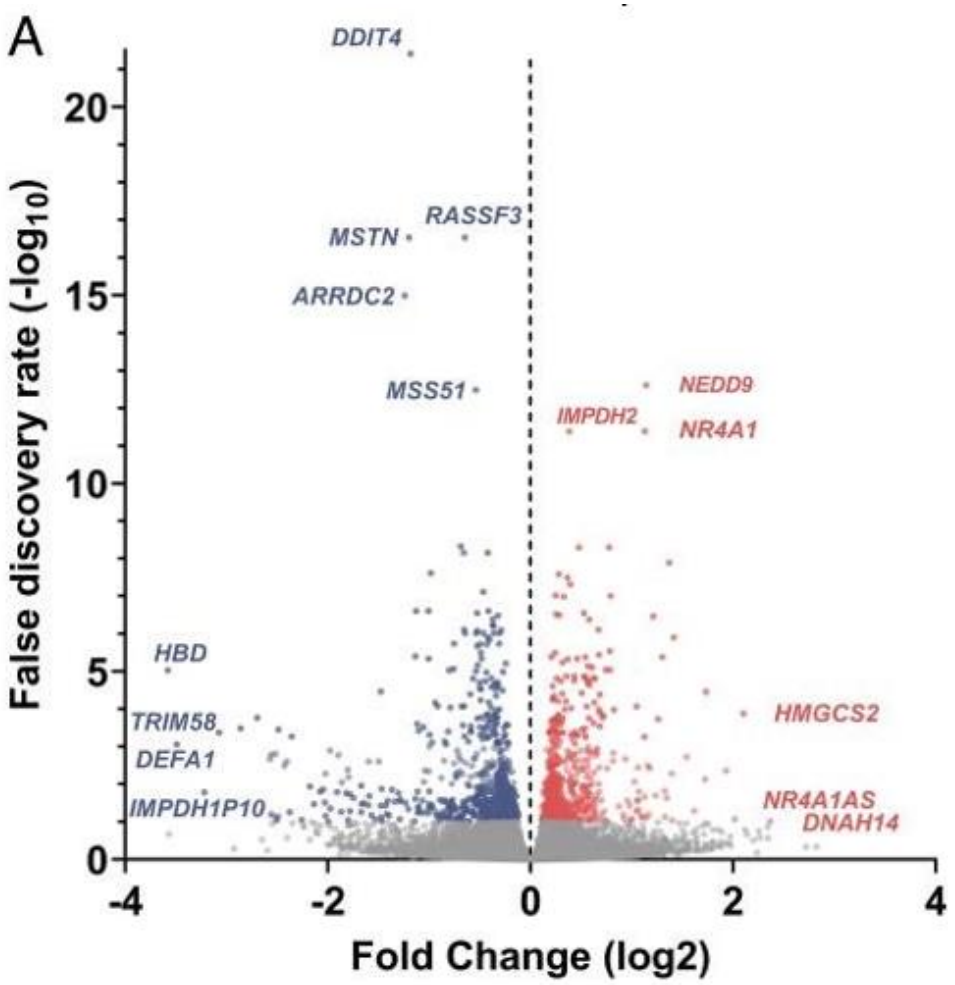


Figure 4. Changes in circulating levels of myokines and their associations with changes in glucose homeostasis and body composition after bariatric surgery. (A) BDNF serum levels. (B) Fractalkine plasma levels. (C) Myostatin plasma levels. *n* = 62, Wilcoxon matched-pairs signed-rank test. ***P < .001. (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.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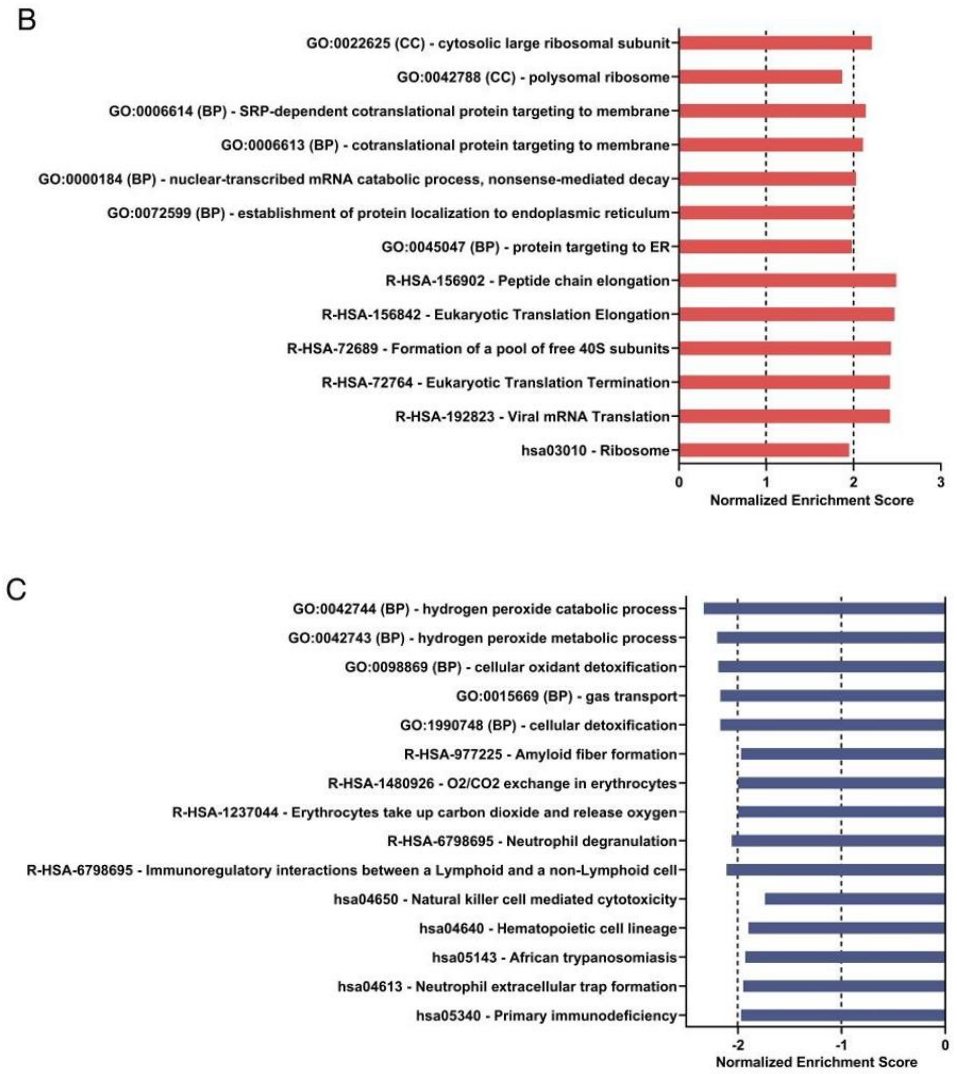
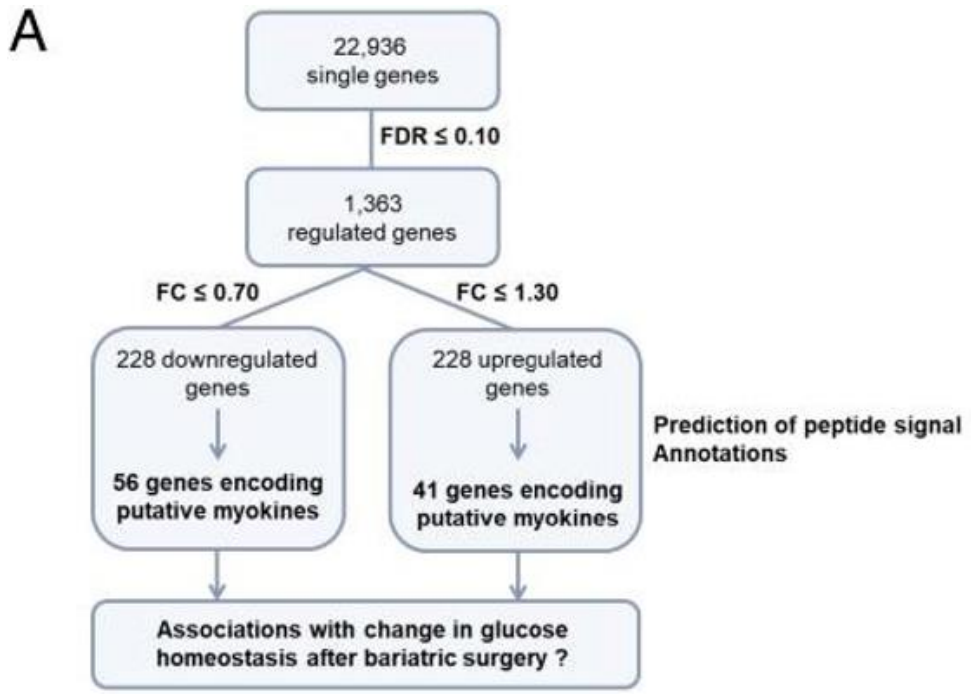


Figure 5. Changes in muscle transcriptome induced by bariatric surgery. (A) RNA-Seq: all regulated single genes after bariatric surgery (FDR ≤ 0.10). Most upregulated and downregulated genes are identified by their Hugo Gene Nomenclature Committee (HGNC) symbol including *MSS51* and *DDIT4*, 2 mammalian 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.

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



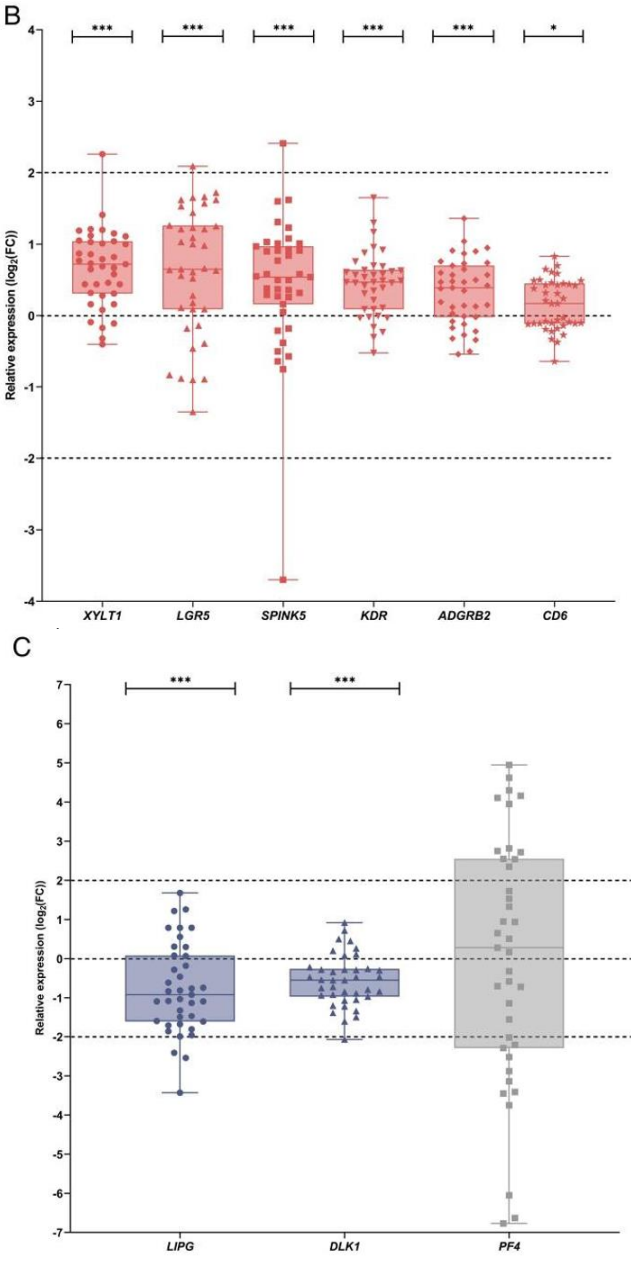


Figure 6. Untargeted study: identification of regulated genes encoding putative myokines. (A) Workflow for the identification of regulated genes encoding putative myokines. (B) Upregulated genes. (C) Downregulated and unchanged genes. n = 39 (RT-qPCR), results presented as median $\log_2$(FC) (min, max). 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.

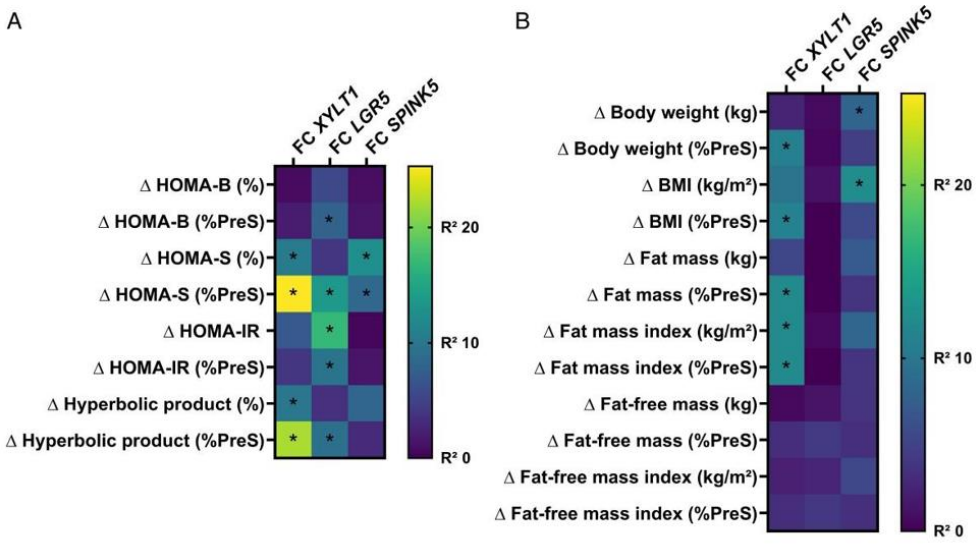


Figure 7. Associations between putative myokines expression, changes in glucose homeostasis, and changes in body composition after bariatric surgery. (A) Univariate linear regression between changes in myokines expression (fold change) and changes in glucose homeostasis (HOMA test). (B) Univariate linear 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 associate	l 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.

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2. Identification of myokines susceptible to improve glucose homeostasis after bariatric surgery using a primary human skeletal muscle cell culture model

2.1. Participants' characteristics

The characteristics of the entire MYDIASECRET cohort have been reported in detail previously (Orioli et al., 2023). In the present study, muscle samples for cell culture were obtained from 16 participants at both time points (Table 1). The participants enrolled in this study were comparable to the rest of the MYDIASECRET cohort (not shown, p>0.05 for all characteristics).

Table 1. Participants' characteristics before (T0) and 3 months (T3) after bariatric surgery (n=16)

Variables	T0	T3	*p*-value
Females/males (n, %)	10/6 (63/37)	/	/
Age (years)	43 [38-52]	/	/
Prediabetes/T2D (n, %)	10/2 (63/13)	/	/
SG/RYGB (n, %)	8/8 (50/50)	/	/
Body weight (kg)	121.2 [110.5-136.0]	100.9 [91.4-111.6]	<0.001
BMI (kg/m²)	44.0 [40.1-45.8]	38.8 [30.9-38.5]	<0.001
Fasting plasma glucose (mmol/L)	5.3 [5.1-5.99]	5.1 [4.6-5.3]	0.012
Fasting insulin (pmol/L)	147 [106-174]	68 [45-102]	0.001
HbA1c (%)	5.8 [5.4-6.2]	5.4 [5.2-5.5]	0.004
HOMA-B (%)	163 [149-206]	115 [93-133]	<0.001
HOMA-S (%)	33 [27-42]	68 [46-107]	0.001
HOMA-IR	3.1 [2.4-3.7]	1.5 [0.9-2.2]	0.001
Hyperbolic product (%)	59 [46-65]	78 [68-122]	0.002

Data expressed as medians [IQR] or numbers and frequencies (%). Abbreviations: 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; SG: sleeve gastrectomy; RYGB: Roux-en-Y gastric bypass; T2D: type 2 diabetes. Hyperbolic product = HOMA-B * HOMA-S. Differences assessed using Wilcoxon signed-rank test. *p* statistically significant < 0.05.

2.2. Isolation of a highly myogenic cell population using FACS

Both sorting techniques allowed to obtain multinucleated cells on the third day of differentiation (Fig. 1A-B). Myosin heavy chain immunostaining showed that both CD56+ and CD56+CD82+ cells expressed myosin heavy chains (Fig. 1C-D). TE7 immunostaining showed multiple positive mononuclear cells among CD56+ cells, but these were nearly absent in CD56+CD82+ cells (Fig. 1E-F). The mean fusion index was 54% in CD56+ cells versus 75% in CD56+CD82+ cells (Fig. 2). RT-q-PCR showed an overall higher expression of myogenic markers in CD56+CD82+ cells compared to CD56+ cells as indicated by higher expression of *Pax7* (fold change = 7.5), *myoD1* (fold change = 3.0), *MYOG* (fold change = 2.8) and *Myf6* (fold change = 5.0) (Fig. 3). Myosin heavy chain expression was also higher in CD56+CD82+ cells than in CD56+ cells, as indicated by higher *MYH1* expression (fold change = 2.9) (Fig. 3). Taken together, these data suggest that FACS allowed the selection of a specific cell population with higher myogenic differentiation potential than that selected by MACS. Further experiments were therefore performed on CD56+CD82+ myotubes.

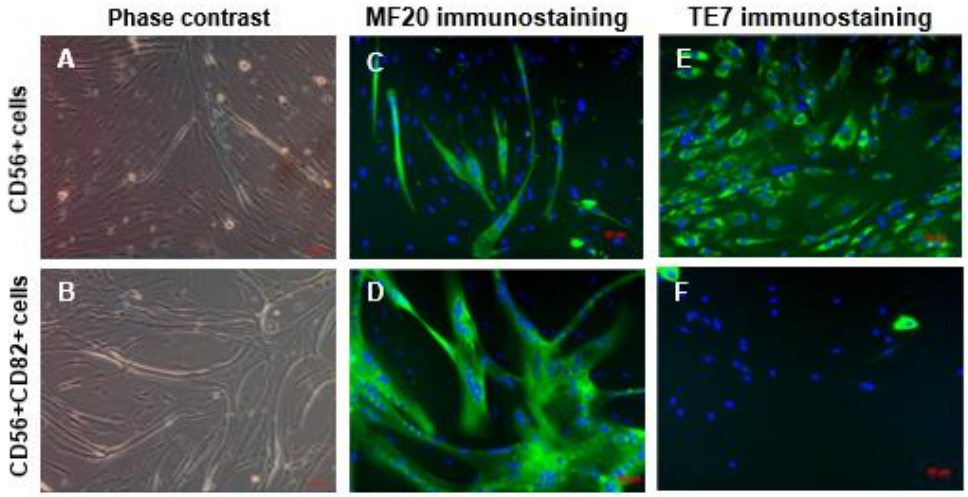


Figure 1. Immunostaining of differentiated muscle cells. A-B. Phase contrast: CD56+ cells (A) and CD56+82+ cells (B). **C-D.** MF20 immunostaining: CD56+ cells (C) and CD56+82+ cells (D). **E-F.** TE7 immunostaining: CD56+ cells (E) and CD56+82+ cells (F). Phase contrast: scale bar, 100 µm; immunostaining: scale bar, 50 µm.

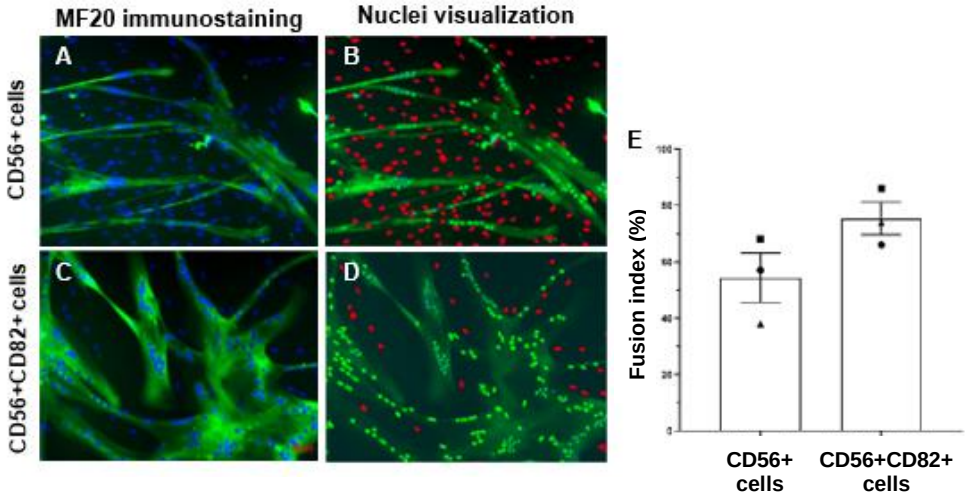


Figure 2. Fusion index. A-B. MF20 immunostaining of CD56+ cells (A) and nuclei of CD56+ cells using Visiopharm analysis (B). **C-D.** MF20 immunostaining of CD56+CD82+ cells (B) and nuclei of CD56+82+ cells using Visiopharm analysis (D). **E.** Fusion index in CD56+CD82+ cells compared to CD56+. Immunostaining: scale bar, 50 µm. n=3 /group, duplicates, 5 fields per slide, mean and standard error of the mean. Blue, green and red indicate nuclei in total, inside and outside MF20+ myotubes, respectively.

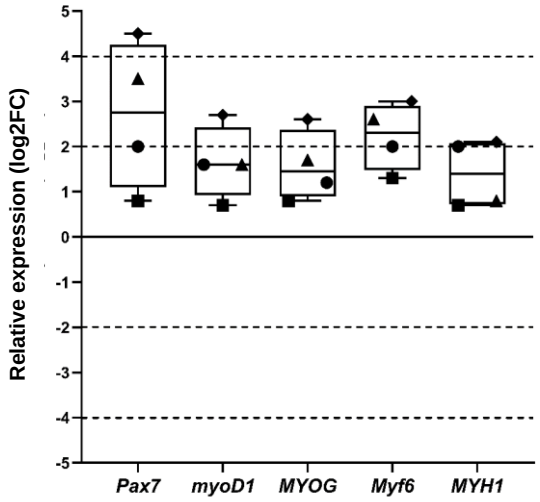


Figure 3. Expression of myogenic markers and myosin heavy chains in CD56+CD82+ cells relative to CD56+ cells. n = 4 per group, results presented as median log_2(FC) [min, max]. CD56+ cells expression arbitrary set at 0 (control value, plain line). Dotted lines for visual support.

