Myokines as potential biomarkers and drivers of sarcopenia in metabolic and endocrine diseases

Short title	**Sarcopenia in metabolic and endocrine diseases**
Acronym / Protocol code	**SARC-ENDO**
Protocol version and date	**V 2.0 dated on 07/03/2026**
Sponsor	**Cliniques Universitaires Saint-Luc Belgium**
Financial/Material support	**N/A**
NCT number	**To be determined**
Investigator-Sponsor	**Pr. Laura Orioli, Service d'Endocrinologie et Nutrition, Cliniques Universitaires Saint-Luc, Belgique**
Co-investigators	**Pr. Audrey Loumaye, Service d'Endocrinologie et Nutrition, Cliniques Universitaires Saint-Luc, Belgique Dr. Fabian Lurquin, Service d'Endocrinologie et Nutrition, Cliniques Universitaires Saint-Luc, Belgique Pr. Etienne Danse, Service d'Imagerie Médicale, Cliniques Universitaires Saint-Luc, Belgique**

The information contained in this document is the property of the Sponsor/ Investigator-Sponsor and may not be reproduced, published or disclosed to others without written authorization of the Sponsor/ Investigator-Sponsor.

Version History

Version	Approval Date		Changes
1.0	XXX	Original	
2.0		Amendment	Fibroscan added to procedures
3.0		Amendment	
4.0		Amendment	

1. SIGNATURE PAGE

INVESTIGATOR-SPONSOR

_____	_____	_____
Name	Signature	Date

SITE PRINCIPAL INVESTIGATOR

I agree to conduct this study in accordance with the design and specific provisions of this protocol and will only make changes in the protocol after notifying the sponsor.

I understand that I may terminate or suspend enrolment of the study at any time if it becomes necessary to protect the best interests of the study subjects.

I agree to personally conduct or supervise this study and to ensure that all associates, colleagues, and employees assisting in the conduct of this study are informed about their obligations in meeting these commitments.

I will conduct the study in accordance with the protocol, Good Clinical Practice, the Declaration of Helsinki, and the moral, ethical and scientific principles that justify medical research. The study will be conducted in accordance with all relevant laws and regulations relating to clinical experimentation and the protection of patients.

I will ensure that the requirements relating to Ethics Committee review and approval are met.

I agree to maintain adequate and accurate records and to make those records available for audit and inspection in accordance with relevant regulatory requirements including the provision of direct access to data and source documents.

I agree to promptly report to the Ethics Committee any changes in the research activity and all unanticipated problems involving risks to human subjects or others. Additionally, I will not make any changes in the research without Ethics Committee approval, except where necessary to ensure the safety of study participants.

_____	_____	_____
Name	Signature	Date

2. PROTOCOL SYNOPSIS

Title of Study	Myokines as potential biomarkers and drivers of sarcopenia in metabolic and endocrine diseases
Acronym / Protocol code	SARC-ENDO
Sponsor	Cliniques Universitaires Saint-Luc
Investigator-sponsor	Pr. Laura Orioli
Co-Investigators	Pr. Audrey Loumaye, Dr. Fabian Lurquin, Pr. Etienne Danse
Department / Study centre(s) and site(s) principal investigator(s)	Service d'Endocrinologie et Nutrition, Cliniques Universitaires Saint-Luc
Pathology	Metabolic and endocrine diseases (obesity, diabetes, Cushing's syndrome, Grave's disease, acromegaly)
Rationale / Publication (references)	• Cruz-Jentoft AJ et al. Sarcopenia: revised European consensus on definition and diagnosis. Age Ageing. 2019 Jan 1;48(1):16-31. doi: 10.1093/ageing/afy169. • Donini LM et al. Definition and Diagnostic Criteria for Sarcopenic Obesity: ESPEN and EASO Consensus Statement. Obes Facts. 2022;15(3):321-335. doi: 10.1159/000521241. • Loumaye A et al. Role of Activin A and myostatin in human cancer cachexia. J Clin Endocrinol Metab. 2015 May;100(5):2030-8. doi: 10.1210/jc.2014-4318. • Orioli L et al. 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. • Orioli L et al. Circulating myostatin as a biomarker of muscle mass and strength in individuals with cancer or obesity. Clin Nutr. 2024 Jul;43(7):1800-1808. doi: 10.1016/j.clnu.2024.05.046. • Orioli L et al. Myokines as potential mediators of changes in glucose homeostasis and muscle mass after bariatric surgery. Front Endocrinol (Lausanne). 2025 Mar 18;16:1554617. doi: 10.3389/fendo.2025.1554617.
Objectives	**Primary**: • To evaluate the prevalence of sarcopenia / sarcopenic obesity in individuals with metabolic and / or endocrine diseases. • To identify risk factors for sarcopenia / sarcopenic obesity in individuals with metabolic and / or endocrine diseases. • To evaluate the changes in the prevalence of sarcopenia / sarcopenic obesity in individuals with metabolic and/or endocrine diseases after standard-of-care treatment for their condition. • To evaluate the one-, five- and ten-year prognosis of sarcopenia / sarcopenic obesity in individuals with metabolic and / or endocrine diseases. • To test whether plasma myostatin (MSTN) – a circulating myokine - is a biomarker for sarcopenia / sarcopenic obesity in individuals with metabolic and / or endocrine diseases. **Secondary:** • To identify myokines potentially contributing to sarcopenia / sarcopenic obesity in individuals with metabolic and / or endocrine diseases in a targeted and untargeted fashion. • To test whether these myokines are biomarkers of sarcopenia / sarcopenic obesity in individuals with metabolic and / or endocrine diseases. • To test whether these myokines are drivers of muscle atrophy *in vitro*.
Study Design	Prospective, interventional, single-center, non-treatment, non-randomized study; clinical and translational research