2.3. Targeted study of changes in myokine expression

All myokine mRNAs in the targeted study were expressed in CD56+CD82+ myotubes at T0 and T3, except for *CX3CL1*, *APLN*, *LGR5*, and *SPINK5* mRNAs, which were detected at very low levels or not detected in all samples (median Ct > 33). RT-qPCR analysis showed a significant increase in the expression of *FNDC5* (+8%, *p*=0.019) (Fig. 4). Changes in the expression of other myokines were not significant, including *ADAMTS9* (+11%, *p*=0.191), *BDNF* (+11%, *p*=0.755), *XYLT1* (+10%, *p*=0.514), *TRIM72* (+5%, *p*=0.363), *IL-6* (-17%, *p*=0.191), *ANG* (-7%, *p*=0.363), *MSTN* (-9%, *p*=0.570), *TNFRSF11B* (-12%, *p*=0.278), and *SFRP4* (-16%, *p*=0.147) (Fig. 4). One

participant had undetectable expression levels for all myokines at T0, in contrast to the other participants. Statistical analysis showed comparable results with and without this participant.

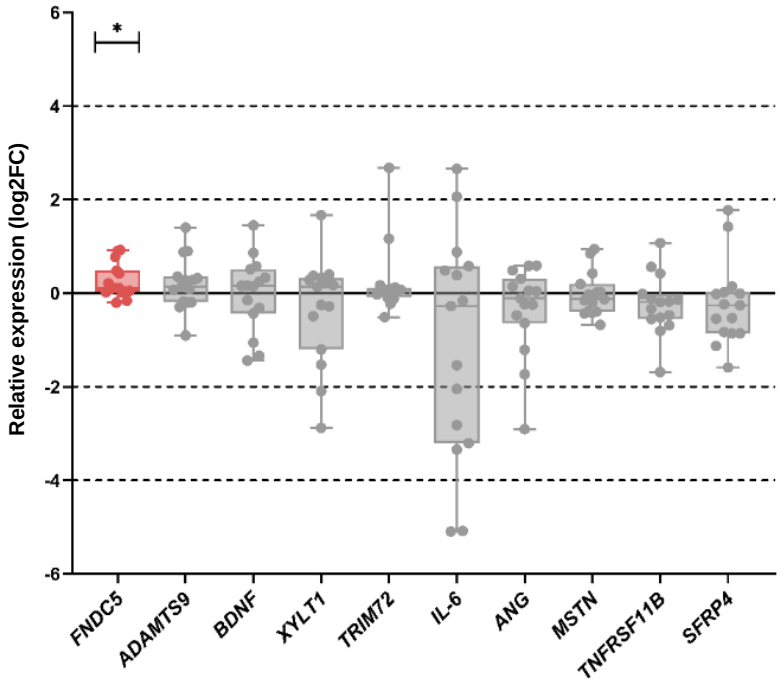


Figure 4. Targeted study of changes in myokine expression. n = 15, results presented as median log_2(FC) [min, max]. T0 expression arbitrary set at 0 (control value). Wilcoxon signed rank test performed on delta CT to assess relative mRNA expression between T0 and T3. Significant changes in red, non-significant changes in grey. * $p < 0.05$.

2.4. Untargeted study of changes in myokine expression

a. Global changes in the transcriptome of CD56+CD82+ myotubes

RNA-Seq was performed on CD56+CD82+ myotubes collected at T0 and T3 from 12 participants. A total of 24,267 individual genes and 97,832 transcripts were sequenced. Five genes and 207 transcripts were differentially expressed after bariatric surgery (FDR ≤ 0.10, Supplementary Figure 1).

b. Pathway enrichment analysis

Gene-based analysis showed that positively enriched gene sets included "adipocytokine signaling pathway" (p=0.075), whereas negatively enriched gene sets included "angiogenesis" (p=0.047) and "tyrosine metabolism" (p=0.075) (Fig. 5A). Transcript-based analysis showed that positively enriched gene sets included "extrinsic component of plasma membrane" (p=0.060), “cytoplasmic side of plasma membrane” (p=0.060), “AMPK signaling pathway” (p=0.069), “presynaptic active zone membrane” (p=0.060), “nitric-oxide synthase binding” (p=0.016), whereas negatively enriched gene sets included "Cul4-RING E3 ubiquitin ligase complex" (p=0.060) and "endoplasmic reticulum to Golgi vesicle-mediated transport" (p=0.072) (Fig. 5).

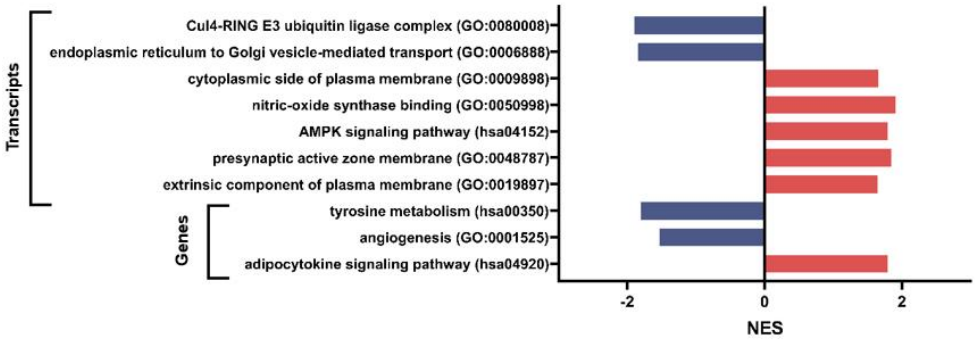


Figure. 5. Gene Set Enrichment Analysis of transcriptomic data. Red and blue indicate positively and negatively enriched gene sets, respectively. A p-value ≤ 0.10 was used as a significant threshold. NES: normalized enrichment score.

c. Differential gene and transcript analysis

None of the 5 differentially expressed genes encoded myokines (Supplementary Table 1). Therefore, we extended the search to genes with a nominal p-value < 0.05 (Fig. 6A). In total, 1321 genes had a nominal p-value < 0.05, including 241 upregulated genes with FC ≥ 1.3 and 113 downregulated genes with FC ≤ 0.7. Thirteen upregulated and 17 downregulated genes encoded myokines (Supplementary Table 2). Most genes had very low expression levels (transcripts per million, TPM < 1.0).

Of the 207 differentially expressed transcripts, 96 were upregulated with FC ≥ 1.3 and 76 were downregulated with FC ≤ 0.70 (Fig. 6B). Eight upregulated and 7 downregulated transcripts encoded myokines (Supplementary Table 3). Most transcripts had very low expression levels (TPM < 1.0).

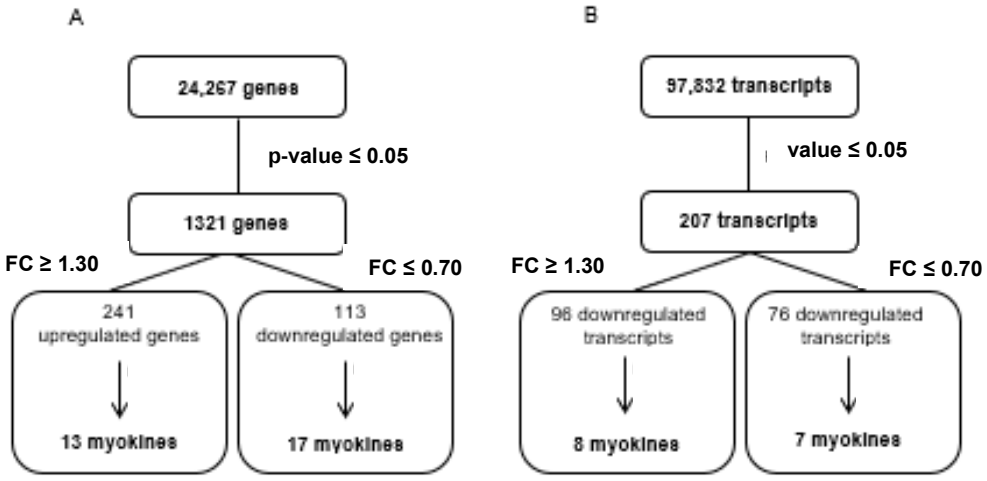


Figure 6. In silico identification of genes and transcripts encoding myokines. **A.** Genes encoding myokines among genes with nominal p-value < 0.05. **B.** Transcripts encoding myokines among differentially expressed transcripts (FDR ≤ 0.10).

d. Changes in expression of selected myokines

Myokines with the most significant changes in expression after bariatric surgery were selected for RT-qPCR analysis (genes and transcripts at the top and bottom of Supplementary Table 2 and 3, respectively). Myokines with the highest expression levels (TPM > 1.0) were also selected. All myokine mRNAs were expressed in CD56+CD82+ myotubes at T0 and T3, except for *ADGRG6*, *CDHR2*, *BRINP2*, and *MEGF11* mRNAs, which were detected at very low levels or not detected in all samples (median Ct > 33). Changes in the expression of other myokines were not significant, including *IGLV5-52* (+21%, *p*=0.307), *FCGR1A* (+10%, *p*=0.776), *THSD8* (+8%, *p*=0.233),

ILR11A (+2%, *p*=0.629), *BTD* (+1%, *p*=0.777), *GDE1* (+1%, *p*=0.955), *TIMP2* (-2%, *p*=0.330), *BACE1* (-5%; *p*=0.233), *COL18A1* (-5%, *p*=0.363), *CD24* (-8%, *p*=1.000), *CTSB* (-12%, *p*=0.078), and *CA11* (-18%; *p*=0.094) (Fig. 7A). One participant had undetectable expression levels for all myokines at T0, in contrast to the other participants. Statistical analysis showed comparable results with and without this participant.

Candidate myokines were also selected from the literature. Fibroblast growth factor (FGF) 18 is a member of the FGF family which comprises 22 structurally related proteins involved in various biological responses, such as tissue remodeling, angiogenesis, and regulation of metabolism (Tsuchiya et al., 2023). FGF18 is a critical growth factor that promotes liver fibrosis (Tsuchiya et al., 2023). In contrast, cardiac-specific overexpression of FGF18 ameliorates fibrosis in a murine model of cardiac hypertrophy (Chen et al, 2023). Protein Wnt-4 **(**WNT4) is one of the key accelerators of pericyte-to-myofibroblast trans-differentiation (Zhang et al., 2021). Myofibroblasts are fibrotic cells involved in response to injury but also in various pathological conditions such as liver fibrosis. In addition, WNT4 is the most abundantly expressed WNT ligand in pancreatic beta cells and its expression is enhanced during the postnatal maturation of human islets (Zhang et al., 2021). WNT4 is upregulated in pancreatic islets of people with T2D (Lee et al., 2008; Bonnefond et al., 2015). Betacellulin (BTC) promotes regeneration of beta cells in experimental

diabetes models and improves glucose metabolism by promoting conversion of intra-islet precursor cells to beta cells in streptozotocin-treated mice (Li et al., 2003). FGF18, WNT4 and BTC therefore seemed to be interesting candidate myokines potentially involved in muscle extracellular matrix remodeling, and muscle-beta cell crosstalk. *FGF18* mRNA was detected at very low levels or not detected in CD56+CD82+ myotubes (median Ct > 33). RT-qPCR analysis showed a significant increase in the expression of *WNT4* (+8%, *p*=0.020) Changes in *BTC* expression were not significant (-3%, *p*=0.820) (Fig. 7B).

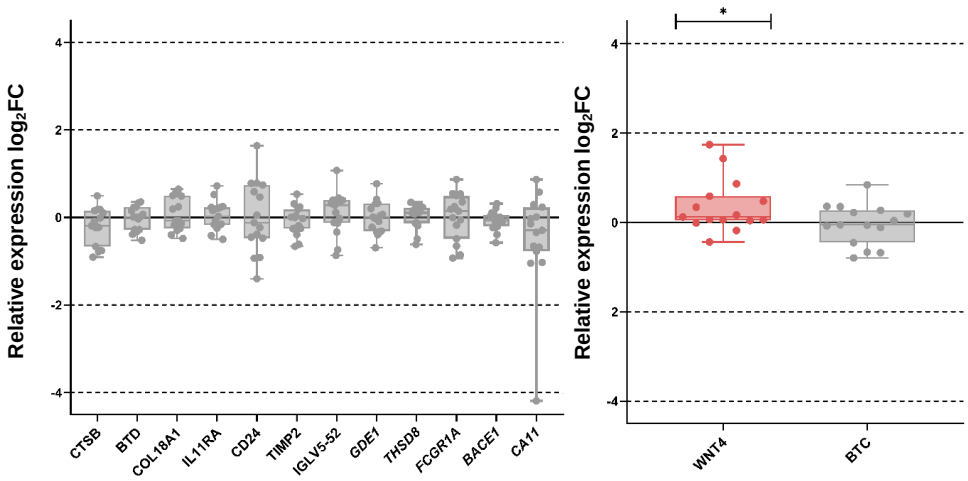


Figure 7. Untargeted study of changes in myokine expression. A. Selected myokines with either the most significant changes in expression after bariatric surgery, or the highest expression levels (Supplementary Tables 2-3). B. Candidate myokines. n = 15, results presented as median log_2(FC) [min, max]. T0 expression arbitrary set at 0 (control value). Wilcoxon signed rank test performed on delta CT to assess relative mRNA expression between T0 and T3. Significant changes in red, non-significant changes in grey. * $p < 0.05$.

2.5. Discussion

We have established a primary human skeletal muscle cell culture model to select a highly myogenic cell population from muscle of people with obesity. Our culture model has allowed us to identify a significant increase in the expression of certain myokines after bariatric surgery (*FNDC5*, *WNT4*). In addition, GSEA revealed changes in several metabolic pathways after bariatric surgery. Changes in these myokines and pathways may contribute to improved glucose and lipid homeostasis after bariatric surgery.

Our primary human skeletal muscle cell culture, adapted from Pakula et al. (Pakula et al., 2019), allows the selection of a highly myogenic cell population from muscle of people with obesity. Specifically, FACS selected a more myogenic population than MACS. Cultured human myotubes share many morphological and biochemical characteristics with human muscle fibers *in vivo* (Aas et al., 2013). Cultured myotubes are therefore considered a valuable model for “bottom-up’ approaches for muscle research. The partial preservation of donor characteristics in cultured myotubes suggests a combination of underlying genetic and epigenetic factors that are transferred to the culture dishes and subcultured cells (Aas et al., 2013). However, overall gene expression redundancy between muscle samples and myotubes from the same cohort is modest for several reasons, including differences in differentiation and lack of the original microenvironment (e.g., hormones,

innervation) (Rutti et al., 2018; Aas et al., 2013; Raymond et al, 2010). RNA-seq of single muscle fibers combined to muscle cell culture should facilitate “bottom-up’ approaches in muscle research (Blackburn et al., 2020).

Our data show that bariatric surgery alters the global transcriptome of myotubes from people with obesity. At the pathway level, enriched gene sets included "adipocytokine signaling pathway", “AMPK signaling pathway”, and “nitric-oxide synthase binding”, among others. Some of these pathways may be relevant to muscle glucose and lipid metabolism after bariatric surgery. The "adipocytokine signaling pathway” and “AMPK signaling pathway” include *CPT1B*, *PPARGC1A*, and *PRKAB1* which respectively encodes carnitine palmitoyltransferase-1 (CPT1, muscle isoform), peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC1A), and 5'-AMP-activated protein kinase (AMPK) subunit beta-1**.** CPT1 is a target of PGC1A and the main regulator of long-chain fatty acid transport across the mitochondrial membrane, while PGC1A, the master regulator of mitochondrial biogenesis, is induced by AMPK (Kim et al., 2000; Tallis et al., 2018). Reduced CPT1 and AMPK activity and PGC1A downregulation have been reported in muscle of people with obesity, which promotes detrimental intramyocellular lipid accumulation and muscle insulin resistance (Kim et al., 2000; Tallis et al., 2018). The nitric-oxide synthase produces nitric oxide, which is involved in mitochondrial biogenesis, with PGC1A as the main signaling

molecule (Tengan et al., 2012). Thus, GSEA on transcriptomic data of myotubes from people with obesity suggest metabolic changes after bariatric surgery that may improve glucose and lipid metabolism in muscle of people with obesity. Direct assessment of the activity of these pathways in skeletal muscle is needed to confirm these data.

We have also shown increased *FNDC5* expression in primary human myotubes after bariatric surgery. *FNDC5* encodes irisin, a contraction-regulated myokine that improves insulin resistance and reduces blood glucose levels (Boström et al., 2012; Barlow et al., 2018). Irisin may have direct effects leading to improved muscle insulin resistance, as muscle cells incubated with irisin show increased glucose uptake and fatty acid oxidation mediated by AMPK activation (Lee et al., 2015; Carson, 2017; Xin et al., 2016). Treatment with recombinant irisin induces a white-to-brown shift in mouse adipose tissue, which increases whole-body energy expenditure (Boström et al., 2012; Eckardt et al., 2014). In addition, insulin-resistant human myotubes secrete higher irisin levels, while recombinant irisin protects human beta cells and islets from palmitate-induced apoptosis (Natalicchio et al., 2017). These data suggest that increased *FNDC5* expression in myotubes after bariatric surgery may positively contribute to muscle glucose and lipid metabolism, and potentially to beta cell function through a muscle-pancreas crosstalk. However, we and others have previously shown that *FDNC5* expression is

decreased in muscle samples after bariatric surgery (Huh et al., 2012; Orioli et al., 2023). Therefore, further studies are needed to characterize the changes and role of irisin in the metabolic improvement induced by bariatric surgery.

We have identified WNT4 as a novel bariatric surgery-regulated myokine and shown increased *WNT4* expression in myotubes of people with obesity after bariatric surgery. WNT4 is involved in the myogenic fate of somites, myogenic proliferation and differentiation of skeletal muscle (Bernardi et al., 2011). Overexpression of *WNT4* in C12C12 myoblasts causes a strong increase in satellite cells and myotube hypertrophy through negative regulation of *MSTN* expression (Bernardi et al., 2011). These data suggest that increased *WNT4* expression in myotubes after bariatric surgery may be associated with decreased *MSTN* expression. Although MSTN was not downregulated in our study, we and others have previously shown that *MSTN* expression is reduced in PWO muscle after bariatric surgery. Decreased *MSTN* expression could improve muscle insulin sensitivity and be interpreted as a protective down-regulatory mechanism to counteract muscle wasting after bariatric surgery (Cleasby et al., 2014; Eilers et al., 2020; Liu et al., 2018). In addition, WNT4 is involved in the postnatal functional maturation of human islets (Zhang et al., 2021), and changes at the muscle level could potentially contribute to a muscle-pancreas crosstalk after bariatric surgery. However, WNT4 is more likely to act in a paracrine manner, as it is not

detected in blood (https://www.proteinatlas.org). We are not aware of any data on WNT4 muscle expression or circulating levels in relation to bariatric surgery.

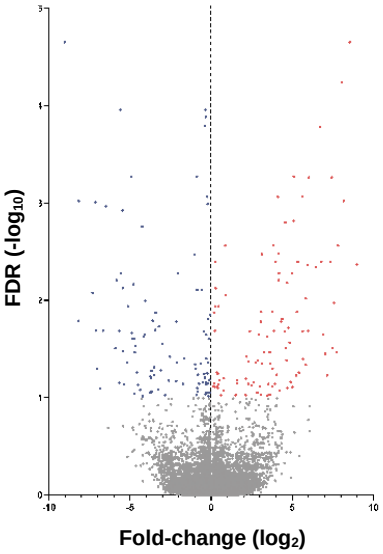
Our study has several limitations. Our population size is small, which limits the power of statistical analysis. This is even more true as there is considerable inter-individual variability in myokine expression changes in response to bariatric surgery. Comparative gene expression profiling between cultured human myotubes and muscle tissue has shown lower expression of genes involved in oxidative metabolism, mitochondria, and muscle contraction in myotubes, while genes related to the extra-cellular matrix and apoptosis had higher expression levels (Raymond et al., 2010). Muscle cell cultures are isolated systems that lack the *in vivo* original microenvironment, innervation and communication with other cells and organs (Aas et al., 2023). Unlike cultured cells, skeletal muscle tissue contains many different cell types such as endothelial cells, neuronal cells, and fibroblasts. This may explain the difference in gene expression between muscle samples and cultured myotubes, as well as low myokine expression in myotubes under basal conditions. The changes in myokine expression that we have previously demonstrated in skeletal muscle (Orioli et al., 2023) were not observed in cultured myotubes. Therefore, extrapolation of our *in vitro* results to the *in vivo* situation is limited. Nevertheless, we are not aware of any other study that has used RNA-Seq to search for bariatric surgery-

regulated myokines on human primary myotubes. Our model has the advantage of allowing meaningful RNA analysis on myotubes despite the small muscle samples obtained from the participants (≈ 10-30 mg) due to our sampling technique (Orioli et al., 2023). We believe that our data is a valuable tool to study changes in the muscle secretome after bariatric surgery.

In conclusion, we have established a primary human skeletal muscle cell culture model that allows the selection of a highly myogenic cell population from muscle of people with obesity. GSEA suggests metabolic changes in myotubes from people with obesity after bariatric surgery that may contribute to improved glucose and lipid homeostasis after bariatric surgery. In addition, we have shown increased expression of FNDC5 and WNT4 in myotubes from people with obesity after bariatric surgery, which may also contribute to improved glucose and lipid homeostasis after bariatric surgery. Our culture model should be a valuable tool to study changes in the muscle secretome after bariatric surgery.

2.6. Supplementary data

a. Supplementary figures



Supplementary Figure 1. Global changes in the transcriptome of CD56+CD82+ myotubes: differentially expressed transcripts (FDR ≤ 0.10) after bariatric surgery. Transcripts with FC > 1 in red (log2FC > 0, n=106) and transcripts with FC ≤ 1 in blue (log2FC

b. Supplementary tables

Supplementary Table 1. Differentially expressed genes encoding proteins after bariatric surgery (FDR ≤ 0.10)

Ensembl gene ID	Gene symbol	Protein name	TPM T0	TPM T3	FC	FDR
ENSG00000108984	MAP2K6	**Dual specificity mitogen-activated protein kinase kinase 6**	0.163	0.321	2.06	1.51E-02
ENSG00000136273	HUS1	**Checkpoint protein HUS1**	15.75	17.51	1.10	1.51E-02
ENSG00000176927	EFCAB5	**EF-hand calcium-binding domain-containing protein 5**	0.118	0.325	2.78	1.51E-02
ENSG00000134198	TSPAN2	**Tetraspanin-2**	4.672	3.848	0.79	6.31E-02
ENSG00000006634	DBF4	**Protein DBF4 homolog A**	4.417	3.737	0.85	9.47E-02

Abbreviations: FC: fold change, FDR: false discovery rate; TPM: transcripts per million.

Supplementary Table 2. Differentially expressed genes encoding myokines (RNA-Seq, nominal p value ≤ 0.05, FC ≥ 1.3 or ≤ 0.70, n = 12 patients)

Ensembl gene ID	Gene symbol	Protein name	TPM T0	TPM T3	FC	*p*-value	FDR
ENSG00000211643	IGLV5-52	Immunoglobulin lambda variable 5-52	1.425	0.503	0.20	1.36E-02	7.07E-01
ENSG00000074276	CDHR2	Cadherin-related family member 2	0.105	0.045	0.31	1.68E-02	7.50E-01
ENSG00000284491	THSD8	Thrombospondin type-1 domain-containing protein 8	0.131	0.071	0.38	4.90E-02	9.13E-01
ENSG00000125872	LRRN4	Leucine-rich repeat neuronal protein 4	0.035	0.016	0.42	2.72E-02	8.48E-01
ENSG00000182585	EPGN	Epigen	0.682	0.303	0.44	1.00E-02	7.01E-01
ENSG00000162894	FCMR	Fas apoptotic inhibitory molecule 3	0.249	0.124	0.47	1.18E-03	3.87E-01
ENSG00000015520	NPC1L1	NPC1-like intracellular cholesterol transporter 1	0.035	0.016	0.49	2.55E-02	8.30E-01
ENSG00000063180	CA11	Carbonic anhydrase-related protein 11	1.244	0.805	0.50	1.78E-02	7.55E-01
ENSG00000288550	**IL17RC**	Interleukin-17 receptor C	0.297	0.137	0.53	2.83E-02	8.53E-01
ENSG00000156427	FGF18	Fibroblast growth factor 18	0.066	0.038	0.53	3.10E-02	8.69E-01
ENSG00000079841	RIMS1	Regulating synaptic membrane exocytosis protein 1	0.101	0.053	0.54	3.81E-02	8.86E-01
ENSG00000242866	STRC	Stereocilin	0.148	0.069	0.54	1.25E-02	7.03E-01
ENSG00000184374	COLEC10	Collectin-10	0.095	0.056	0.60	3.45E-02	8.83E-01
ENSG00000174808	BTC	Probetacellulin	0.337	0.198	0.62	1.50E-02	7.28E-01
ENSG00000115828	QPCT	Glutaminyl-peptide cyclotransferase	0.644	0.442	0.66	3.92E-02	8.86E-01
ENSG00000123243	ITIH5	Inter-alpha-trypsin inhibitor heavy chain H5	0.903	0.563	0.68	3.36E-02	8.78E-01
ENSG00000068078	FGFR3	Fibroblast growth factor receptor 3	0.262	0.173	0.69	5.70E-03	5.63E-01
ENSG00000145864	GABRB2	Gamma-aminobutyric acid receptor subunit beta-2	0.156	0.220	1.35	1.99E-02	7.85E-01
ENSG00000007062	PROM1	Prominin-1	0.258	0.342	1.38	1.81E-02	7.58E-01
ENSG00000162552	WNT4	Protein Wnt-4	0.692	0.945	1.38	1.20E-03	3.87E-01
ENSG00000143768	LEFTY2	Left-right determination factor 2	0.283	0.443	1.51	1.65E-02	7.50E-01
ENSG00000081985	IL12RB2	Interleukin-12 receptor subunit beta-2	0.102	0.150	1.52	3.86E-02	8.86E-01
ENSG00000213443	NPM1P7	Nucleophosmin 1 Pseudogene 7	0.565	0.915	1.60	1.06E-03	3.87E-01
ENSG00000108231	LGI1	Leucine-rich glioma-inactivated protein 1	0.560	0.749	1.60	1.26E-02	7.03E-01
ENSG00000143512	HHIPL2	HHIP-like protein 2	0.086	0.155	1.67	9.19E-03	6.95E-01
ENSG00000115138	POMC	Pro-opiomelanocortin	0.298	0.373	1.76	2.64E-02	8.36-01

ENSG00000124091	GCNT7	Beta-1,3-galactosyl-O-glycosyl-glycoprotein beta-1,6-N-acetylglucosaminyl-transferase 7	0.020	0.048	2.24	3.73E-02	8.86E-01
ENSG00000150337	FCGR1A	High affinity immunoglobulin gamma Fc receptor I	0.120	0.217	2.39	3.06E-02	8.68E-01
ENSG00000198797	BRINP2	BMP/retinoic acid-inducible neural-specific protein 2	0.028	0.053	3.31	3.19E-03	5.50E-01
ENSG00000157890	MEGF11	Multiple epidermal growth factor-like domains protein 11	0.027	0.079	3.45	6.78E-04	3.23E-01

Abbreviations: FC: fold change, FDR: false discovery rate; TPM: transcripts per million. Grey indicates myokines selected from RT-qPCR analysis.

Supplementary Table 3. Differentially expressed transcripts encoding myokines (RNA-Seq, FDR ≤ 0.10, FC ≥ 1.3 or ≤ 0.70, n = 12 patients)

Ensembl transcript ID	Ensembl gene ID	Gene symbol	Protein name	TPM T0	TPM T3	FC	p-value	FDR
ENST00000355480	ENSG00000182871	COL18A1	Collagen alpha-1(XVIII) chain	0.108	0.060	0.019	1.66E-05	2.05E-02
ENST00000690286	ENSG00000137070	IL11RA	Interleukin-11 receptor subunit alpha	0.370	0.065	0.022	3.76E-06	7.51E-03
ENST00000230173	ENSG00000112414	ADGRG6	Adhesion G-protein coupled receptor G6	0.180	0.059	0.048	1.57E-04	8.31E-02
ENST00000504264	ENSG00000155893	PXYLP1	2-phosphoxylose phosphatase 1	0.422	0.143	0.052	4.47E-07	1.75E-03
ENST00000677418	ENSG00000164733	CTSB	Cathepsin B	0.415	0.219	0.077	9.55E-05	6.31E-02
ENST00000466945	ENSG00000184719	RNLS	Renalase	0.288	0.060	0.093	8.92E-06	1.34E-02
ENST00000356955	ENSG00000143537	ADAM15	Disintegrin and metalloproteinase domain-containing protein 15	0.898	0.435	0.225	1.07E-04	6.76E-02
ENST00000563645	ENSG00000006007	GDE1	Glycerophosphodiester phosphodiesterase 1	7.687	10.15	1.324	1.48E-04	7.98E-02
ENST00000509916	ENSG00000186318	BACE1	Beta-secretase 1	1.231	2.334	1.873	4.86E-06	8.81E-03
ENST00000340882	ENSG00000110400	NECTIN1	Nectin-1	0.441	1.003	2.853	1.95E-04	9.41E-02
ENST00000556674	ENSG00000119699	TGFB3	Transforming growth factor beta-3 proprotein	0.125	0.294	5.680	1.31E-04	7.44E-02
ENST00000343457	ENSG00000188993	LRRC66	Leucine-rich repeat-containing protein 66	0.106	0.345	12.21	1.87E-04	9.32E-02
ENST00000615659	ENSG00000272398	CD24	Signal transducer CD24	0.210	0.455	13.72	8.47E-06	1.31E-02
ENST00000383778	ENSG00000169814	BTD	Biotinidase	0.235	0.205	18.69	1.61E-04	8.47E-02
ENST00000536189	ENSG00000035862	TIMP2	Metalloproteinase inhibitor 2	0.062	0.221	57.13	6.02E-05	4.60E-02

Abbreviations: FC: fold change, FDR: false discovery rate; TPM: transcripts per million. Grey indicates myokines selected from RT-qPCR analysis.

3. Identification of myokines susceptible to improve glucose homeostasis after bariatric surgery using multiplex immunoassays on plasma samples

3.1. Results

a. Participants' characteristics

The characteristics of the MYDIASECRET cohort have been reported in detail previously (Orioli et al., 2023). Briefly, the study included 62 Caucasian participants of whom 26 (42%) were male and 39 (63%) had SG. The median age was 44 (25-62) years. Body weight and BMI were reduced as expected after bariatric surgery (-18% and -17%, respectively, both $p<0.001$). Skeletal muscle mass, expressed as skeletal muscle mass index (SMMI) and appendicular skeletal muscle mass index (ASMMI), were also reduced after bariatric surgery (-8% and -11%, respectively, both $p<0.001$). HOMA-IR was reduced by 53% ($p<0.001$) and HOMA-S increased by 112% ($p<0.001$), indicating improved insulin sensitivity. HOMA-B was reduced by 35% ($p<0.001$), indicating less beta cell compensation associated with improved insulin sensitivity. HOMA-BxS was increased by 39% ($p<0.001$), showing an overall improved beta cell function adjusted for insulin sensitivity.

b. Changes in circulating myokine levels

Plasma levels at T0 and T3, as well as the magnitude of changes, were highly variable among myokines and between participants (Table 1). Levels of most myokines were significantly decreased at T3. In contrast, FABP3 and FGF-21 levels were significantly increased at T3, whereas IL-6 levels were unchanged. We chose to exclude from further analysis myokines for which the median level was below the sensitivity threshold of the immunoassay. These myokines included IL-15 and oncostatin M.

Table 1. Plasma myokine levels before (T0) and after (T3) bariatric surgery and changes in plasma myokine levels (n=62)

Myokines	T0 (pg/mL)	T3 (pg/mL)	T0-T3 (pg/mL)	T0-T3 (%T0)	*p*-value
SPARC	128 851 [88 270 ; 198 588]	118 816 [83 088 ; 205 963]	-7 754 [-50 256 ; 48 625]	-5 [-33 ; 32]	0.002
FSTL-1	6 826 [3 424 ; 13 334]	5 755 [3 108 ; 10 191]	-824 [-4 286 ; 1 496]	-14 [-53 ; 25]	<0.001
EPO	4 575 [2 825 ; 7 631]	4 059 [2 575 ; 6 580]	-418 [-2 502 ; 368]	-7 [-44 ; 11]	<0.001
FABP3	1 816 [919 ; 3 638]	2 181 [1 016 ; 3 956]	270 [-1 176 ; 1 359]	+18 [-32 ; 83]	0.001
Irisin	1 219 [208 ; 3 157]	903 [217 ; 3 423]	-240 [-1 762 ; 416]	-28 [-82 ; 96]	<0.001
Musclin	783 [506 ; 1 080]	694 [419 ; 1 060]	-59 [-361 ; 152]	-7 [-46 ; 29]	<0.001
APLN	213 [89 ; 365]	174 [86 ; 371]	-28 [-172 ; 58]	-15 [-57 ; 26]	<0.001
FGF21	53 [9 ; 174]	74 [15 ; 557]	25 [-48 ; 371]	+65 [-54 ; 599]	<0.001
LIF	33 [10 ; 72]	23 [4 ; 57]	-7 [-45 ; 8]	-24 [76 ; 38]	<0.001
IL-6	5.5 [1.2 ; 16.1]	4.8 [1.2 ; 20.4]	-0.4 [-5.6 ; 7.4]	-6 [-71 ; 113]	0.446

Data expressed as medians [95%CI]. Abbreviations: APLN: apelin; EPO, erythropoietin; FABP3, fatty acid-binding protein 3; FSTL-1, follistatin-related protein 1; IL-6, interleukin-6; FGF-21, fibroblast growth factor 21; LIF, leukemia inhibitory factor; SPARC: secreted protein acidic and rich in cysteine. Differences assessed using Wilcoxon signed-rank test. *p* statistically significant < 0.05.

c. Changes in myokines leading to changes in glucose homeostasis

We first tested whether changes in circulating myokine levels were associated with changes in glucose homeostasis after bariatric surgery (Table 2). In the multivariate analysis, the percentage decrease in LIF and FSLT-1 (for both, %T0) was positively associated with the percentage decrease in HOMA-B (%T0). The absolute decrease in APLN (pg/mL) was negatively associated with the percentage decrease in HOMA-IR (%T0). The absolute and percentage increase in FABP3 (pg/mL and %T0, respectively) were positively associated with the absolute and percentage increase in the hyperbolic product (% and %T0, respectively). All the associations remained significant in Model 2 and Model 3 (not shown).

d. Changes in myokines leading to changes in muscle mass

We tested whether changes in circulating myokine levels could lead to changes in muscle mass after bariatric surgery (Table 3). In the multivariate analysis, the absolute and percentage decrease in SPARC (pg/mL and %T0) were negatively associated with changes in absolute and percentage decrease in SMMI and ASMMI (for both, kg/m^2 and %T0, respectively). The absolute increase in FGF21 (pg/mL) was negatively associated with the percentage decrease in ASMMI (%T0).

Table 2. Associations between changes in myokines and changes in glucose homeostasis (multivariate linear regression analyses, adjusted for age and sex)

Dependent variables	Independent variables	N	Unadjusted estimate [95% CI]	Adjusted estimate [95% CI]	*p*-value
HOMA-B (%T0)	LIF (%T0)	62	0.14 [0.01 ; 0.28]	0.16 [0.02 ; 0.30]	0.026
	FSTL-1 (%T0)	62	0.20 [-0.01 ; 0.42]	0.22 [0.01 ; 0.43]	0.043
HOMA-IR (%T0)	APLN (pg/mL)	62	-0.002 [-0.004 ; -0.0001]	-0.06 [-0.11 ; -0.005]	0.031
HOMA-BxS (%)	FABP3 (pg/mL)	62	0.01 [0.002 ; 0.01]	0.01 [0.003 ; 0.015]	0.006
	FABP3 (%T0)	62	0.10 [0.03 ; 0.18]	0.10 [0.03 ; 0.18]	0.007
HOMA-BxS (%T0)	FABP3 (pg/mL)	62	0.01 [0.002 ; 0.02]	0.01 [0.002 ; 0.03]	0.018
	FABP3 (%T0)	62	0.16 [0.02 ; 0.31]	0.15 [0.01 ; 0.29]	0.035

Abbreviations: 95CI, 95% confidence interval; %T0: percentage change from baseline; APLN: apelin; FSTL-1: follistatin-related protein 1; LIF: leukemia inhibitory factor; FABP3: fatty acid-binding protein 3. Adjusted estimate should be interpreted as the mean change in the dependent variable for one unit of change in the independent variable after adjustment for age and sex.

Table 3. Associations between changes in myokines and changes in muscle mass (multivariate linear regression analyses, adjusted for age and sex)

Dependent variables	Independent variables	N	Unadjusted estimate [95% CI]	Adjusted estimate [95% CI]	*p*-value
SMMI (kg/m²)	SPARC (pg/mL)	62	-4.3E-6 [-6.0E-6 ; -2.7E-6]	-6.5E-6 [-9.3E-6 ; -3.7E-6]	<0.001
	SPARC (%T0)	62	-6.2E-3 [-8.5E-3 ; -3.8E-3]	-9.2E-3 [-13.2E-3 ; - 5.2E-3]	<0.001
SMMI (%T0)	SPARC (pg/mL)	62	-2.7E-5 [-4.3E-5 ; -1.1E-5]	-3.4E-5 [-6.0E-5 ; -1.0E-4]	0.007
	SPARC (%T0)	62	-3.9E-2 [-6.0E-2 ; -1.6E-2]	-4.8E-2 [-8.3E-2 ; -1.3E-2]	0.007
ASMMI (kg/m²)	SPARC (pg/mL)	62	-3.4E- [5.9E-5 ; -1.0E-5]	-4.3E-6 [-6.0E-6 ; -2.6E-6]	<0.001
	SPARC (%T0)	62	-4.8E-2 [-8.3E-2; -1.3E-2]	-6.2E-3 [-8.6E-3; -3.0E-3]	<0.001
ASMMI (%T0)	SPARC (pg/mL)	62	-2.7E-5 [-4.3E-5 ; 1.0E-5]	-2.7E-5 [-4.3E-5 ; -1.0E-5]	0.002
	SPARC (%T0)	62	-3.8E-2 [-6.2E-2 ; -1.4E-2]	-3.8E-2 [-6.2E-2 ; -1.4E-2]	0.002
	FGF21 (pg/mL)	62	-6.7E-3 [-13.E-3 ; -2.0E-3]	-6.7E-3 [-13.E-3 ; 2.0E-4]	0.043

Abbreviations: 95CI, 95% confidence interval; %T0: percentage change from baseline; ASSMI: appendicular skeletal muscle mass index; FGF21: fibroblast growth factor 21; SMMI: skeletal muscle mass index; SPARC: secreted protein acidic and rich in cysteine.Adjusted estimate should be interpreted as the mean change in the dependent variable for one unit of change in the independent variable after adjustment for age and sex.

3.2. Discussion

Our study shows that bariatric surgery is associated with changes in circulating levels of many myokines, most of which are decreased after bariatric surgery. Changes in circulating LIF, FSLT-1, APLN, and FABP3 levels are associated with changes in glucose homeostasis. Changes in muscle mass are associated with changes in circulating SPARC and FGF21 levels. Our study suggests that certain myokines may serve as mediators of changes in glucose homeostasis and muscle mass after bariatric surgery.

We and others have shown that changes in circulating levels of some myokines, such as BDNF and SPARC, are associated with changes in glucose homeostasis after bariatric surgery (Orioli et al., 2023; Lee et al., 2014). We are now extending this observation to other circulating myokines including LIF, FSLT-1, APLN and FABP3.

LIF is a cytokine that belongs to the IL-6 family (Brandt et al., 2015). LIF and its receptor are expressed by skeletal muscle and pancreatic duct cells, to induce the proliferation of progenitor cells (Broholm et al., 2012; De Breuck et al., 2006). Several observations support the potential role of LIF in glucose homeostasis. Like IL-6, LIF acutely increases muscle glucose uptake in mice (Brandt et al., 2015). In addition, incubation of adult pancreatic exocrine cells with LIF results in their transdifferentiation into functional beta cells (Baeyens

et al., 2005). In our study, circulating LIF levels decrease after bariatric surgery, and decreased LIF levels after bariatric surgery are associated with decreased HOMA-B. These data, combined with the literature, may suggest that LIF mediates a muscle-pancreas crosstalk contributing to the adaptation of insulin secretion to improved insulin sensitivity after bariatric surgery. However, LIF is a ubiquitously expressed cytokine. Therefore, changes in circulating LIF levels may not originate from muscle, as suggested by the lack of changes in circulating LIF levels after exercise while its muscle expression is increased (https://www.proteinatlas.org ;Broholm et al., 2011). We are not aware of any report of circulating LIF levels after bariatric surgery.

FSTL-1 is a small glycoprotein that belongs to the same family as follistatin and SPARC (Xu et al., 2020). FSTL-1 is secreted by human myotubes in response to contraction and by other tissues, including adipose tissue (Görgens et al., 2013; Xu et al., 2020). Several observations support the potential role of FSTL-1 in glucose homeostasis. FSTL-1 stimulates glucose uptake in muscle cells by inducing GLUT4 protein expression and translocation in an AMPK-dependent manner (Lee et al., 2017). On one hand, circulating FSTL-1 levels are higher in people with type 2 diabetes than in healthy people, and positively associated with body mass index and HOMA-IR, suggesting either FSLT-1 resistance or increased secretion by adipose tissue (Xu et al., 2020). On the other hand, circulating FSTL-1 levels increase in response to exercise

in healthy people. Together these observations suggest that acutely and chronically elevated FSTL-1 may result from physiological (exercise) or pathogenic (type 2 diabetes) stimuli, as with other myokines (e.g., IL-6) (Xu et al., 2020). In fact, FSTL-1 is induced at both gene and circulating level in various inflammatory conditions to promote IL-1β secretion by immune cells (Chaly et al., 2014; Xu et al., 2020). FSTL-1 secretion by primary human myotubes is increased in response to proinflammatory cytokines, suggesting that muscle may contribute to increased circulating FSTL-1 levels in people with obesity and type 2 diabetes, both conditions being associated with chronic low-grade inflammation (Gorgens et al., 2013; Xu et al., 2020). In our study, circulating FSTL-1 levels decrease after bariatric surgery, as reported by others (Santos et al., 2024). In addition, decreased circulating FSTL-1 levels after bariatric surgery are associated with decreased HOMA-B, as observed for LIF. However, we are not aware of any report on FSTL-1 and beta cell function. In fact, decreased FSTL-1 may reflect the reduced inflammatory state or the reduced adipose tissue mass after bariatric surgery, which is also associated with less beta cell compensation.

APLN is a 36 amino acid peptide and the endogenous ligand of the G protein-coupled receptor APJ receptor. APLN is secreted by human muscle cells and various tissues including adipose tissue (Besse-Patin et al., 2014; Krist et al., 2013). APLN expression increases in response to insulin in adipocytes and to

contraction in human muscle cells and tissue (Boucher et al., 2005; Besse-Patin et al., 2014). Several observations support the potential role of APLN in glucose homeostasis. Muscle APLN expression increases after exercise and associates with improved whole-body insulin sensitivity (Besse-Patin et al., 2014). In addition, treatment of APLN-null mice or obese insulin-resistant mice with APLN restores glucose tolerance by improving muscle glucose uptake and oxidation (Yue et al., 2010; Dray et al., 2008; Attané et al. 2012). However, circulating APLN levels are higher in people with obesity and type 2 diabetes and inversely associated with insulin sensitivity, suggesting either APLN resistance or increased secretion by adipose tissue (Boucher et al., 2005; Krist et al., 2013; Soriguer et al., 2009; Heinonen et al., 2005). In fact, muscle APLN expression is similar between healthy people and people with type 2 diabetes, but adipose tissue APLN expression is increased in people with type 2 diabetes, suggesting that adipose tissue is responsible for increased APLN circulating levels during type 2 diabetes (Dray et al., 2010; Krist et al., 2013). In our study, circulating APLN levels decrease after bariatric surgery and decreased APLN levels are negatively associated with decreased in insulin sensitivity, as reported by others (Krist et al., 2013; Soriguer et al., 2009; Heinonen et al., 2005). However, we found no significant changes in *APLN* mRNA expression in muscle after bariatric surgery in our previous study (Orioli et al., 2023). In contrast, APLN expression in adipose tissue is decreased after bariatric surgery, which likely alters circulating APLN levels and may

associate with improved glucose homeostasis after bariatric surgery (Krist et al., 2013). Further research is needed to determine the role of APLN in the context of bariatric surgery.

FABP3 is the major isoform of cytosolic fatty acid binding protein (FABP) expressed in human skeletal muscle and heart (Rosa et al., 2007). FABPs are small proteins that facilitate fatty acid influx across the plasma membrane and transport to the mitochondrial beta-oxidation system (Storch et al., 2023). Several observations support the potential role of FABP3 in glucose and lipid metabolism. FABP3-deficient mice have increased plasma fatty acid levels and muscle fatigue during exercise due to impaired muscle fatty acid uptake (Furuhashi et al., 2008). In fact, muscle FABP3 expression increases during exercise and fatty acid exposure due to increased fatty acid demand or availability (Tunstall et al., 2002; Furuhashi et al., 2008). FABP3 is also upregulated in the muscle of obese insulin-resistant mice where it may stimulate fatty acid uptake and oxidation to prevent lipotoxicity (Kusudo et al., 2011). In addition, muscle cells overexpressing FABP3 show enhanced basal and insulin-stimulated glucose uptake (Kusudo et al., 2011). These data suggest that FABP3 has positive effects on muscle oxidative metabolism and insulin sensitivity. In contrast, FABP3 inhibition protects beta cells from lipotoxicity, suggesting different FABP3 effects depending on the cell type (Hyder et al., 2024). In our study, circulating FABP3 levels increase after bariatric surgery

and increased FABP3 levels after bariatric surgery are associated with increased HOMA-BxS, which shows an overall improved beta cell function adjusted for insulin sensitivity. Increased circulating FABP3 levels can be interpreted as a mechanism aimed at preventing lipotoxicity, at least at the muscle level, since adipose tissue lipolysis is increased early after bariatric surgery (Gancheva et al., 2019). Rosa et al. have shown that muscle FABP3 mRNA expression is decreased 3 years after bariatric surgery, suggesting that muscle may not be responsible for changes in circulating FABP3 (Rosa et al., 2007). However, this late time point may coincide with the attenuation of lipolysis in adipose tissue.

We have shown that changes in circulating myostatin levels are associated with changes in muscle mass after bariatric surgery (Orioli et al, 2023). We are now extending this observation to other circulating myokines, including SPARC and FGF21. SPARC is a glycoprotein that exerts profibrotic effects in various tissues, including skeletal muscle (Wu et al., 2011; Lee et al., 2014). Its circulating levels and muscle mRNA expression are higher in people with obesity and type 2 diabetes than in healthy people, suggesting that SPARC may contribute to muscle fibrosis during obesity (Wu et al., 2011). Consistent with this, circulating SPARC levels decrease after bariatric surgery and correlate with improved insulin sensitivity (Lee et al., 2014). In our study, we also find decreased circulating SPARC levels after bariatric surgery, but we

did not find an association with glucose homeostasis. However, decreased circulating SPARC levels are negatively associated with decreased muscle mass. SPARC is expressed in satellite cells and stimulates myogenesis (Jørgensen et al., 2009; Cho et al., 2000; Nakamura et al., 2012). SPARC knock-out mice have decreased muscle mass and strength, suggesting that SPARC deficiency leads to sarcopenia (Bradshaw et al., 2003; Nakamura et al., 2012; Jørgensen et al., 2017). Increased circulating SPARC levels in people with sarcopenia suggest either SPARC resistance, or a mechanism to counter muscle wasting (Kwak et al., 2018). Thus, decreased circulating SPARC levels after bariatric surgery could be involved in muscle mass loss and serve as a biomarker of muscle mass.

FGF21, a member of the FGF19 subfamily, is mainly a hepatokine, but also an adipomyokine (Itoh, 2014; Faramia et al., 2020). FGF21 binds to the beta-Klotho complex, an FGF co-receptor that is highly expressed in metabolically active tissues (Gao et al., 2019). Several observations support that FGF21 may control muscle mass in certain conditions. Basal FGF21 expression is very low in healthy muscle, but cellular stressors including fasting induce FGF-21 release from muscle (Oost et al., 2019; Vandanmagsar et al., 2016). Muscle-specific FGF21 knockout mice have similar muscle fiber size than control mice, indicating that FGF21 does not control muscle mass under basal conditions (Oost et al., 2019). In contrast, FGF21 knock-out mice are protected against

starvation-induced muscle atrophy, indicating that FGF21 contributes to muscle atrophy in the context of cellular stress (Oost et al., 2019). Consistent with this, people with sarcopenia have higher circulating FGF21 levels compared to controls (Jung et al. Bone., 2021). Circulating FGF21 levels are also higher in people with type 2 and thought to result from metabolic imbalance and/or FGF21 resistance (Gao et al., 2019; Fisher et al., 2010; Mashili et al., 2011). However, muscle FGF21 mRNA expression is similar in people with type 2 diabetes and healthy people (Mashili et al., 2011), suggesting that increased circulating FGF-21 levels in people with type 2 diabetes do not originate from muscle. A steep increase in circulating FGF21 levels has been observed within weeks after bariatric surgery, which is consistent with our current findings (Crujeiras et al., 2017; Gomez-Ambrosi et al., 2017; Lips et al., 2014). We also show that changes in circulating FGF21 levels are associated with changes in muscle mass, which may be related to muscle mass loss in response to caloric restriction.

Our study has several limitations. Our cohort includes both sleeve gastrectomy and Roux-en-Y gastric bypass. Although Roux-en-Y-gastric bypass may be slightly more effective than sleeve gastrectomy for early improvement of glucose homeostasis (Cummings et al., 2018), sleeve gastrectomy and Roux-en-Y-gastric bypass have the same time course for changes in body weight and insulin sensitivity (Gancheva et al., 2019). In

addition, we chose a unique early time point while participants are in a dynamic phase of weight loss and catabolism. Our data may therefore not be representative of later time points. Our data do not allow us to conclude that changes in circulating myokine levels originate from muscle, since there is a large overlap between adipokines, hepatokines, and myokines (Raschke et al., 2013; Faramia et al., 2020). In addition, changes in myokine expression in muscle samples were not investigated in this study. Analysis of the arteriovenous difference would be necessary to establish that muscle is the source of changes in circulating myokine levels (Whitham et al., 2018). Increased physical activity after bariatric surgery may be a confounding factor because many myokines are regulated by muscle contraction. Finally, the associations we have found do not establish a causal relationship between changes in myokines and changes in glucose homeostasis or muscle mass. Mechanistic studies are needed to determine the role of the myokines identified in our study in the changes in glucose homeostasis and muscle mass after bariatric surgery. Nevertheless, we are convinced that the longitudinal design and a relatively large population are the main strengths of our study.

In conclusion, our study shows that bariatric surgery is associated with changes in circulating levels of many myokines. Changes in some of these myokines, including LIF, FSLT-1, APLN, and FABP3, are associated with

changes in glucose homeostasis. In addition, changes in SPARC and FGF21 are associated with muscle mass. Thus, these myokines may serve as mediators of changes in glucose homeostasis and muscle mass after bariatric surgery.

4. Myostatin as a biomarker of muscle mass and strength in people with obesity undergoing bariatric surgery

4.1. Published manuscript

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4.2. Abstract

Background & aims: Our study aims to determine whether myostatin is associated with muscle mass and strength in individuals with cancer or obesity, as well as with cancer cachexia (CC) or sarcopenic obesity.

Methods: The ACTICA study included individuals with CC (n=70) or without CC (NC, n=73). The MYDIASECRET study included individuals with obesity evaluated before (T0) and 3 months (T3) after bariatric surgery (n=62). Body composition was assessed using bioelectrical impedance analysis. Skeletal muscle mass (SMM) and appendicular SMM (ASMM) were calculated from Janssen's and Sergi's equations, respectively, and expressed as indexes (SMMI and ASMMI). Handgrip strength (HGS) was assessed using a Jamar hand-held dynamometer. Myostatin plasma levels were measured using

ELISA. Spearman's coefficient was used to correlate plasma myostatin levels with muscle mass and strength. Receiver operating characteristic (ROC) curve analysis was performed to identify an optimal myostatin cutoff level for the prediction of CC or sarcopenic obesity.

Results: In the ACTICA study, muscle mass and strength were lower in CC individuals than in NC individuals (SMMI: 8.0 kg/m² vs 9.0 kg/m², p=0.004; ASMMI: 6.2 kg/m² vs 7.2 kg/m², p<0.001; HGS: 28 kg vs 38 kg, p<0.001). Myostatin was also lower in CC individuals than in NC individuals (1434 pg/mL vs 2149 pg/mL, p<0.001). Muscle mass and strength were positively correlated with myostatin (SMMI: R=0.500, p<0.001; ASMMI: R=0.479, p<0.001; HGS: R=0.495, p<0.001). ROC curve analysis showed a myostatin cutoff level of 1548 pg/mL (AUC 0.684, sensitivity 57%, specificity 75%, p<0.001) for the prediction of CC. In the MYDIASECRET study, muscle mass and strength were reduced at T3 (SMMI: -8%, p< 0.001; ASMMI: -12%, p<0.001; HGS: -6%, p=0.005). Myostatin was also reduced at T3 (1773 pg/mL vs2582 pg/mL, p<0.001). Muscle mass and strength were positively correlated with myostatin at T0 and T3 (SMMI-T0: R=0.388, p=0.002; SMMI-T3: R=0.435, p<0.001; HGS-T0: R=0.337, p=0.007; HGS-T3: R=0.313, p=0.013). ROC curve analysis showed a myostatin cutoff level of 4225 pg/mL (AUC 0.835, sensitivity 98%, specificity 100%, p=0.014) for the prediction of sarcopenic obesity at T3.

Conclusions: Myostatin is positively correlated with muscle mass and strength in individuals with cancer or obesity, suggesting its potential use as a biomarker of muscle mass and strength. The ROC curve analysis suggests the potential use of myostatin as a screening tool for CC and sarcopenic obesity.

4.3. Significance of the data

Our data reinforce previous observations of positive associations between myostatin and muscle mass in humans (Alexopoulos et al., 2021; Nishikawa et al., 2022; Kurose et al., 2021). In addition, our study extends this observation to people with obesity undergoing bariatric surgery and to individuals with cancer cachexia, although both cohorts were different. Interestingly, myostatin was also positively correlated with muscle strength in the two cohorts, which is relevant for clinical practice since low muscle strength is overtaking the role of low muscle mass as a principal determinant of sarcopenia (Cruz-Jentoft et al., 2019; Donini et al., 2022).

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Original article

Circulating myostatin as a biomarker of muscle mass and strength in individuals with cancer or obesity

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SUMMARY

Background & aims: Our study aims to determine whether myostatin (MSTN) is associated with muscle mass and strength in individuals with cancer or obesity, as well as with cancer cachexia (CC) or sarcopenic obesity (SO).

Methods: The ACTICA study included individuals with CC (n = 70) or without CC (NC, n = 73). The MYDIASECRET study included individuals with obesity evaluated before (T0) and 3 months (T3) after bariatric surgery (n = 62). Body composition was assessed using bioelectrical impedance analysis (BIA). Skeletal muscle mass (SMM) and appendicular SMM (ASMM) were calculated from Janssen's and Sergi's equations, respectively, and expressed as indexes (SMMI and ASMMI). Handgrip strength (HGS) was assessed using a Jamar hand-held dynamometer. MSTN plasma levels were measured using ELISA. Spearman's coefficient was used to correlate MSTN with muscle mass and strength. Receiver operating characteristic (ROC) curve analysis was performed to identify an optimal MSTN cutoff level for the prediction of CC or SO.

Results: In the ACTICA study, muscle mass and strength were lower in CC individuals than in NC individuals (SMMI: 8.0 kg/m^2 *vs* 9.0 kg/m^2, $p = 0.004$; ASMMI: 6.2 kg/m^2 *vs* 7.2 kg/m^2, $p < 0.001$; HGS: 28 kg *vs* 38 kg, $p < 0.001$). MSTN was also lower in CC individuals than in NC individuals (1434 pg/mL *vs* 2149 pg/mL, $p < 0.001$). Muscle mass and strength were positively correlated with MSTN (SMMI: $R = 0.500$, $p < 0.001$; ASMMI: $R = 0.479$, $p < 0.001$; HGS: $R = 0.495$, $p < 0.001$). ROC curve analysis showed a MSTN cutoff level of 1548 pg/mL (AUC 0.684, sensitivity 57%, specificity 75%, $p < 0.001$) for the prediction of CC. In the MYDIASECRET study, muscle mass and strength were reduced at T3 (SMMI: −8%, $p < 0.001$; ASMMI: −12%, $p < 0.001$; HGS: −6%, $p = 0.005$). MSTN was also reduced at T3 (1773 pg/mL *vs* 2582 pg/mL, $p < 0.001$). Muscle mass and strength were positively correlated with MSTN at T0 and T3 (SMMI-T0: $R = 0.388$, $p = 0.002$; SMMI-T3: $R = 0.435$, $p < 0.001$; HGS-T0: $R = 0.337$, $p = 0.007$; HGS-T3: $R = 0.313$, $p = 0.013$). ROC curve analysis showed a MSTN cutoff level of 4225 pg/mL (AUC 0.835, sensitivity 98%, specificity 100%, $p = 0.014$) for the prediction of SO at T3.

Conclusions: MSTN is positively correlated with muscle mass and strength in individuals with cancer or obesity, suggesting its potential use as a biomarker of muscle mass and strength. The ROC curve analysis suggests the potential use of MSTN as a screening tool for CC and SO.

Introduction

Skeletal muscle, the largest tissue in the human body, is a dynamic metabolic tissue essential for vital functions such as posture, locomotion, and breathing [1]. Muscle wasting is therefore associated with poor outcomes such as functional impairment and increased mortality [2e4]. Measurement of muscle mass is required to identify individuals with sarcopenia, a muscle disease that can be primary (e.g., age-related) or secondary (e.g., cancer- or obesity-related) [4,5]. Direct measurement of muscle mass is based on one of several imaging modalities, including dual-energy x-ray absorptiometry (DXA), computed tomography (CT), or magnetic resonance imaging (MRI) [6,7]. However, these imaging modalities are impractical and expensive for routine clinical use, whereas bioimpedance analysis (BIA) can easily provide an indirect estimation of muscle mass as long as validated equations are used [8,9]. In this context, the identification of biomarkers of muscle mass, easily obtained from a single blood sample, would facilitate the screening of sarcopenia [10e13].

Myostatin (MSTN), also referred to as growth-differentiation factor 8 (GDF-8), is a member of the transforming-growth factor beta (TGF-Beta) superfamily, mainly expressed in and secreted from skeletal muscle [14]. This myokine acts as a direct negative regulator of muscle growth through activin type II receptors and SMAD2-3 transcription factors [14]. MSTN gene knock-out in mice leads to muscle hypertrophy whereas transgenic mice that overexpress MSTN in skeletal muscle have muscle atrophy [14]. In line with these data, several studies have suggested that MSTN could contribute to muscle wasting in humans [15e18]. However, recent studies have found a positive correlation between circulating MSTN and muscle mass, suggesting that this myokine could be used as a biomarker of muscle mass [10,11].

Cancer and obesity are growing public health issues that adversely affect quality of life and life expectancy [19e22]. Both conditions can be associated with muscle wasting. Cancer cachexia (CC) is a multifactorial syndrome defined by an ongoing loss of muscle mass, with or without fat mass loss, that cannot be fully reversed by conventional nutritional support [23]. Sarcopenic obesity (SO) is characterized by the coexistence of excess fat mass and a relative reduction of muscle mass adjusted to body weight [5,24]. In both conditions, muscle wasting results from a combination of decreased food intake, physical inactivity, hormonal changes, protein catabolism, inflammation, anabolic resistance, and mitochondrial dysfunction [1,5,25e28]. In addition, bariatric surgery, which remains the most effective intervention for achieving long-term weight-loss [29], inevitably leads to muscle wasting [30,31]. This unintended muscle wasting is suggested to counteract the long-term benefits of bariatric surgery, due to the potential occurrence of sarcopenia [31]. To the best of our knowledge, it has not been investigated whether

MSTN could be a biomarker of muscle mass in individuals with CC or SO, before or after bariatric surgery.

In this study, we first investigated the association of circulating MSTN with muscle mass and strength in two independent cohorts, one of individuals with cancer and another of individuals with obesity undergoing bariatric surgery. We then investigated whether MSTN was associated with CC or SO.

2. Materials & methods

2.1. Study designs

The ACTICA study is a prospective observational study investigating the role of activin A in CC (NCT2011/19AVR/157) [32]. Briefly, individuals with colorectal or lung cancer were enrolled at diagnosis or relapse before any therapeutic intervention. Individuals with cachexia (CC individuals) were compared to those without cachexia (NC individuals).

The MYDIASECRET study is a prospective, longitudinal, and interventional study investigating the potential role of myokines in glucose homeostasis after bariatric surgery (NCT03341793) [33]. Briefly, individuals with obesity were evaluated before (T0) and 3 months (T3) after bariatric surgery which consisted of either a sleeve gastrectomy or a Roux-en-Y gastric bypass.

Both studies were approved by the institutional review board and conducted in accordance with the principles of the Declaration of Helsinki. Patients were enrolled after obtaining written informed consent.

2.2. Muscle mass assessment using CT

In the ACTICA study, muscle mass was assessed at inclusion using CT imaging performed for standard cancer care. Briefly, skeletal muscle area was quantified on a transverse CT image taken at the level of the third lumbar vertebrae using the Slice-O-Matic software, version 4.3 (Tomovision) [32]. Hounsfield unit thresholds ranged from 29 to 150 units for skeletal muscle. Crosssectional area was normalized for the height and expressed as skeletal muscle mass index (SMMI-CT, cm^2/m^2).

2.3. Muscle mass assessment using BIA

In both studies, body composition was assessed using BIA (BIA101 device, Akern®, Pisa, Italy). Whole body resistance and reactance were measured with the subject in a supine position, with arms and legs slightly separated. Surface electrodes were placed on the right side of the body on the dorsal surface of the hand and foot. Fat

mass (FM) was estimated using Bodygram® software and proprietary manufacturer algorithms (Akern®, Pisa, Italy). Skeletal muscle mass (SMM) was calculated using Janssen's equation which is based on MRI as reference method [8]. Appendicular SMM (ASMM) was calculated using Sergi's equation which is based on DXA as reference method [9]. FM, SMM, and ASMM were expressed as absolute values (kg), percentage values (percentage of body weight, %BW), and indexes (I, kg/m^2). In the MYDIASECRET study, longitudinal changes from baseline (T0) were expressed as absolute changes (kg or kg/m^2) or percentage changes (percentage change from T0, %T0). Individuals from the original ACTICA study in whom BIA data were not available were not included in the present study (n ¼ 9/152) [32].

2.4. Muscle strength assessment

In both studies, handgrip strength (HGS) was assessed using a Jamar hand-held dynamometer. Three measures were made on the nondominant side in a time interval of 30 seconds. The highest value (kg) was used.

2.5. Definition of CC and SO

In the ACTICA study, CC was defined as either involuntary weight loss >5% over the past 6 months or weight loss >2% with body mass index (BMI) < 20 kg/m^2 or weight loss >2% with low muscle mass, as previously reported [32,34]. Low muscle mass was defined as SMMI-CT < 55 cm^2/m in men and 39 cm^2/m^2 in women.

In the MYDIASECRET study, SO was considered in individuals with obesity (BMI 30 kg/m^2 and/or percentage FM 25% in men and 35% in women) and low muscle mass. Low muscle mass was defined as SMM adjusted for body weight (SMM/BW, %) <37.7% in men and <26.7% in women [5]. Class I and class II SO were defined as SMM/BW between 31.5 and 37.7% in men and 22.1e27.6% in women, and <31.5% in men and <22.1% in women, respectively [5]. 2.6. Plasma MSTN measurement

In both studies, blood samples (EDTA) were taken in standardized conditions and centrifuged for 10 min at 4 C, 2.000g. Plasma was transferred to microtubes and stored at 80 C. Mature MSTN levels were measured using quantitative sandwich ELISA (DGDF80, R&D Systems, Minneapolis, USA) according to the manufacturer's recommendations, as previously reported [32,33]. Plasma levels were expressed as absolute values (pg/mL). In the MYDIASECRET study, longitudinal changes from baseline (T0) were expressed as absolute changes (pg/mL) or percentage changes (percentage change from T0, %T0).

2.7. Statistical analysis

Continuous and discrete variables are reported as medians with 95% confidence interval (95%CI) and numbers with frequencies, respectively. Cross-sectional and longitudinal differences in continuous variables were assessed using the non-parametric Mann-Whitney U or the Wilcoxon signed rank test, respectively. Cross-sectional and longitudinal differences in discrete variables were assessed using the Chi-Carre test and McNemar test, respectively. Correlations were estimated using Spearman's Rank correlation coefficient. Associations between MSTN, muscle mass, and muscle strength were assessed using uni- and multivariate linear regression. Multivariate analysis was adjusted for age and sex (Model 1), and for age, sex, and BMI (Model 2). Results were expressed as estimates with 95%CI. Associations between MSTN and CC or SO were assessed using uni- and multivariate logistic regression. Multivariate analysis was adjusted using Model 1 and Model 2, unless there was a collinearity issue. Results were expressed as odds ratio (OR) with 95%CI. Receiver operating characteristic (ROC) curve analysis was performed to identify an optimal MSTN cutoff level for the prediction of CC or SO. Statistical significance was set at $p < 0.05$. Statistical analyses were performed using SPSS Statistics software (IBM SPSS Statistics, Version 27.0. Armonk, NY, USA) and SAS software (version 9.4; SAS Institute Inc, Cary, NC, USA). Graphs were made with GraphPad Prism (version 8.0.0 for Windows, GraphPad Software, San Diego, California USA, www.graphpad.com).

3. Results

3.1. Patients' characteristics

The ACTICA study included 143 individuals of whom 70 (49%) had CC and 87 (61%) had colorectal cancer (Table 1). The median age was 66 (39e84) years. Most were men (57%). Age and sex ratio were comparable between CC and NC individuals. As expected, body weight and BMI were lower in CC individuals than in NC individuals (for both, $p < 0.001$). CC was unrelated to cancer site, tumor staging, or presence of metastases [32].

The MYDIASECRET study included 62 individuals of whom 26 (42%) were males and 39 (63%) underwent a sleeve gastrectomy (Table 2). The median age was 44 (25e62) years. BW and BMI were reduced after bariatric surgery (18% and 17%, respectively, $p < 0.001$), as reported in detail earlier [33].

3.2. Muscle mass assessment

In the ACTICA study, muscle mass was lower in CC individuals than in NC individuals, whatever the technique used to estimate it, when expressed as absolute values and

indexes (Table 1). In contrast, percentage muscle mass (%BW) was similar in CC and NC individuals, suggesting muscle and fat mass loss of similar amplitude during CC. CT, considered as the reference method, was available in 130 individuals. SMMI-CT was 6% lower in CC individuals than in NC individuals (p ¼ 0.004). SMMI-CT was positively and strongly correlated with SMMI (R 0.635, $p < 0.001$), and ASMMI (R 0.702, $p < 0.001$).

In the MYDIASECRET study, SMM and ASMM were reduced after surgery, when expressed as absolute values and indexes (Table 2). Specifically, SMMI and ASMMI were reduced by 8% and 12%, respectively (for both, $p < 0.001$). However, percentage muscle mass (%BW) was increased after bariatric surgery, suggesting a predominant loss of FM.

3.3. Muscle strength assessment

In the ACTICA study, HGS was lower in CC individuals than in NC individuals (Table 1). HGS was positively and strongly correlated with SMMI-CT (0.623, $p < 0.001$), SMMI (R 0.718, $p < 0.001$), and ASMMI (R 0.670, $p < 0.001$).

In the MYDIASECRET study, HGS was significantly reduced after bariatric surgery (p ¼ 0.005) (Table 2). However, HGS adjusted for BMI (HGS/BMI) or muscle mass (HGS/ASMMI) was increased, suggesting improved muscle performance after bariatric surgery, despite muscle mass loss. HGS was positively and well correlated with SMMI (T0: R 0.623, $p < 0.001$ and T3: R 0.627, $p < 0.001$) and ASMMI (T0: R 0.521, $p < 0.001$ and T3: R 0.450, $p < 0.001$). In contrast, no significant correlation was found between muscle mass and HGS changes after bariatric surgery (not shown).

3.4. MSTN plasma levels

In the ACTICA study, MSTN was lower in CC individuals than in NC individuals (33%, $p < 0.001$) (Table 1). MSTN was positively correlated with muscle mass (Fig.1A), whatever the technique used to estimate it, as well as with HGS, in CC and NC individuals (Table 3).

In the MYDIASECRET study, MSTN was reduced after bariatric surgery (32%, $p < 0.001$) (Table 2), as previously reported [33]. MSTN was positively correlated with SMMI at both timepoints (Fig. 1B). A positive correlation was also found between MSTN and muscle mass changes (Table 3). In addition, MSTN was positively correlated with HGS at both timepoints, whereas no significant correlation was found between MSTN and HGS changes. MSTN was also correlated with SMM/BW (Table 3). Interestingly, MSTN adjusted for FM (MSTN/FM) was more strongly

correlated with SMM/BW than MSTN alone. Therefore, MSTN adjusted for adiposity correlated well with muscle mass adjusted for body mass, the parameter defining SO.

3.5. Associations of MSTN with muscle mass and CC

Using linear regression analysis, MSTN was positively associated with SMMI and ASMMI, in both models (Table 4). In addition, an inverse association was found between MSTN and CC using univariate logistic regression. The association remained significant in both models. ROC curve analysis of the relationship between MSTN and CC showed an AUC of 0.684 ($p < 0.001$) (Fig. 2A). The MSTN threshold with the best sensitivity (57%) and specificity (75%) was 1548 pg/mL.

3.6. Associations of MSTN with muscle mass and SO

Using linear regression analysis, MSTN was positively associated with SMMI at both timepoints (Table 5), but not with ASMMI (not shown). However, the association was no longer significant in Models 1 and 2. Changes in MSTN were positively associated with changes in ASMMI, but not with changes in SMMI (not shown). Here, the association remained significant in both models. In addition, MSTN/FM was positively associated with SMM/BW (Table 5). This association was observed in uni- and multivariate analysis at both timepoints. The prevalence of SO was significantly decreased after bariatric surgery, particularly with fewer individuals having class II SO (26% vs 79%, $p < 0.001$) (Supplementary Table 1). Using logistic regression analysis, we found an inverse association between MSTN and SO at T3 (Table 5), but not at T0 (not shown). The association was no longer significant in Model 1. We also found an inverse association between MSTN/FM and SO at T3 (Table 5) which remained significant in Model 1.

ROC curve analysis of the relationship between MSTN and SO at T3 showed an AUC of 0.835 (p 0.014) relationship between MSTN/FM and SO at T3 showed an AUC of (Fig. 2B). The MSTN threshold with the best sensitivity (98%) and 0.971 ($p < 0.001$) (Fig .2C). The MSTN/FM threshold with the best specificity (100%) was 4225 pg/mL. ROC curve analysis of the sensitivity (94%) and specificity (100%) was 8374 pg/mL/kg.

4. Discussion

Our study shows that circulating MSTN is positively correlated with muscle mass and strength in individuals with cancer or obesity. In addition, MSTN was inversely

associated with CC or SO. Taken together, our data suggest the potential use of MSTN as a biomarker of muscle mass and strength in individuals with cancer or obesity, as well as a screening tool for CC and SO.

4.1. MSTN as a biomarker of muscle mass

Our data reinforce previous observations of positive associations between MSTN and muscle mass in humans [10,11,35]. In addition, our study extends this observation to individuals with CC or individuals with obesity undergoing bariatric surgery. Indeed, among these, we also found a positive correlation between MSTN and muscle mass changes. In addition, MSTN was interestingly positively correlated with muscle strength in the two cohorts, which is relevant for clinical practice since low muscle strength is overtaking the role of low muscle mass as a principal determinant of sarcopenia [4,5]. However, the positive association of MSTN with muscle mass and strength seems at first counter-intuitive given that MSTN is an established negative regulator of muscle mass [14]. MSTN indeed induces myotubes atrophy in vitro [36]. Similarly, muscle overexpression or systemic administration of MSTN induce muscle atrophy in rodents whereas MSTN inhibition induce muscle hypertrophy [37e39]. However, the role of MSTN in muscle wasting in humans in unclear. As ours, other studies reported positive associations between MSTN and muscle mass in various muscle wasting conditions that differ in their mechanisms, including liver cirrhosis [10], chronic kidney disease [40], peritoneal dialysis or hemodialysis [41,42], older age [12], genetic muscle diseases [43], and bedrest due to hospitalization [44]. In addition, this positive association between MSTN and muscle mass seems independent of age, sex, and sarcopenia [45]. Taken together, these data suggest the potential use of circulating MSTN as a biomarker of muscle mass and strength in humans.

4.2. MSTN as a biomarker of CC

In our study, an inverse association between circulating MSTN and CC was observed even after adjustment for co-factors. The ROC curve analysis suggests that a MSTN cutoff value could be established for the prediction of CC in clinical practice. The association of MSTN, as a surrogate of muscle mass, with clinical variables such as involuntary weight loss [34], could facilitate the screening of CC. A timely diagnosis of CC is critical since this syndrome is associated with poorer outcomes [25].

However, measuring muscle mass in individuals with cancer remain challenging owing to limited accessibility and repeatability of imaging modalities or limited availability of simpler but indirect methods [46]. Therefore, identification of biomarkers of muscle mass would facilitate the screening of CC [46]. Our results suggest that MSTN could be one of these biomarkers and support the need for validation studies in larger cohorts.

4.3. MSTN as a biomarker of SO

To the best of our knowledge, our study investigated for the first time the association between circulating MSTN and SO in individuals undergoing bariatric surgery. We found that the prevalence of SO was reduced after bariatric surgery, despite muscle mass loss. In fact, bariatric surgery was associated with increased percentage muscle mass, that is an improved muscle mass to body mass ratio. Thus, the predominant loss of FM could explain the reduction in SO after bariatric surgery since SO is defined as a relative reduction of muscle mass in combination with excess adiposity [47]. In addition, muscle strength adjusted for muscle mass was also improved after bariatric surgery. Taken together, these findings suggest an improved body composition (i.e., increased muscle to fat ratio), improved muscle function (i.e., strength to muscle mass ratio), and, overall, improved muscle health after bariatric surgery despite muscle mass loss. The ROC curve analysis suggests that a MSTN cutoff value could be established for the prediction of SO in clinical practice. In addition, the observation that the correlations between MSTN and muscle mass were stronger after adjusting MSTN for adiposity suggests that the MSTN/FM ratio could be used as a marker of SO. Taken together, our results suggest that MSTN, alone or adjusted for adiposity, could be useful to monitor changes in muscle mass and screen for SO after bariatric surgery.

4.4. Rationale for decreased MSTN in muscle wasting conditions

Several hypotheses can be put forward to explain the decreased circulating MSTN in muscle wasting conditions. On the one hand, decreased MSTN could merely reflects decreased muscle mass since MSTN is mainly secreted by skeletal muscle [14], as observed for leptin and adipose tissue [48]. On the other hand, decreased circulating MSTN could result from decreased muscle MSTN expression, as we previously reported after bariatric surgery [33]. In this case, decreased MSTN expression and circulating levels could represent a counter-regulatory mechanism

to preserve muscle mass during acute caloric restriction [33,49]. However, this hypothesis could extend to other muscle wasting conditions. In neuromuscular diseases, MSTN receptor and signaling pathway are, counter-intuitively, downregulated and preclude good clinical efficacy of anti-myostatin approaches [50]. In various types of cancers, decreased circulating MSTN or MSTN pro-peptide levels which reflect decreased MSTN production were reported in individuals with low muscle mass [32,51]. These data together with ours suggest that circulating MSTN does not play a major pathogenic role in muscle wasting during CC or after bariatric surgery. On the contrary, decreased MSTN could even be interpreted as a protective down-regulatory mechanism to counteract muscle wasting.

4.5. Preanalytics and other factors influencing MSTN determination and interpretation

Several pre-analytical factors need to be taken into account when considering circulating MSTN levels for clinical decision-making. These factors can include age, gender, several common diseases, smoking, physical exercise, and fasting [52]. Contradictory results have been reported concerning the effect of age and gender on circulating MSTN levels. Earlier studies have reported an increase in circulating MSTN levels with age [53,54], whereas recent studies have shown no correlation between MSTN and age [55]. Similarly, some studies have reported higher circulating MSTN levels in men, while others have found higher levels in women, or no difference between genders [55]. Common diseases such as type 2 diabetes and insulin-resistance are associated with higher circulating MSTN levels [55]. In addition, active smoking [54] and exercise training decrease circulating MSTN levels [56]. Although overnight fasting is routinely recommended to minimize variability in laboratory tests [52], short-term fasting does not affect circulating MSTN [57]. As with many laboratory tests, these factors should be managed by appropriate reference limits for each population and standardized preparation of individuals before sampling [52,58]. Finally, circulating MSTN levels could differ between immunoassays that detect either mature MSTN only, or different forms of MSTN or cross-react with other TGF-Beta members (e.g., GDF-11) [46,55]. The immunoassay must therefore be carefully selected according to the specific use clinicians intend to make of it.

4.6. Limitations

Our study has several limitations. In both cohorts, we used an immunoassay that measures only mature MSTN levels. Our result could therefore differ from studies using other immunoassays [46]. In the ACTICA study, CT was available only at inclusion, precluding longitudinal muscle mass assessment using this gold standard

method. However, muscle mass indexes determined using BIA and CT correlated well. In the MYDIASECRET study, BIA could have overestimated muscle mass since obesity violates the constant hydration assumption [5,31]. In addition, the equations used to estimate muscle mass were not originally designed for individuals with cancer or obesity. However, they were established from data of individuals from both sexes, with a wide range of age and BMI [8,9]. In addition, our multivariate analyses were adjusted for age, gender and BMI (where applicable), factors likely to influence circulating MSTN levels and muscle mass. In this study, SO was defined using muscle mass alone while adding muscle strength is currently suggested [5]. However, none of the individuals of the MYDIASECRET study reached the cutoff values suggested for low muscle strength [5]. Indeed, individuals with obesity commonly have comparable or even higher absolute muscle mass and strength compared to lean individuals [4,5]. The strengths of our study include consistent findings in two very different populations, relatively large population of individuals with cancer, and longitudinal assessment in those with obesity undergoing bariatric surgery.

5. Conclusion

Our study shows that circulating MSTN mirrors muscle mass and strength in individuals with cancer or obesity. In individuals with obesity undergoing bariatric surgery, MSTN changes also mirror those of muscle mass. In addition, MSTN is inversely associated with CC and SO, especially after adjustment for fat mass in the latter case. Taken together, our data suggest the potential use of MSTN as a biomarker of muscle mass and strength in individuals with cancer or obesity, as well as a screening tool for CC and SO.

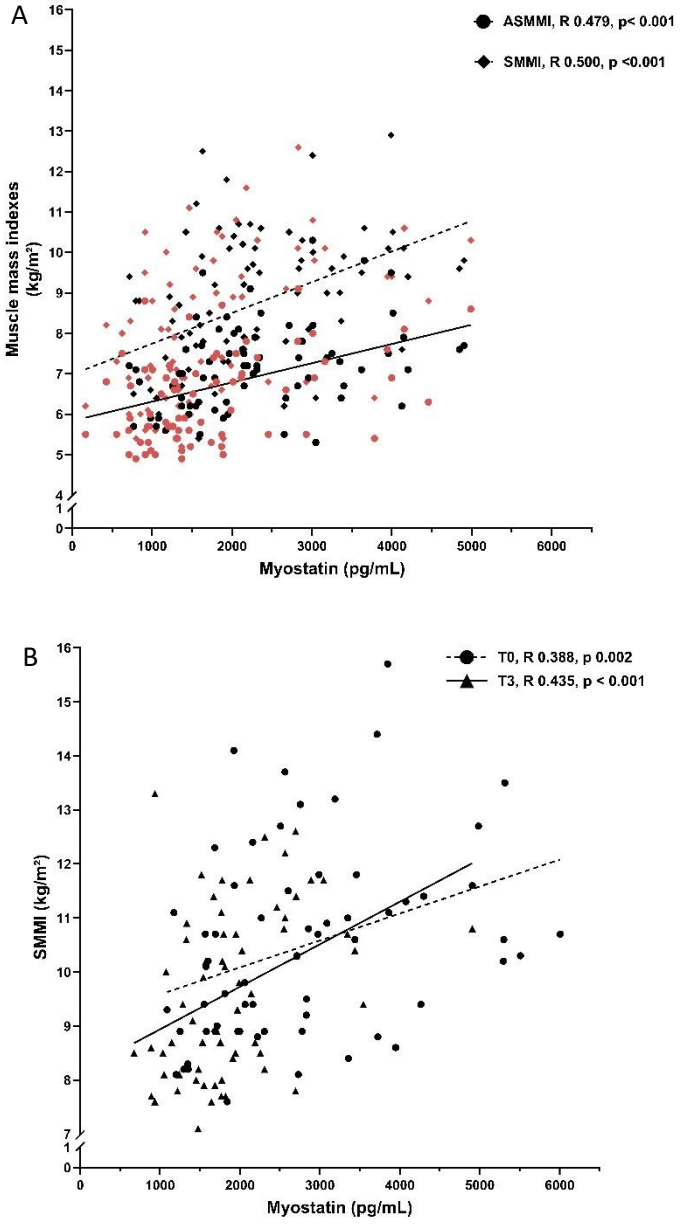

Figure 1. Relationships between MSTN and muscle mass. A. ACTICA study: correlation between MSTN and muscle mass indexes, ASMMI: circles and plain line, SMMI: squares and dotted line, NC individuals: black, CC individuals: red, n = 143, Spearman's correlation. **B.** MYDIASECRET study: correlation between MSTN and SMMI, T0: black circles and dotted line, T3: black triangles and plain line, n = 62, Spearman's correlation. ASMMI: appendicular skeletal muscle mass index, MSTN: myostatin; CC: with cancer cachexia; NC: without cancer cachexia; SMMI: skeletal muscle mass index, T0: before bariatric surgery, T3: after bariatric surgery.

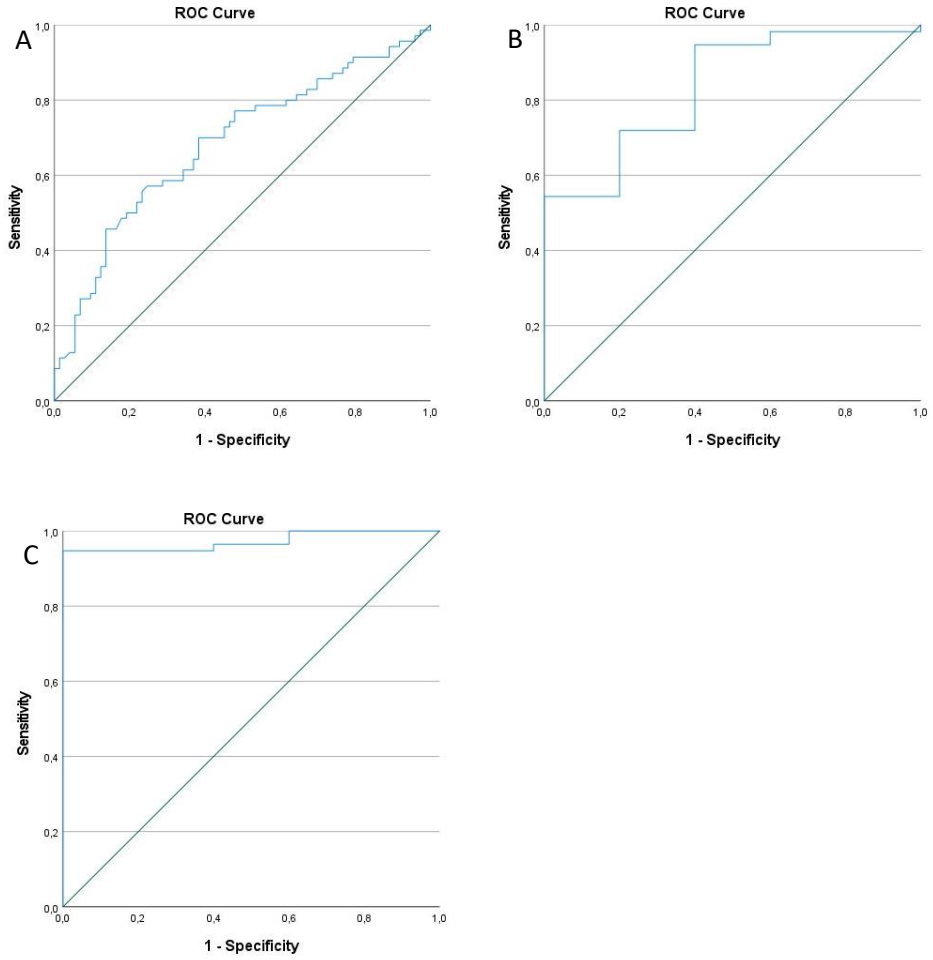

Figure 2. ROC curve analysis. A. ACTICA study: relationship between MSTN and CC. **B.** MYDIASECRET study: relationship between MSTN and SO after bariatric surgery. **C**. MYDIASECRET study: relationship between MSTN/FM and SO after bariatric surgery. MSTN: myostatin; CC: cancer cachexia; FM: fat mass; SO: sarcopenic obesity.

Table 1. Clinical characteristics and body composition in NC and CC individuals (ACTICA)

	Study population (n = 143)	**NC (n = 73)**	**CC (n = 70)**	***p* value (CC vs NC)**
General characteristics				
Male sex (n, %)	81 (57)	45 (62)	36 (51)	0.218
Age (years)	66 [39-84]	64 [38-82]	68 [40-86]	0.458
Colon cancer (n, %)	87 (61)	47 (64)	40 (57)	0.375
Anthropometry				
Body weight (kg)	72.0 [49.2-102.4]	75.7 [52.7-103.9]	64.2 [48.1-99.5]	<0.001
BMI (kg/m²)	24.9 [19.1-34.5]	26.6 [20.7-34.7]	23.5 [18.4-34.1]	<0.001
CT scan				
SMMI-CT (cm²/m²)	48.6 [35.2-69.1]	50.5 [37.9-69.1]	47.3 [33.0-67.6]	0.004
BIA				
SMM (kg)	25.2 [14.5-35.2]	25.8 [16.7-35.8]	22.8 [14.2-35.2]	0.018
SMM, percentage (%BW)	33.0 [24.4-44.7]	33.0 [23.1-43.3]	33.4 [26.1-45.3]	0.541
SMMI (kg/m²)	8.6 [5.8-11.2]	9.0 [6.3-12.0]	8.0 [5.5-10.9]	0.004
ASMM (kg)	19.3 [13.1-27.1]	20.4 [14.1-28.1]	18.0 [12.5-26.2]	0.002
ASMM, percentage (%BW)	26.6 [22.0-31.9]	26.6 [31.3-31.5]	26.5 [23.4-32.0]	0.663
ASMMI (kg/m²)	6.9 [5.1-8.8]	7.2 [5.6-9.5]	6.2 [5.0-8.6]	<0.001
FM (kg)	18.5 [10.1-30.1]	20.8 [12.3-31.0]	16.8 [8.9-27.3]	0.001
FM, percentage (%BW)	26.5 [15.7-36.1]	27.6 [18.4-38.7]	26.3 [14.5-33.8]	0.117
FMI (kg/m²)	6.6 [3.3-10.6]	7.4 [4.4-11.0]	6.1 [3.2-9.7]	0.001
Muscle strength				
HGS (kg)	30 [18-52]	38 [20-52]	28 [14-45]	<0.001
MSTN plasma levels				
MSTN (pg/mL)	1811 [718-4144]	2149 [829-4165]	1434 [594-4070]	<0.001

Data expressed as medians [95%CI] or numbers (frequencies). Abbreviations: ASMM: appendicular skeletal muscle mass; ASMMI: appendicular skeletal muscle mass index; BIA: bioelectrical impedance analysis; BMI: body mass index; BW: body weight; CC: with cancer cachexia; FM: fat mass; FMI: fat mass index; HGS: handgrip strength; MSTN: myostatin; NC: without cancer cachexia; SMMI-CT: skeletal muscle mass index using CT-scan; SMM: skeletal muscle mass; SMMI: skeletal muscle mass index; %BW: percentage of body weight. Differences assessed using the Mann-Whitney U test, Chi-carré test. *p* statistically significant < 0.05.

Table 2. Clinical characteristics and body composition before (T0) and 3 months (T3) after bariatric surgery (MYDIASECRET)

Study population (n= 62)	T0	T3	*p* value
General characteristics			
Male sex (n, %)	26 (42)	-	-
Age (years)	44 [25-62]	-	-
Sleeve gastrectomy (n, %)	39 (63)	-	-
Anthropometry			
Body weight (kg)	124.8 [96.6-168.2]	103.4 [75.8-141.1]	<0.001
BMI (kg/m²)	43 [40-55]	35 [29-47]	<0.001
BIA			
SMM (kg)	28.1 [20.4-46.6]	26.0 [19.2-42.6]	<0.001
SMM, percentage (%BW)	22.9 [18.6-32.7]	25.9 [20.8-37.5]	<0.001
SMMI (kg/m²)	10.3 [8.1-14.0]	9.5 [7.6-12.5]	<0.001
ASMM (kg)	25.9 [19.1-37.9]	22.8 [16.7-33.5]	<0.001
ASMM, percentage (%BW)	20.8 [18.8-25.2]	21.9 [19.7-27.5]	<0.001
ASMMI (kg/m²)	8.9 [7.8-11.3]	7.9 [6.7-9.7]	<0.001
FM (kg)	56.2 [42.2-78.2]	41.6 [27.4-62.2]	<0.001
FM, percentage (%BW)	48.4 [34.8-53.8]	42.1 [27.3-49.6]	<0.001
FMI (kg/m²)	20.5 [13.4-26.3]	14.9 [8.2-20.1]	<0.001
Muscle strength			
HGS (kg)	34 [20-56]	32 [18-56]	0.005
HGS (kg) adjusted to BMI (kg/m²)	79 [46-133]	90 [51-173]	<0.001
HGS (kg) adjusted to ASMMI (kg/m²)	367 [241-579]	404 [231-647]	<0.001
MSTN plasma levels			
MSTN (pg/mL)	2582 [1209-5311]	1773 [896-3424]	<0.001

Data expressed as medians [95%CI] or numbers (frequencies). Abbreviations: ASMM: appendicular skeletal muscle mass; ASMMI: appendicular skeletal muscle mass index; BIA: bioelectrical impedance; BMI: body mass index; BW: body weight; FM: fat mass; FM: fat mass; FMI: fat mass index; SMM: skeletal muscle mass; HGS: handgrip strength; MSTN: myostatin; SMMI: skeletal muscle mass index; %BW: percentage of body weight. Differences assessed using the Wilcoxon signed-rank test. *p* statistically significant < 0.05.

Table 3. Correlations estimate with myostatin plasma levels (Spearman's Rank correlation coefficient)

		SMMI-CT (cm^2/m^2)	SMMI (kg/m^2)	ASMMI (kg/m^2)	HGS (kg)	SMM/BW (kg/kg)
ACTICA study						
MSTN (pg/mL), study population (n = 143)	r Spearman	0.391	0.500	0.479	0.495	-
	p value	<0.001	<0.001	<0.001	<0.001	-
MSTN (pg/mL), CC patients (n = 70)	r Spearman	0.247	0.468	0.383	0.330	-
	p value	0.046	<0.001	0.001	0.005	-
MSTN (pg/mL), NC patients (n = 73)	r Spearman	0.336	0.398	0.406	0.478	-
	p value	0.007	<0.001	<0.001	<0.001	-
MYDIASECRET study (n = 62)						
MSTN-T0 (pg/mL)	r Spearman	-	0.388[$]	0.282[$]	0.337[$]	0.428[$]
	p value	-	0.002	0.026	0.007	<0.001
MSTN-T3 (pg/mL)	r Spearman	-	0.435[$]	0.238[$]	0.313[$]	0.455[$]
	p value	-	<0.001	0.062	0.013	<0.001
MSTN, T0-T3 (pg/mL)	r Spearman	-	0.309*	0.295*	- 0.016*	-
	p value	-	0.014	0.020	0.905	-
MSTN, T0-T3 (%T0)	r Spearman	-	0.340*	0.169*	- 0.016*	-
	p value	-	0.007	0.189	0.904	-
MSTN/FM-T0 (pg/mL)	r Spearman	-	-	-	-	0.610[$]
	p value	-	-	-	-	<0.001
MSTN/FM-T3 (pg/mL)	r Spearman	-	-	-	-	0.726[$]
	p value	-	-	-	-	<0.001

Abbreviations: ASMMI: appendicular skeletal muscle mass index; BW: body weight; CC: with cancer cachexia; HGS: handgrip strength; NC: without cancer cachexia; SMM: skeletal muscle mass; SMMI: skeletal muscle mass index. Correlations assessed using Spearman's Rank correlation. T0 and T3 refer to before and after surgery, respectively. [$] T0 or T3, as appropriate in the MYDIASECRET study. * Muscle indexes or HGS expressed as absolute changes (T0-T3, pg/mL) and percentage changes from baseline (%T0), as appropriate.

Table 4. Associations between myostatin, muscle mass indexes, and cancer cachexia (ACTICA, uni- and multivariate analysis)

Dependent variable	Independent variables	Unadjusted OR/estimate (95%CI)	Unadjusted *p* value	Model 1 Adjusted OR/estimate (95%CI)	Adjusted *p* value	Model 2 Adjusted OR/estimate (95%CI)	Adjusted *p* value
Linear regression analysis							
SMMI	**MSTN**	7.59.10E-4 (5.11.10E-4-10.06.10E-4)	<0.001	3.25.10E-4 (1.55.10E-4 -4.96.10E-4)	< 0.001	1.60.10E-4 (0.27.10E-4 -2.93.10E-4)	0.019
ASMMI	**MSTN**	4.75.10E-4 (3.12.10E-4 - 6.38.10E-4)	<0.001	2.66.10E-4 (1.26.10E-4 -4.06.10E-4)	< 0.001	0.83.10E-4 (0.18.10E-4 -1.49.10E-4)	0.013
Logistic regression analysis							
CC	**MSTN**	0.999443 (0.999089-0.999797)	0.002	0.999464 (0.999089-0.999840)	0.005	0.999577 (0.999182-0.999972)	0.036

Abbreviations: 95CI, 95% confidence interval; ASMMI: appendicular skeletal muscle mass index; BMI: body mass index; CC: cancer cachexia; MSTN: myostatin plasma levels; OR: odds ratio; SMMI: skeletal muscle mass index. Model 1 adjusted for age and sex. Model 2 adjusted for age, sex, and BMI.

Table 5. Associations between myostatin, muscle mass indexes, and sarcopenic obesity (MYDIASECRET, uni- and multivariate analysis)

Dependent variable	Independent variables	Unadjusted OR/estimate (95%CI)	*p* value	Model 1 Adjusted OR/estimate (95%CI)	*p* value	Model 2 Adjusted OR/estimate (95%CI)	*p* value
Linear regression analysis							
SMMI-T0	**MSTN-T0**	5.04.10E-4 (1.58.10E-4 - 8.50.10E-4)	0.005	1.14.10E-4 (-1.92.10E-4 - 4.21.10E-4)	0.459	1.58.10E-4 (-1.24.10E-4- 4.40.10E-4)	0.267
SMMI-T3	**MSTN-T3**	7.87.10E-4 (3.00.10E-4 - 12.74.10E-4)	0.002	1.39.10E-4 (-2.05.10E-4 - 4.84.10E-4)	0.421	1.96.10E-4 (-0.99.10E - 4-4.91.10E-4)	0.189
ASMMI, T0-T3	**MSTN, T0-T3**	0.0001.74 .10E-4 (0.00.10E-4 - 3.48.10E-4)	0.040	1.87.10E-4 (0.04.10E-4 - 3.70.10E-4)	0.046	1.90.10E-4 (0.07.10E-4 -3.73.10E-4)	0.043
ASMMI, %T0	**MSTN, T0-T3**	1.74.10E-4 (0.10E-4 - 3.48.10E-4)	0.040	1.8710E-4 (0.0410E-4 -3.7010E-4)	0.046	1.9010E-4 (0.0710E-4 -3.7310E-4)	0.043
SMM/BW-T0	**MSTN/FM-T0**	9.61.10E-4 (6.15.10E-4 -13.07.10E-4)	<0.001	4.23.10E-4 (1.35.10E-4 -7.10.10E-4)	0.005	-	-
SMM/BW-T3	**MSTN/FM-T3**	14.55.10E-4 (1.19.10E-4 - 17.9.10E-4)	<0.001	8.34.10E-4 (5.64.10E-4 -1.03.10E-4)	<0.001	-	-
Logistic regression analysis							
SO-T3	**MSTN**	0.999776 (0.997680-0.999874)	0.029	0.998997 (0.997830-1.000165)	0.092	-	-
SO-T3	**MSTN/FM-T3**	0.998768 (0.997677-0.999860)	0.027	0.998417 (0.996971-0.999864)	0.032	-	-
SO-T3, class 2	**MSTN/FM-T3**	0.999704 (0.999411-0.999997)	0.003	0.999479 (0.999114-0.999845)	0.005	-	-

Abbreviations: 95CI, 95% confidence interval; ASMMI: appendicular skeletal muscle mass index; BMI: body mass index; BW: body weight; MSTN: myostatin plasma levels; OR: odds ratio; SMMI: skeletal muscle mass index; SO: sarcopenic obesity. Model 1 adjusted for age and sex. Model 2 adjusted for age, sex, and BMI.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.clnu.2024.05.046.

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Conflict of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

Authors contribution

Laura Orioli: Conceptualization, Methodology, Formal analysis, Investigation, Data curation, Writing, Visualization, Funding acquisition. **Sofia Samaras**: Methodology, Software, Formal analysis, Investigation. **Kiswendsida Sawadogo**: Formal analysis. **Pascale Lause**: Resources. **Marie de Barsy**: Investigation. **Yannick Deswysen**: resources. **Benoit Navez:** resources. **Jean-Paul Thissen:** Conceptualization, Methodology, Resources, Writing, Visualization, Supervision, Project administration, Funding acquisition. **Audrey Loumaye**: Conceptualization, Methodology, Investigation, Writing, Visualization, Supervision, Project administration.

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V. GENERAL DISCUSSION

1. General overview

In this work, we used three complementary approaches based on muscle samples, primary human myotubes, and plasma samples to identify myokines that are regulated by bariatric surgery and that may contribute to changes in glucose homeostasis and muscle mass after bariatric surgery. All data (e.g., body composition, glucose homeostasis) and samples were collected before and 3 months after bariatric surgery during a prospective, longitudinal, and interventional study called the MYDIASECRET study (Orioli et al., 2023). We used two complementary approaches, literature-based (targeted study) and RNA-seq-based (untargeted study), to identify myokines regulated by bariatric surgery in both muscle samples (*muscle* study) and primary human myotubes (*in vitro* study). The combined analysis of myokine expression in muscle samples and primary human myotubes was necessary because the muscle tissue is made of many cell populations in addition to myogenic cells. Therefore, changes observed in muscle tissue might not result from changes in myogenic cells. We also used multiplex immunoassays or ELISA on plasma/serum samples (*plasma/serum* study) to identify myokines regulated by bariatric surgery at the circulating level. Indeed, changes in myokine expression observed in vastus lateralis muscle samples may not be representative of changes occurring in other muscles.

Thus, our *muscle* study may have missed myokines of interest. On the other hand, many myokines are not specific of skeletal muscle. Therefore, changes in muscle expression of myokines may not be reflected in changes in circulating levels, although these are critical for interorgan crosstalk. Finally, we looked for relationships between changes in myokines and changes in glucose homeostasis or muscle mass using linear regression analysis. Taken together, our data show that bariatric surgery alters the expression of some myokines at the muscle and circulating levels and suggest that some myokines may contribute to changes in glucose homeostasis and muscle mass after bariatric surgery, as we found significant relationships between changes in these myokines and changes in glucose homeostasis or muscle mass.

The main changes in myokine expression and circulating levels observed in our work are summarized in table 1.

Table 1. Changes in myokines expression and circulating levels after bariatric surgery across our studies

	Changes in gene expression				Changes in circulating levels	
Myokine	**Muscle samples**		**Primary myotubes**		**Plasma/serum**	
	RNA-seq (n=12, FDR≤0.10)	**RT-qPCR** (n=39, p≤0.05)	**RNA-seq** (n=12, FDR≤0.10)	**RT-qPCR** (n=15, p≤0.05)	**ELISA** (n=62, p≤0.05)	**MIA** (n=62, p≤0.05)
BDNF	↔	↑	↔	↔	↓	N/A
Fractalkine	↑	↑	N/E	N/E	↑	N/A
MSTN	↓	↓	↔	↔	↓	N/A
Irisin	↔	↓	↔	↑	N/A	↓
ADAMTS9	↑	↑	↔	↔	N/A	N/A
ANG	↔	↑	↔	↔	N/A	N/A
IL6	↔	↑	↔	↔	N/A	↔
TNFRSF11B	↔	↑	↔	↔	N/A	N/A
TRIM72	↔	↑	↔	↔	N/A	N/A
SFRP4	↓	↓	↔	↔	N/A	N/A
APLN	↔	↔	↔	↔	N/A	↓
XYLT1	↑	↑	↔	↔	N/A	N/A
LGR5	↑	↑	↔	N/E	N/A	N/A
SPINK5	↑	↑	N/E	N/E	N/A	N/A
WNT4	↔	↔	↔	↑	N/A	N/A
SPARC	↓	↔	N/A	N/A	N/A	↓
FSTL1	↔	↔	N/A	N/A	N/A	↓
EPO	N/A	N/A	N/A	N/A	N/A	↓
FABP3	↔	↔	N/A	N/A	N/A	↑
Musclin	N/A	N/A	N/A	N/A	N/A	↓
FGF21	↔	N/E	N/A	N/A	N/A	↑
LIF	↔	N/E	N/A	N/A	N/A	↓

Abbreviations: ADAMTS9: ADAM metallopeptidase with thrombospondin type 1 motif 9; ANG: angiogenin; APLN: apelin; BDNF: brain-derived neurotrophic factor; EPO: erythropoietin; FABP3: fatty acid-binding protein 3; FGF21: fibroblast growth factor 21; FSTL1: follistatin related protein 1; IL: interleukin; LGR5: leucine-rich repeat-containing G-protein coupled receptor 5; LIF: leukemia inhibitory factor; MIA : multiplex immunoassays, N/A: not available, N/E: not expressed; TRIM72: tripartite motif containing 72 (or MG53, mitsugumin); TNFRSF11B: osteoprotegerin; SFRP4: secreted frizzled-related protein 4 ; SPARC: secreted protein acidic and rich in cysteine (osteonectin); SPINK5: serine protease inhibitor Kazal-type 5; XYLT1: xylosyltransferase 1.

2. Changes in myokines susceptible to contribute to improved glucose homeostasis after bariatric surgery

Among the myokines significantly regulated by bariatric surgery in our work, several may contribute to improved glucose homeostasis.

In the *muscle* study (muscle samples), we identified four myokines that may contribute to improved glucose homeostasis after bariatric surgery: BDNF in the targeted study and XYLT1, LGR5, and SPINK5 in the untargeted study (Orioli et al., 2023). Changes in the expression of these myokines are associated with improved insulin sensitivity, as assessed by the HOMA test. It is worth noting that XYLT1, LGR5, and SPINK5 have never been studied in the context of bariatric surgery. Taken together, our results suggest that these myokines may play a role in improved glucose homeostasis after bariatric surgery, as we hypothesized.

BDNF is a contraction-regulated myokine that is upregulated in human muscle after exercise (Fulgenzi et al., 2020; Matthews et al., 2009). We show an upregulation of BDNF in muscle after bariatric surgery, with an amplitude comparable to that observed after acute exercise in vastus lateralis samples (Matthews et al., 2009). In contrast, circulating BDNF levels were decreased after bariatric surgery in our study, as reported by others (Muñoz-Rodríguez et al., 2018; Merhi et al., 2009). Muscle BDNF is thought to act locally to regulate

positively muscle insulin sensitivity, whereas circulating BDNF is mainly released from platelets and the brain and enhances glucose-stimulated insulin secretion (Fulgenzi et al., 2020). The discrepancy between changes in circulating levels and gene expression in our study suggests that some myokines such as BDNF may act in an autocrine/paracrine manner rather than an endocrine manner. Consequently, changes in mRNA levels do not necessarily translate into changes in circulating levels (Raschke et al., 2013). Nevertheless, changes in BDNF reported in our study, although occurring in opposite directions at the gene and circulating levels, may still contribute to improved glucose homeostasis after bariatric surgery (i.e., improved insulin sensitivity and reduced compensatory hyperinsulinemia reflecting improved insulin sensitivity) (Orioli et al., 2023). We are not aware of any human BDNF analogs or agonists of its receptors. Interestingly, treatment with recombinant BDNF has been shown to improve glucose homeostasis in rodents, demonstrating its therapeutic potential (Fulgenzi et al., 2020).

We have identified XYLT1, LGR5 and SPINK5 as putative myokines that may contribute to improved glucose homeostasis, particularly insulin sensitivity, after bariatric surgery. XYLT1 is expressed in many tissues, including pancreas, muscle, and adipose tissue, and encodes xylosyltransferase 1, which is involved in the biosynthesis of extracellular matrix glycosaminoglycan chains (Uhlén et al., 2019). LGR5 is highly expressed in

skeletal muscle and encodes the leucine-rich repeat-containing G protein-coupled receptor 5 involved in the canonical Wnt signaling pathway associated with myogenic differentiation (Uhlén et al., 2019; Bernardi et al., 2011). SPINK5 encodes serine peptidase inhibitor Kazal type 5, which is a serine protease inhibitor involved in anti-inflammatory protection (Uhlén et al., 2019). These myokines may therefore be involved in extra-cellular matrix remodeling, improved myogenesis and decreased inflammation in muscle after bariatric surgery. Few data are available on the involvement of these myokines in glucose homeostasis. However, XYLT1 and LGR5 variants have been reported to be associated with type 2 diabetes in genome-wide association studies, suggesting a link between these three myokines and glucose homeostasis (Buniello et al., 2019; Zeggini et al., 2008; Xue et al., 2018). Further studies are clearly needed to characterize the role these myokines in glucose homeostasis.

We expected to find associations between changes in some myokines known to regulate glucose homeostasis (e.g., fractalkine, myostatin, IL-6) and changes in the HOMA test after bariatric surgery. The fact that we did not find an association between improved glucose homeostasis after bariatric surgery and these myokines may be related (among several possibilities) to the technique used to assess glucose homeostasis, namely the HOMA test (see infra).

In the *in vitro* study (primary human myotubes), we identified two myokines, namely irisin (encoded by *FNDC5*) and WNT4, that may contribute to improved glucose homeostasis after bariatric surgery. As we hypothesized, bariatric surgery alters the expression of myokines in primary human myotubes of people with obesity undergoing bariatric surgery. Based on the literature, irisin and WNT4 could contribute to improved glucose homeostasis after bariatric surgery. Specifically, *FNDC5*, which encodes irisin, was upregulated in myotubes after bariatric surgery in our targeted study, whereas, using RNA-seq, we have identified WNT4 as a novel bariatric surgery-regulated myokine. Irisin is a contraction-regulated myokine, which increases glucose uptake and fatty acid oxidation in muscle cells, thereby exerting anti-diabetic effects (Lee et al., 2015; Carson, 2017; Xin et al., 2016). In addition, recombinant irisin protects human beta cells from palmitate-induced apoptosis (Natalicchio et al., 2017). These data suggest that upregulation of irisin in myotubes after bariatric surgery may positively contribute to muscle glucose and lipid metabolism, and potentially to beta cell function through a muscle-pancreas crosstalk. However, we and others have previously shown that *FDNC5* expression is decreased in muscle samples after bariatric surgery (Huh et al., 2012; Orioli et al., 2023). Overall, current experimental data on irisin suggests its potential for therapeutic purposes, especially in type 2 diabetes and obesity. However, considerable uncertainty remains regarding the reliability and accuracy of methods used for the dosage of circulating

irisin, although irisin has been detected and quantified in human serum by tandem mass spectrometry (Flori et al., 2021; Marrano et al., 2021; Jedrychowski et al., 2015). The lack of a gold standard quantification method can partially explain the discrepancies reported by different research groups, the unclear pattern of changes in circulating irisin after bariatric surgery, and the cautious approach of pharmaceutical companies to this myokine which shares some metabolic actions with incretin hormones (e.g., stimulation of insulin biosynthesis, accretion of beta cell mass in rodents) (Marrano et al., 2021).

WNT4 was also upregulated in myotubes of people with obesity after bariatric surgery. WNT4 is involved in myogenic proliferation and differentiation of skeletal muscle (Bernardi et al., 2011). Interestingly, overexpression of WNT4 in muscle cells causes muscle hypertrophy through downregulation of myostatin expression (Bernardi et al., 2011). Although myostatin was not downregulated in our myotubes, we and others have previously shown that myostatin expression is downregulated in muscle after bariatric surgery (Orioli et al., 2023; Orioli et al., 2024; Milan et al., 2004). In addition, WNT4 is involved in the functional maturation of human islets (Zhang et al., 2021). Therefore, changes at the muscle level could potentially contribute to a muscle-pancreas crosstalk after bariatric surgery. However, WNT4 is more likely to act in a paracrine manner, as it is not detected in blood (https://www.proteinatlas.org). More broadly, the Wnt signaling pathway is an ancient and evolutionarily

conserved pathway that regulates virtually every aspect of embryonic development and controls homeostatic self-renewal in many adult tissues (Komiya et al., 2008; Clevers, 2006). The Wnts are secreted glycoproteins and two major signaling pathways have been identified including a canonical or Wnt/β-catenin dependent pathway and the non-canonical or β-catenin-independent pathway (Komiya et al., 2008). Somatic mutations in the Wnt pathway are associated with cancer of the intestine and diverse tissues (e.g., leukemia), underlining a potential limitation of the Wnt pathway activation for therapeutic purposes. Both small-molecule inhibitors and agonists of the Wnt pathway, such as small-molecule inhibitors of GSK-3 (glycogen synthase kinase-3), have become available (Clevers, 2006). To our knowledge, their metabolic effects in obesity and type 2 diabetes are unknown. Due to the small number of individuals in whom myotubes were collected in our study, we did not assess the relationships between changes in myokines (irisin and WNT4) and changes in the HOMA test.

We and others identified changes in the circulating levels of some myokines, including BDNF, SPARC, APLN and Metrnl, which are associated with improved glucose homeostasis after bariatric surgery (Orioli et al., 2023; Lee et al., 2014; Krist et al., 2013; Soriguer et al., 2009; Heinonen et al., 2005; Pellitero et al., 2018). In our *plasma* study, we extend the observation to other circulating myokines including LIF, FSLT-1, and FABP3, and we also found an association with

APLN. Overall, LIF, FSLT-1, APLN and FABP3 increase glucose uptake in muscle cells which is expected to associate with muscle insulin sensitivity (Brandt et al., 2015; Lee et al., 2017; Kusudo et al., 2011; Yue et al., 2010; Dray et al., 2008; Attané et al. 2012). In addition, muscle FABP3 expression is upregulated during fatty acid exposure, which suggests that FABP3 may protect muscle cells from lipotoxicity (Tunstall et al., 2002; Furuhashi et al., 2008; Kusudo et al., 2011). However, LIF, FSTL-1 and APLN were decreased after bariatric surgery and were either not associated with insulin sensitivity (LIF, FSTL-1), or negatively associated with improved insulin sensitivity (APLN). Decreased FSTL-1 and LIF were also positively associated with decreased HOMA-B, suggesting that these myokines may be involved in a muscle-pancreas crosstalk after bariatric surgery. However, we hypothesize that this association rather reflects a coordinated decrease in circulating factors associated with decreased low-grade adipose tissue inflammation after bariatric surgery. Decreased beta cell compensation (HOMA-B) in this context reflects improved insulin sensitivity (HOMA-S). On the other hand, FABP3 was increased after bariatric surgery and positively associated with increased hyperbolic product (HOMA-BxS), which shows an overall improved beta cell function adjusted for insulin sensitivity. Increased FABP3 can be interpreted as a mechanism aimed at preventing lipotoxicity in muscle level since adipose tissue lipolysis is increased early after bariatric surgery (Gancheva et al., 2019). In conclusion, our study on plasma samples shows that bariatric

surgery is associated with changes in circulating levels of many myokines using multiplex immunoassays, as we hypothesized. Thus, changes in some of myokines, including BDNF (see above), LIF, FSLT-1, APLN, and FABP3, are associated with changes in glucose homeostasis, as we hypothesized.

3. Changes in myokines susceptible to contribute to changes in muscle mass after bariatric surgery

Based on the analysis of muscle and plasma samples, we identified three myokines, namely myostatin, SPARC and FGF-21, which are associated with changes in muscle mass after bariatric surgery.

In the *muscle* study (muscle samples), we observed decreased muscle expression of myostatin (Orioli et al., 2023). This decrease was associated with decreased circulating levels of myostatin using ELISA on plasma samples (Orioli et al., 2023). Furthermore, circulating myostatin was positively correlated with muscle mass and strength in people with obesity, whereas myostatin was inversely associated with sarcopenic obesity (Orioli et al., 2024). Taken together, our data suggest the potential use of myostatin as a biomarker of muscle mass and strength in people with obesity and as a screening tool for sarcopenic obesity (Orioli et al., 2024). Our data reinforce previous observations of a positive association between circulating myostatin levels and muscle mass in humans (Alexopoulos et al., 2021; Nishikawa et al., 2022; Kurose et al., 2021).

In addition, changes in myostatin levels after bariatric surgery were also positively correlated with changes in muscle mass. Interestingly, myostatin was positively correlated with muscle strength, which is relevant to clinical practice because low muscle strength is overtaking the role of low muscle mass as a major determinant of sarcopenia (Cruz-Jentoft et al., 2019; Donini et al., 2022). In our published manuscript, we also report a positive association between myostatin and muscle mass in people with cancer cachexia from a previous study lead in our lab (Orioli et al., 2024), suggesting the potential use of myostatin as a biomarker of muscle mass in muscle wasting diseases with different etiologies.

To the best of our knowledge, our study investigated for the first time the association between circulating myostatin and sarcopenic obesity in people undergoing bariatric surgery (Orioli et al., 2024). Sarcopenic obesity is a distinct phenotype of obesity which associates both excessive fat and sarcopenia, at any age (Donini et al., 2022). Sarcopenic obesity is considered a secondary form of sarcopenia, as opposed to age-related sarcopenia, which is considered primary (Donini et al., 2022; Cruz-Jentof et al., 2019). In addition, the diagnosis of sarcopenic obesity requires a low muscle mass relative to body weight, as opposed to age-related sarcopenia, which requires a low muscle mass index (Donini et al., 2022; Cruz-Jentof et al., 2019). We found that the prevalence of sarcopenic obesity was reduced after bariatric surgery, despite muscle mass

loss (Orioli et al., 2024). In fact, bariatric surgery was associated with increased percentage muscle mass that is an improved muscle mass to body mass ratio. Thus, the predominant loss of fat mass could explain the reduction in sarcopenic obesity after bariatric surgery. In addition, muscle strength adjusted for muscle mass was also improved after bariatric surgery. Taken together, these findings suggest an improved body composition (i.e., increased muscle to fat ratio), improved muscle function (i.e., strength to muscle mass ratio), and, overall, improved muscle health after bariatric surgery despite muscle mass loss. The ROC curve analysis suggests that a myostatin cutoff value could be established for the prediction of sarcopenic obesity in clinical practice (Orioli et al, 2024).

The positive association of myostatin with muscle mass and strength seems at first counter-intuitive given that myostatin is an established negative regulator of muscle mass that avoids pathological muscle hypertrophy (Lee, 2021). Myostatin indeed induces myotubes atrophy *in vitro* (Trendelenburg et al., 2009). Similarly, muscle overexpression or systemic administration of myostatin induces muscle atrophy in rodents whereas myostatin inhibition induces muscle hypertrophy (Durieux et al., 2007; Zimmers et al., 2002; McPherron et al., 1997). However, the role of myostatin in muscle wasting in humans is unclear. As our, other studies reported positive associations between myostatin and muscle mass in various muscle wasting conditions that differ

in their mechanisms, including liver cirrhosis (Alexopoulos et al., 2021), chronic kidney disease (Zhou et al., 2021), peritoneal dialysis or hemodialysis (Yamada et al., 2016; Delanaye et al., 2019), older age (Arrieta et al., 2019), genetic muscle diseases (Burch et al., 2017), and bedrest due to hospitalization (Echeverria et al., 2021). In addition, this positive association between myostatin and muscle mass seems independent of age, sex, and sarcopenia (Bergen et al., 2015). Several hypotheses can be put forward to explain the decreased circulating myostatin in muscle wasting conditions. On the one hand, decreased myostatin could merely reflect decreased muscle mass since myostatin is mainly secreted by skeletal muscle (Lee et al., 2021), as observed for leptin and fat mass (Considine et al., 1996). On the other hand, decreased circulating myostatin could result from decreased muscle myostatin expression, as we previously reported after bariatric surgery (Orioli et al., 2023). Downregulation of myostatin expression and circulating levels could represent a counter-regulatory mechanism to preserve muscle mass during acute caloric restriction (Orioli et al., 2023; Milan et al., 2004). This hypothesis could extend to other muscle wasting conditions. In neuromuscular diseases, myostatin receptor and signaling pathway are, counter-intuitively, downregulated and preclude good clinical efficacy of anti-myostatin approaches (Mariot et al., 2017). In various types of cancers, decreased circulating myostatin or myostatin pro-peptide levels which reflect decreased myostatin production were also reported in people with low muscle mass (Loumaye et al., 2015: Choi

et al., 2020). These data together with ours suggest that circulating myostatin does not play a major pathogenic role in muscle mass loss after bariatric surgery.

Our demonstration of an association between myostatin and muscle mass has several limitations. First, the multivariate analyses were adjusted for age, gender and BMI (where applicable), factors likely to influence circulating myostatin levels and muscle mass. In fact, contradictory results have been reported concerning the effect of age and gender on circulating myostatin levels. Earlier studies have reported an increase in circulating myostatin levels with age (Yarasheski et al., 2002; Szulc et al., 2012), whereas recent studies have shown no correlation between myostatin and age (Baczek et al., 2020). Similarly, some studies have reported higher circulating myostatin levels in men, while others have found higher levels in women, or no difference between genders (Baczek et al., 2020). Common diseases such as type 2 diabetes and insulin-resistance are associated with higher circulating myostatin levels (Baczek et al., 2020). Second, exercise training, which probably increases after weight loss, could also decrease circulating myostatin levels (Hittel et al., 2010). Third, although overnight fasting is routinely recommended to minimize variability in laboratory tests (Irjala et al., 1998), short-term fasting does not affect circulating myostatin (Larsen et al., 2006). Finally, we defined sarcopenic obesity using muscle mass alone while adding muscle strength

is currently suggested (Donini et al., 2022). However, none of the participants reached the cutoff values suggested for low muscle strength (not shown) (Donini et al., 2022). The strengths of our study include consistent findings in two different cohorts (obesity and cancer cachexia), and the longitudinal assessment in those with obesity undergoing bariatric surgery.

In conclusion, we show that circulating myostatin mirrors muscle mass and strength in people with obesity. Changes in myostatin also mirror those of muscle mass after bariatric surgery. In addition, myostatin is inversely associated with sarcopenic obesity. These data suggest the potential use of myostatin as a biomarker of muscle mass and strength in people with obesity, as well as a screening tool for sarcopenic obesity.

Because of this association between myostatin and muscle mass, we looked for associations between changes in muscle mass and circulating levels of other myokines quantified by multiplex immunoassays. We found significant associations between changes in two myokines, SPARC and FGF21, and changes in muscle mass, extending our observation of a relationship between myostatin and muscle mass. SPARC is expressed in satellite cells and stimulates myogenesis (Jørgensen et al., 2009; Cho et al., 2000; Nakamura et al., 2012). SPARC knock-out mice have decreased muscle mass and strength, suggesting that SPARC deficiency leads to sarcopenia (Bradshaw et al., 2003; Nakamura et al., 2012; Jørgensen et al., 2017). Increased circulating SPARC levels

in people with sarcopenia suggest either SPARC resistance, or a mechanism to counter muscle wasting (Kwak et al., 2018). Thus, decreased circulating SPARC levels after bariatric surgery could be involved in muscle mass loss. Basal FGF21 expression is very low in healthy muscle, but cellular stressors including fasting induce FGF-21 release from muscle (Oost et al., 2019; Vandanmagsar et al., 2016). FGF21 knockout mice are protected against starvation-induced muscle atrophy, indicating that FGF21 contributes to muscle atrophy in the context of cellular stress (Oost et al., 2019). Consistent with this, people with sarcopenia have higher circulating FGF21 levels compared to controls (Jung et al. Bone., 2021). We show that changes in circulating FGF21 levels are associated with changes in muscle mass, which may be related to muscle mass loss in response to caloric restriction.

4. Amplitude of changes in myokines

Some of the changes observed at the gene or circulating level appear to be relatively modest in magnitude. However, these changes are similar in magnitude to those observed after exercise or bariatric surgery in other studies, either at the muscle or circulating levels (Pourteymour et al., 2017; Catoire et al., 2014; Kumar et al., 2019). In addition, some of these changes are significantly associated with changes in glucose homeostasis and muscle mass after bariatric surgery. This suggests that these changes, although

modest in magnitude, may contribute to (or reflect) changes in glucose homeostasis and muscle mass after bariatric surgery.

5. Changes in myokine expression versus circulating levels

Skeletal muscle has the potential to be a primary source of myokines at the systemic level, as skeletal muscle is the largest tissue in the human body (Sartori, 2021). However, most myokines are not specifically expressed and secreted by skeletal muscle, making it difficult to determine the role of muscle in regulating circulating myokine levels, especially in humans. There is indeed a large overlap between adipokines, hepatokines, and myokines (Raschke et al., 2013; Faramia et al., 2020). In fact, changes in circulating myokines levels may not originate from muscle. Analysis of the arteriovenous difference would be necessary to establish that muscle is the source of changes in myokines observed in the circulation (Whitham et al., 2018), but this is difficult to perform. In addition, the extent to which muscle-derived factors alter circulating levels to influence distant organs remains to be determined (Barlow et al., 2018).

6. Changes in myokine expression *in vivo* versus *in vitro*

The RNA analysis by RTq-PCR and RNA-Seq performed on muscle samples does not allow us to determine which muscle cell populations contribute the most to gene expression changes. For this reason, we established a primary

human skeletal muscle cell culture model as a complementary approach to identify myokines directly produced by myogenic cells and susceptible to improve glucose homeostasis after bariatric surgery. Primary human muscle cell culture offers the advantage of isolating muscle cells that partially retain the metabolic characteristics of the donor (Aas et al., 2013; Batista et al., 2020). This suggests a combination of genetic and epigenetic factors that are transferred to the culture dishes and subcultured cells (Aas et al., 2013). The development of a primary muscle cell culture model therefore seemed to be a valuable complementary tool to our study performed on muscle samples. However, overall gene expression redundancy between muscle samples and myotubes from the same cohort is modest for several reasons (Rutti et al., 2018; Aas et al., 2013; Raymond et al, 2010). First, the participants from whom muscle samples and myotubes were collected in our work were different individuals, adding to the overall difference between muscle tissue and muscle cells. Second, the less advanced differentiation of myotubes *in vitro* compared to *in vivo* muscle may be related to the timepoint we chose, i.e. 3 days of differentiation. Cell viability was indeed lower beyond this timepoint, and myogenic markers expression was maximal before 6 days of differentiation (not shown). In previous studies, comparative gene expression profiling between cultured human myotubes and muscle tissue from the same individuals has shown a lower expression of genes involved in oxidative metabolism, mitochondria, glucose metabolism, and muscle contraction in

myotubes, while genes related to the extra-cellular matrix and apoptosis had higher expression levels (Raymond et al., 2010). Third, the muscle phenotype *in vitro* could differ from the phenotype observed *in vivo*. Indeed, muscle cells *in vitro* seem to be rather glycolytic (Aas et al., 2013). However, we did not compare myosin heavy chains and PGC1A expression between our muscle samples and our myotubes. Fourth, low contractile activity *in vitro* may preclude the expression or secretion of myokines. Indeed, most myokines are expressed at low levels at rest, making their detection difficult under basal conditions, as realized in our study. Electric pulse stimulation can help specify fiber type *in vitro* (Hennig et al., 2023) and detect myokines in primary human muscle cell culture at the gene or protein level by enhancing their expression or secretion (Mengeste et al., 2022). The absence of electric pulse stimulation in our study contrasts with the increased physical activity observed after bariatric surgery (Steele et al., 2015; Herring et al., 2016). Fifth, the muscle bed includes other cell populations than myofibers and muscle satellite cells that influence all measurements made on intact tissue (Sylow et al., 2021). Sixth, since muscle from people with obesity is infiltrated by adipocytes and immune cells, the absence of these cells *in vitro*, although desired, may cause differences between measurements made on myotubes and muscle samples. Finally, human muscle cells have circadian rhythms (Perrin et al., al, 2018). A large part of the *in vivo* mRNA rhythmicity is lost in primary cultured myotubes which alters the expression of genes involved in

glucose homeostasis and lipid metabolism as well as basal myokine secretion (e.g., IL-6) (Perrin et al., 2015; Perrin et al., 2018). For all these reasons, it is not unexpected that the changes in myokine expression that we previously demonstrated in muscle samples (Orioli et al., 2023) were not observed in our cultured myotubes (e.g., irisin expression changed in opposite directions in myotubes and muscle samples). Therefore, the extrapolation of our *in vitro* data to the *in vivo* situation is limited. In addition, we were unable to study changes in the secretome of myotubes in our model due to low cell viability after serum withdrawal (not shown). Nevertheless, we are not aware of any other study that has used RNA-seq to search for bariatric surgery-regulated myokines on human primary myotubes.

7. Three-month assessment of glucose homeostasis

In our work, we chose to test the impact of bariatric surgery at a unique early timepoint of 3 months, when participants are still in a dynamic phase of weight loss and catabolism. Therefore, our data may not be representative of later time points because of attenuation of weight and muscle mass loss or even because of weight regain. To our knowledge, the association between weight regain after bariatric surgery and myokines has never been studied. We believe that a 3-month assessment is valid given the significant weight loss and improved glucose homeostasis were observed in our cohort. Furthermore, we expect that muscle insulin resistance was improved in our

study because improved muscle insulin resistance has been reported in individuals with similar weight loss 3 months after bariatric surgery using gold standard methods (i.e., hyperinsulinemic-euglycemic clamp and 3-stage hyperinsulinemic-euglycemic pancreatic clamp) (Yoshino et al., 2020; Bojsen-Møller et al., 2014).

8. Impact of physical activity on myokines

Muscle gene expression is influenced among others by contraction and insulin (Pourteymour et al., 2017; Catoire et al., 2014; Ducluzeau et al., 2001). Therefore, 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 (Pourteymour et al., 2017; Raschke et al., 2013; Catoire et al., 2014; Dantas et al., 2019). Increases in both objectively recorded and self-reported physical activity were demonstrated after bariatric surgery with a shift towards a greater amount of active time, but of a lower intensity within the first 6 months of bariatric surgery (Steele et al., 2015; Herring et al., 2016). Since physical activity levels were not assessed in our study, the possibility remains that some observed changes in myokines might result from increased physical activity after bariatric surgery.

9. Impact of muscle phenotype on myokines

Changes in muscle fiber distribution may affect myokine expression and secretion. Indeed, some myokines are fiber-specific, including IL-6, angiogenin, and osteoprotegerin (encoded by TNFRSF11B), which are more highly expressed by type 2 muscle fibers (Rutti et al., 2018; Hiscock et al., 2004). In our targeted study on muscle samples, we showed an upregulation of IL-6, angiogenin, and osteoprotegerin after bariatric surgery (Orioli et al., 2023). Although none of these myokines were associated with changes in glucose homeostasis, their changes might result of changes in muscle phenotype. However, this is unlikely to be the case, as the muscle phenotype after bariatric surgery appears to evolve toward a more insulin-sensitive and oxidative nature with fewer type 2 fibers. Indeed, compared to control subjects, our participants before surgery were characterized by higher expression of type 2x muscle fibers (*MYH1*) and type 2a muscle fibers (*MYH2*). These data are consistent with the literature showing an increased proportion of type 2 muscle fibers, particularly type 2x muscle fibers, in people with obesity compared to lean people (Damer et al., 2022; Tallis et al., 2018; Tanner et al., 2002; Serrano et al., 2023). In line with this, differences in the muscle phenotype may explain changes in myokine expression and secretion in people with obesity compared to lean people, but this was not analyzed in our study. This “slow-to-fast” shift in muscle fiber-type distribution observed

in people with obesity is thought to contribute to impaired glucose uptake and oxidative metabolism in skeletal muscle (Tanner et al., 2002; Serrano et al., 2023; Egan et al., 2013; Tallis et al., 2018; Albers et al., 2015, a). Few studies have investigated the changes in muscle phenotype after bariatric surgery. We show that the expression of type 2x muscle fibers (*MYH1*) is decreased after bariatric surgery, whereas the expression of type 2a muscle fibers (*MYH2*) is increased. Compared to control individuals, people with obesity have comparable expression of type 2x (*MYH1*) muscle fibers after bariatric surgery. In addition, the expression of PGC1A was unchanged after bariatric surgery in our study, as reported by others (Campbell et al., 2016). On the other hand, upregulation of myokines involved in fatty acid oxidation, such as BDNF in muscle samples, may promote a more oxidative muscle phenotype. However, this is not consistent with our pathway analysis (Orioli et al., 2023) and with the downregulation of other myokines in muscle samples such as irisin which is known to promote oxidative metabolism. Investigating associations between changes in myokine and myosin heavy chains expression would document the potential contribution of myokines to changes in muscle phenotype. Overall, the type of muscle analyzed, and more specifically the proportion of type I and type II muscle fibers, may influence the myokine response to bariatric surgery. Our data obtained on samples from vastus lateralis which is a mixed muscle may therefore not be representative of changes occurring in other muscles and may explain

discrepant results between changes in myokine expression and circulating levels which may originate from other muscles.

10. Impact of body composition on myokines

Body weight loss is the primary driver of the metabolic benefits of bariatric surgery (Yoshino et al., 2020). Fat mass loss contributes most to body weight loss after bariatric surgery, but fat-free mass loss is also expected (Davidson et al., 2018). As we hypothesized, changes in body composition may contribute to changes in myokines after bariatric surgery. Indeed, we show that upregulation of myokines including BDNF, CX3CL1 (fractalkine), ADAMTS9, and SPINK5 in muscle is associated with the magnitude of body weight loss (Orioli et al., 2023). Specifically, upregulation of BDNF, CX3CL1, and ADAMTS9 is associated with the magnitude of fat-free mass loss. In addition, fat-free mass loss is an independent predictor of decreased circulating levels of myostatin in our study, as reported by others (Milan et al., 2004). Therefore, fat-free mass loss, which is primarily skeletal muscle mass loss, seems to mediate most of the effects of bariatric surgery on certain myokines. The relationship between specific myokines such as myostatin, SPARC and FGF-21 and muscle mass have been discussed above.

11. Impact of the nutritional status on myokines

Changes in diet and nutrient intake after bariatric surgery may influence circulating levels of certain myokines. As an example, runners with low carbohydrate intake have lower BDNF levels, whereas those with adequate carbohydrate intake have higher myostatin levels (Sierra et al., 2022). Higher cholesterol and lower fiber intake also result in lower irisin, apelin, and BDNF levels in runners. In people with overweight or obesity, ketogenic and/or hypocaloric diet increases circulating BDNF levels, but decreases irisin and FGF21 (Mohorko et al., 2019; Kackley et al., 2022; Tok et al., 2021). Zinc supplementation also increases BDNF levels in people with obesity (Solati et al. 2015). To our knowledge, the impact of changes in micro and macronutrient intake on myokine levels or expression in muscle after bariatric surgery has not been studied. Therefore, it is not completely excluded that some observed changes in myokines in our work might result from dietary changes after bariatric surgery.

12. Changes in metabolic pathways that may regulate glucose homeostasis

Despite improved glucose homeostasis, glucose homeostatic pathways were not enriched in muscle or myotubes after bariatric surgery in our study (Orioli et al., 2023). Nevertheless, changes in other metabolic pathways

susceptible to contribute to improved metabolism were observed and, in some cases, associated with changes in myokines, particularly myostatin and irisin. Specifically, the downregulation of MSS51, an effector of myostatin on metabolic processes including fatty acid oxidation, glycolysis, and oxidative phosphorylation (Moyer et al., 2015; Gonzalez et al., 2019), should result in increased mitochondrial function, as expected after bariatric surgery (Barberio et al., 2021; Campbell et al., 2016; Gastaldi et al., 2007). However, our data show a downregulation of mitochondrial pathways after bariatric surgery, as reported by others at early timepoints (~3 months) (Gancheva et al., 2019; Campbell et al., 2016). It is therefore possible that decreased mitochondrial activity results from an energy economy phenomenon essentially due to low lipid oxidation and transient lipotoxicity due to increased lipolysis of adipose tissue (Bertoni et al., 2021; Bobbioni-Harsch et al., 2000). In addition, decreased mitochondrial activity may result from decreased expression of irisin (encoded by *FDNC5*) in muscle early after bariatric surgery, as we report together with others (Huh et al., 2012; Orioli et al., 2023), as well as from decreased circulating levels of irisin (Kumar et al., 2019). Our *in vitro* observations also pinpointed some pathways enriched in upregulated genes included the "adipocytokine signaling pathway" and “AMPK signaling pathway” which include *CPT1B*, *PPARGC1A*, and *PRKAB1*. These genes respectively encode CPT1 (muscle isoform), PGC1A, and AMPK subunit beta-1. CPT1 is a target of PGC1A and the main regulator of long-chain fatty acid transport

across the mitochondrial membrane, while PGC1A, the master regulator of mitochondrial biogenesis, is induced by AMPK (Kim et al., 2000; Tallis et al., 2018). Reduced CPT1 and AMPK activity as well as PGC1A downregulation have been reported in muscle of people with obesity. These defects promote detrimental intramyocellular lipid accumulation and muscle insulin resistance (Kim et al., 2000; Tallis et al., 2018). In addition, the "nitric-oxide synthase binding" was also enriched in upregulated genes. The nitric-oxide synthase produces nitric oxide, which is involved in mitochondrial biogenesis, with PGC1A as the main signaling molecule (Tengan et al., 2012). Thus, changes in these metabolic pathways induced by bariatric surgery may contribute to improved glucose and lipid metabolism. Direct assessment of the activity of these pathways in muscle is needed to confirm these data.

13. Changes in metabolic pathways that may regulate muscle mass

Changes in metabolic pathways associated with changes in myokines, particularly myostatin, may be involved in the regulation of skeletal mass in response to bariatric surgery. Indeed, we show that pathways involved in muscle protein synthesis (e.g., ribosomal and translational pathways) are enriched in upregulated genes, which may represent a counterregulatory mechanism to preserve muscle mass after bariatric surgery (Orioli et al. 2023), as suggested by others (Campbell et al., 2016; Milan et al, 2004). Our transcriptomic

data show downregulation of DDIT4 (or REDD1), a potent inhibitor of mTORC1 activity (Britto et al., 2020), which may be driven by decreased myostatin. Interestingly, overexpression of WNT4 in muscle cells causes muscle hypertrophy through downregulation of myostatin expression (Bernardi et al., 2011). Although myostatin was not downregulated in our myotubes, others and we have previously shown that myostatin expression is downregulated in muscle from people with obesity after bariatric surgery (Orioli et al. 2023). Finally, our study suggests that myokines, such as myostatin, may be involved in metabolic pathways altered by bariatric surgery, including the dynamics of protein synthesis and mitochondrial pathways (Orioli et al., 2023).

14. Design limitations

14.1. Type of surgery

Our cohort includes both sleeve gastrectomy and Roux-en-Y gastric bypass. Although Roux-en-Y gastric bypass may be slightly more effective than sleeve gastrectomy for early improvement of glucose homeostasis (Cummings et al., 2018), both procedures have the same time course for changes in body weight, muscle insulin sensitivity, and muscle transcriptome (Gancheva et al., 2019). In addition, we found no significant association between myokines and type of surgery using regression analysis (not shown).

14.2. Gender

Some myokines could be influenced by sex hormones, as shown for BDNF in rats and myostatin in humans (Yang et al., 2019; Yarasheski et al., 2002; Szulc et al., 2012; Baczek et al., 2020). Nevertheless, multivariate regression analyses were adjusted for sex.

14.3. Control subjects

When comparing the muscle phenotype of our participants to control subjects, several limitations should be kept in mind. Firstly, the control group included only men, unlike our study. Secondly, some control individuals had a BMI consistent with being overweight (25.0-29.9 kg/m²), which may still result in “higher or lower than normal” myosin heavy chain expression. Thirdly, muscle samples and muscle RNA from control individuals were obtained using different techniques to ours (De Groote et al., 2021). These factors may influence measurements made on muscle. Fourthly, RT-qPCR provides relative changes in the genes encoding myosin heavy chains, which may give different results compared to the quantification of muscle fiber types by immunochemistry.

14.4. Discrepancies with previous studies

The observed discrepancies in changes in myokine circulating levels after bariatric surgery between our study and others may be due to several factors,

including different assay methods and physical activity levels. Of note, most studies investigating the effects of bariatric surgery including ours mainly include premenopausal and Caucasian women, whereas age, sex, ethnicity, and body composition influence the muscle transcriptome, proteome, phenotype, and thereby myokine expression and secretion (Barberio et al., 2021; Tanner et al., 2002). Finally, most people who undergo bariatric surgery remain in the obese range early after bariatric surgery (BMI >30kg/m²), which may still result in “higher or lower than normal” myokine levels. The relative contribution of bariatric surgery itself versus weight loss and lifestyle changes (e.g., increased physical activity and reduced energy intake) in inducing changes in circulating myokine levels after bariatric surgery is difficult to determine. Despite these limitations, we are convinced the longitudinal analysis of a broad array of myokines at the muscle and circulating levels in a relatively large cohort are the main strengths of our study.

15. Technical limitations

15.1. Assessment of glucose homeostasis

The hyperinsulinemic-euglycemic clamp and/or the oral glucose tolerance test would have provided a better assessment of glucose homeostasis than the HOMA test. In line with this, we only have a steady state assessment of the beta cell function. The assessment of beta-cell function, defined as the relationship between insulin secretion rate and plasma glucose, by other

indices (e.g., plasma insulin-to-plasma glucose concentration areas under the curve ratio, disposition index) (Cao et al., 2024) may have yield different associations between changes in beta cell function and myokines. Notably, the mathematical assumptions underlying these models have not been developed for people with obesity undergoing bariatric surgery. Indeed, bariatric surgery increases the rate of delivery of ingested glucose into the systemic circulation, causing a spike in plasma glucose concentrations that influences the rapidity and magnitude of the insulin secretory response to glucose or mixed meal ingestion (Bradley et al., 2012). The alteration in glucose absorption rate complicates the interpretation of the surgical effect on beta cell function. Globally, it seems that Roux-en-Y bypass does not significantly affect early insulin secretion but increases the disposition index after marked weight loss, whereas sleeve gastrectomy does not affect early insulin secretion in people without diabetes but increases early insulin secretion in people with type 2 diabetes (Bradley et al. 2012).

15.2. RTq-PCR and RNA-seq

The RNA analysis on muscle samples does not allow us to determine which muscle cell populations contribute the most to gene expression, as discussed earlier.

15.3. Body composition analysis

Bioelectrical impedance could have overestimated muscle mass since obesity violates the constant hydration assumption (Donini et al., 2022; Nuijten et al., 2020). In addition, the equations used to estimate muscle mass were not originally designed for people with obesity. However, they were established from data of individuals from both sexes, with a wide range of age and BMI (Janssen, 2000, b; Sergi, 2015).

15.4. Myostatin ELISA

We used an immunoassay that measures only mature myostatin. Our result could therefore differ from studies using other immunoassays targeting different forms of myostatin or cross-reacting with other TGF-b members (e.g., GDF-11) (Loumaye et al., 2017; Baczek et al., 2020).

16. Advantages and limitations of the omic techniques used in our studies

The omics technique, i.e. RNA-Seq, used in our work has several limitations which may explain some of the discrepancies observed between our muscle and myotubes studies. In addition, other omic techniques, such as proteomics on muscle cells or conditioned media, could have provided additional data on changes in myokine expression and secretion after bariatric surgery.

16.1. mRNA-Seq

a. RNA-Seq versus RT-qPCR

We used high-throughput next-generation sequencing (NGS) to perform mRNA-Seq (referred to as RNA-Seq in the manuscript) to identify myokines regulated by bariatric surgery in an untargeted, discovery-driven approach. RNA-Seq is a highly sensitive and accurate means of quantifying gene expression and identifying both known and novel transcript isoforms (Deshpande et al., 2023). Thus, RNA-Seq captures the entire coding transcriptome while requiring small amounts of RNA (Wang et al., 2009). This was an important point in our work because our muscle samples were small, and our culture model yielded a limited number of muscle cells. In line with our hypothesis, RNA-Seq of muscle samples and muscle cells allowed us to identify novel myokines regulated by bariatric surgery (e.g., XYLT1, WNT4). In addition, RNA-Seq allows the analysis of functional relationships between regulated genes or transcripts (Reimand et al., 2019). Thus, RNA-Seq allowed us to identify changes in relevant metabolic pathways after bariatric surgery. Compared to RT-qPCR, RNA-Seq has the advantage of not being dependent on one or more reporter genes, as reads (sequenced fragments of cDNA) are directly quantified and normalized to gene length and sequencing depth (Deshpande et al., 2023).

b. Single-cell RNA-Seq versus total RNA-Seq

However, RNA-Seq on muscle samples is limited to bulk transcriptome analysis, which is influenced by other cell populations for muscle tissue (Cai et al., 2023). This is particularly relevant in people with obesity, whose muscle is infiltrated by immune cells and adipocytes (Varma et al., 2009; Henin et al., 2023). This may explain some of the observed differences between the results of RNA-seq performed on muscle samples and on muscle cells in our work, as mentioned earlier. The same limitation applies to muscle cells, where RNA-seq is performed globally on a mixed population of type I and type II myotubes (Rubenstein et al., 2020). Single-cell RNA-seq would therefore offer greater precision in analyzing the response of type I and type II myotubes to bariatric surgery, as shown for exercise (Rubenstein et al., 2020). This is of particular interest in the study of myokines after bariatric surgery, as some of them are fiber-specific (IL-6, angiogenin, osteoprotegerin, myonectin) (Rutti et al., 2018; Hiscock et al., 2004). Single-cell RNA-seq would be feasible in myotubes but may be more difficult to perform on human muscle fibers because it would require obtaining a tendon-to-tendon (Rubinstein et al., 2020; Agley et al., 2015). Nevertheless, muscle fiber isolation can be performed on muscle samples to generate fiber type-specific samples (Rubinstein et al., 2020). As discussed earlier, some of the discrepancies observed in our work may result from differences in phenotype and differentiation between human muscle fibers

(muscle samples) and myotubes. This limitation also applies to our RT-qPCR analysis.

c. Statistical limitation of RNA-Seq

The large number of genes or transcripts measured by RNA-Seq has a statistical limitation. Indeed, it is necessary to apply a correction for multiple comparisons to control the rate of false positive results (Deshpande et al., 2023). In our work, the false discovery rate, which is the proportion of Type I errors among all the rejected null hypotheses (Benjamini et al., 2001), was used for this purpose. However, this can mask significant changes in gene expression that are otherwise significant when examined by a specific, targeted technique such as RT-qPCR (Rachinger et al., 2021). This explains, at least in part, why some genes that were not altered after bariatric surgery in the RNA-Seq analysis were significantly altered in the RT-qPCR analysis. In addition, this is why we extended the analysis to genes whose nominal (uncorrected) p-value was < 0.05 in RNA-Seq performed on muscle cells, as RT-qPCR would serve as validation step.

d. Impact of the mRNA size on RNA-Seq

Finally, although RNA-Seq is generally considered unbiased, it is important to note that fragmentation and library construction can introduce some biases into RNA-Seq results (Ma et al., 2019). Briefly, the number of reads

corresponding to each transcript is proportional to the number of cDNA fragments rather than the number of transcripts. Therefore, more reads will be assigned to longer transcripts. Consequently, differentially expressed genes are more likely to be enriched for longer than shorter transcripts (Ma et al., 2019). This is another reason for discrepancies between RNA-Seq and targeted analysis provided by RT-qPCR. Recently, new 3' RNA-Seq methods, such as Tag-seq, have been developed to minimize this bias (Weng et al., 2022; Ma et al., 2019). In the 3' RNA-Seq method, mRNAs are not fragmented before reverse transcription. Instead, the cDNAs are only reverse transcribed from the 3′ end of the mRNAs (tag read), and only one copy of cDNA is generated for each transcript (Ma et al., 2019). Thus, when the cDNAs are sequenced, the number of reads directly reflects the number of transcripts of a certain gene, and the longer and shorter transcripts should have the same coverage of reads.

e. Transcript versus gene analysis

In addition to the limitations of RNA-Seq, the transcriptome of protein-coding loci appears to be dominated by one transcript per gene, and not all transcripts that contribute to transcriptome diversity are equally likely to contribute to protein diversity (Gonzàlez-Porta et al., 2013). This is the main reason why we chose to analyze both genes and transcripts in our *in vitro* study, considering that transcript analysis would probably provide an

additional list of myokines. Transcript analysis was not performed on the muscle samples, as differential gene analysis had already identified many genes of interest.

16.2. Proteomic analysis of conditioned culture media

The lack of analysis of conditioned media from primary myotubes is the main limitation of our *in vitro* study. Untargeted proteomic analysis of conditioned media by mass spectrometry, the most accurate and specific method currently available for secretome analysis, requires serum starvation (Weigert et al., 2021). Indeed, serum proteins would mask the true secretome and low abundance proteins secreted by muscle cells, precluding meaningful analysis (Weigert et al., 2021). However, serum starvation is a cellular stressor that can alter muscle cell viability, metabolism, and differentiation (Jang et al., 2022). Indeed, we were unable to maintain sufficient viability of our myotubes under serum-free conditions (not shown). As a result, the conditioned media would have been contaminated by proteins derived from cell lysis. Analysis of the conditioned media, either by untargeted (mass spectrometry) or targeted (ELISA, western blot) methods seems therefore mandatory to confirm that the changes observed in myokine expression are reflected in changes in myokine secretion. Indeed, the putative myokines we identified, namely XYLT1, LGR5, and SPINK5, are computationally predicted secreted proteins. However, to the best of our knowledge, these proteins have not

been reported in conditioned media from muscle cells, so we cannot confirm that they are “true” myokines. Furthermore, post-translational modifications can affect protein secretion (Zhong et al., 2023). Thus, a functional protein may not be produced despite changes in gene expression. In addition, translation is a key regulatory node in gene expression (King et al., 2016). Methods have been developed to infer the translatome, which refers to the entirety of mRNAs associated with ribosomes for protein synthesis. These methods include polysomal profiling, ribosomal profiling, and ribosome affinity purification techniques (King et al., 2016). In addition, some myokines exist in a plasma membrane-bound form and in a soluble form resulting from extracellular proteolytic cleavage (e.g., irisin, fractalkine) (Boström et al., 2012; Rutti et al., 2014). Therefore, it is possible that a myokine whose expression is altered at the gene or even protein level remains unchanged in culture media depending on extracellular proteolytic cleavage. To overcome the limitations of serum-free conditions, bead-based multiplex immunoassays are an efficient and sensitive means of analyzing protein signatures in serum-containing media (Weigert et al., 2021). A sample of unconditioned medium would be required to ensure that the immunoassay does not cross-react with serum proteins. Finally, the native protein concentration is low, so culture media must be concentrated to achieve a working concentration for subsequent analysis (Weigert et al., 2021).

VI. CONCLUSION

Bariatric surgery changes the muscle expression and circulating levels of several myokines. Changes in some of these myokines are associated with changes in glucose homeostasis and muscle mass after bariatric surgery. Firstly, upregulation of BDNF and putative myokines, XYLT1, LGR5, and SPINK5 in muscle are associated with improved glucose homeostasis after bariatric surgery. Secondly, changes in circulating levels of LIF, FSTL-1, APLN, and FABP3 are associated with changes in glucose homeostasis. In addition, bariatric surgery upregulates irisin and WNT4 in human primary myotubes and alters several metabolic pathways, which may contribute to improved glucose homeostasis. Thirdly, myostatin is downregulated at the muscle and circulating level after bariatric surgery, and positively associates with muscle mass, suggesting the potential interest of myostatin as a biomarker of muscle mass in people with obesity undergoing bariatric surgery. Conversely, circulating myostatin levels were negatively associated with sarcopenic obesity, suggesting the potential use of myostatin as a screening tool for sarcopenic obesity. In addition, changes in circulating levels of SPARC and FGF-21 are associated with changes in muscle mass after bariatric surgery. Taken together, our data suggest that myokines may play a role in improved glucose homeostasis and changes in muscle mass after bariatric surgery.

VII. PERSPECTIVES

We believe that our work opens several avenues for future research.

1. Biological effects of myokines

We have identified some myokines that may regulate glucose homeostasis and muscle mass after bariatric surgery. However, most of our work has been based on association studies, which does not allow us to establish a causal relationship between changes in myokines and changes in glucose homeostasis or muscle mass. Thus, the next step would be to test the biological effects of these myokines on glucose homeostasis and muscle mass. For example, the effects of XYLT1, LGR5 and SPINK5 on muscle cell insulin sensitivity and size and glucose-stimulated insulin secretion could be assessed in *in vitro* models. In addition, XYLT1 and SPINK5 knockout mice are viable (https://www.jax.org/) and could allow the characterization of the effects of these myokines on glucose homeostasis, muscle mass and function in *in vivo* models. In addition, we expected an association of fractalkine with changes in glucose homeostasis, particularly at the beta cell level. Analysis of the metabolic and muscle phenotype of fractalkine knockout mice (Cook et al., 2001) would help characterize the role of this myokine in glucose homeostasis and muscle function.

2. Presence of myokines in the circulation and conditioned culture media

The effects of plasma collected before and after bariatric surgery on glucose-stimulated insulin secretion, muscle cell insulin sensitivity and muscle cell size could be tested in *in vitro* models. Similarly, the effect of culture media conditioned by myotubes obtained before and after bariatric surgery could be tested. These approaches would provide proof-of-concept that circulating factors or factors secreted by muscle cells and regulated by bariatric surgery alter insulin secretion as well as muscle insulin sensitivity and mass. Inhibitory antibodies targeting specific myokines could be used to study their specific effects, as reported by others (Natalicchio et al., 2017; Hittel et al., 2009).

3. Characterization of the muscle protein secretome

Efforts should be made to study changes in the muscle secretome after bariatric surgery by proteomic approaches. Firstly, a mouse model of bariatric surgery (Yin et al., 2012) should allow larger amounts of muscle cells to be obtained from muscle samples. This model could allow the study of changes in the muscle secretome using mass spectrometry on conditioned culture media from myotubes collected before and after bariatric surgery. In addition, muscle cell culture could be combined with stable isotope labeling by amino acids in culture (SILAC), which allows for the identification of

muscle-specific proteins and for the quantification of proteins between samples (Hittel et al., 2009). The bariatric surgery-regulated myokines identified by this approach could be studied in muscle and plasma samples from people with obesity undergoing bariatric surgery. Secondly, our studies could be repeated by taking larger muscle samples or by pooling culture media from several participants, to identify changes in the human muscle secretome after bariatric surgery. Gradual serum withdrawal could improve cell viability, as previously reported in our lab (Gueugneau et al., 2018), to perform mass spectrometry on conditioned culture media of primary human myotubes taken before and after bariatric surgery. Alternatively, a multiplex immunoassay or ELISA approach on culture media containing serum could be used, as reported by others (Weigert et al., 2021). It would be of particular interest to test for XYLT1, LGR5 and SPINK5 under these conditions.

4. Characterization of the muscle secretome at large

The muscle secretome is not limited to peptides (Barlow et al., 2018), and the potential contribution of muscle metabolites and microRNAs to changes in glucose homeostasis and muscle mass after bariatric surgery requires further study. Specifically, many circulating proteins are packaged in extracellular vesicles (Whitham et al., 2018). An approach combining quantitative proteomic techniques and arteriovenous balance studies after

bariatric surgery could identify novel candidate myokines released into circulation independently of classical secretion (Whitham et al., 2018).

5. Confirmation of the interest of myostatin as a marker of muscle mass

More in line with the clinical practice, our work suggests that myostatin could be used as a biomarker of muscle mass and sarcopenia in people with obesity. Extending this observation to other muscle wasting conditions such as people with Cushing's syndrome, a disease characterized by corticosteroid-induced muscle wasting (Fleseriu et al., 2021), would be feasible. In addition, type 2 diabetes promotes sarcopenia (Park et al., 2006). However, no study has evaluated the association between myostatin and muscle mass in people with type 2 diabetes. The combination of unbiased proteomics on plasma samples obtained from people with sarcopenia and artificial intelligence should allow the identification of novel biomarkers of sarcopenia. Artificial intelligence is already being used for identification of sarcopenia from muscle transcriptomic and imaging data (Chung et al., 2021; Blanc-Durand et al., 2020).

6. Potential role of myokines on metabolic dysfunction-associated steatotic liver disease

Myosteatosis is strongly and independently associated with liver stiffness in people with obesity and metabolic dysfunction-associated steatotic liver

disease (Nachit et al., 2021). The study of myokines at the muscle and circulating levels in these people would document the potential contribution of myokines to metabolic dysfunction-associated steatotic liver disease as well as to the muscle function itself according to the severity of the liver disease. Notably, a study is currently ongoing on this topic in our clinics.

7. Impact of weight loss drugs on myokines

Finally, it would also be interesting to repeat our study on muscle samples taken in people with obesity before and after weight loss induced by new drugs for obesity such as tirzepatide or semaglutide. The comparison of changes in myokines in people matched for weight loss achieved either through diet, bariatric surgery, or drugs would bring additional data on the relative contribution of weight loss and bariatric surgery by itself in changes in myokines.

In the longer term, understanding the role of myokines in the regulation of glucose homeostasis and muscle mass after bariatric surgery may enable the development of new treatments and biomarkers for obesity, type 2 diabetes and sarcopenia.

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