Number of patients	Estimated number: 250 participants, including participants with rare diseases
Main criteria for inclusion (inclusion/exclusion criteria)	**Inclusion:** • Age ≥ 18 years • At least one of the following metabolic or endocrine diseases: obesity (BMI ≥ 30 kg/m^2), diabetes (including type 1 diabetes, type 2 diabetes, secondary diabetes due to total pancreatectomy), Cushing's syndrome, Grave's disease or acromegaly. • Able to give informed consent **Exclusion:** • Hemorrhagic risk due to anticoagulants/antiplatelet agents (contraindication to muscle biopsy only) • Known myopathy • Systemic steroid use (> 1 mg/kg hydrocortisone) or exogenous Cushing's syndrome • Psychological, family, sociological or geographical conditions that could potentially limit compliance with the study protocol • History of cancer within the five years prior to the study or Cushing's syndrome due to ACTH-secreting tumor
Total study duration: - date of planned first enrolment - date of planned last completed	First enrolment: 01/01/2026; last completed: 01/01/2056
Study procedures	Assessment/measurement of: • Anthropometric parameters (body weight, height, BMI) • Body composition (bioelectrical impedance analysis, BIA, & Bioelectrical Impedance Vector Analysis, BIVA) • Muscle mass (BIA and CT scan) • Muscle function (dynamometer, gait speed, sit-to-stand test) • Muscle quality (CT scan) • Liver steatotis and fibrosis (fibroscan) Collection of: • Continuous glucose monitoring data (diabetes) • Blood samples (HOMA test, circulating myokines levels) • Muscle samples (myokine expression) Human muscle cell culture Common statistical procedures

3. SCHEDULE OF ACTIVITIES (STUDY FLOWCHART)

Table 1. Study flowchart

	Screening - Inclusion	**Baseline**	**3-month follow-up**	**One-year follow-up**	**Five-year follow-up**	**Ten-year follow-up**
Applicable if			SOC treatment			
	V0	V1	V2	V3	V4	V5
Time	≤3 months before baseline	1 month ± 1 week before SOC treatment (if any)	3 ± 1 month after SOC treatment (if any)	12 ± 3 months after inclusion	60 ± 3 months after inclusion	120 ± 3 months after inclusion
Status		Overnight fast	Overnight fast	Overnight fast	Overnight fast	Overnight fast
	Standard-of-care	Standard-of-care	Standard-of-care	Standard-of-care	Standard-of-care	Standard-of-care
Informed consent	X					
Medical history	X	X	X	X	X	X
Current medications	X	X	X	X	X	X
Body weight	X	X	X	X	X	X
Height	X	X				
Abdominal circumference	X	X	X	X	X	X
Hip circumference	X	X	X	X	X	X
Blood pressure	X	X	X	X	X	X
BIA/BIVA		X	X	X	X	X
Fibroscan		X	X	X	X	X
CT-scan*		X	X	X	X	X
Muscle function		X	X	X	X	X
Blood samples*		X	X	X	X	X
HOMA test		X	X (except T1D)	X (except T1D)	X (except T1D)	X (except T1D)
Muscle sample*		X	X	X	X	X
CGM data		X (for participants with diabetes)	X (for participants with diabetes)	X (for participants with diabetes)	X (for participants with diabetes)	X (for participants with diabetes)
Adverse events		X	X	X	X	X
Phone call		X (if muscle biopsy)	X (if muscle biopsy)	X (if muscle biopsy)	X (if muscle biopsy)	X (if muscle biopsy)

Abbreviations: BIA: bioelectrical impedance analysis; BIVA: bioelectrical Impedance Vector Analysis; CT: computed tomography; HOMA: Homeostasis Model Assessment; SOC Standard-of-care; T1D: type 1 diabetes; V: visit. * In willing participants.

Table of content

1. SIGNATURE PAGE 3

2. PROTOCOL SYNOPSIS 4

3. SCHEDULE OF ACTIVITIES (STUDY FLOWCHART) 6

4. LIST OF ABBREVIATIONS AND DEFINITIONS 8

5. ETHICS 9

6. OBJECTIVES 10

6.1. PRIMARY 10
6.2. SECONDARY 10

7. ENDPOINTS 10

7.1. PRIMARY 10
7.2. SECONDARY 10

8. BACKGROUND INFORMATION AND SCIENTIFIC RATIONALE 11

9. INVESTIGATIONAL PLAN 12

9.1. DESIGN 12
A. STUDY DESIGN 12
9.2. DESCRIPTION OF POPULATION 17
9.3. STRATEGIES FOR PARTICIPANT RECRUITMENT 20

10. STUDY INTERVENTIONS AND PROCEDURES 20

10.1. METHOD OF ASSIGNING PARTICIPANT TO INTERVENTIONS GROUPS ..... **ERREUR ! SIGNET NON DEFINI.**
10.2. SAMPLE LAB COLLECTION 22
10.3. PROTOCOL AMENDEMENTS 22
10.4. PROTOCOL DEVIATIONS 23

11. SAFETY REPORTING 23

11.1. DEFINITIONS AND REPORTING PROCESS 23
11.2. ASSESSING, RECORDING, AND ANALYZING SAFETY PARAMETERS 23

12. DATA MANAGEMENT RESPONSIBILITIES 25

12.1. DATA QUALITY ASSURANCE 25
12.2. STATISTICAL ANALYSIS 25
12.3. DATA HANDLING AND RECORD KEEPING 26
12.4. CASE REPORT FORM 26
12.5. DATA STORAGE 27
12.6. ACCESS TO DATA 27

13. INSURANCE 27

14. END OF STUDY 27

14.1. FOR AN INDIVIDUAL SUBJECT 27
14.2. FOR THE WHOLE STUDY 27

15. DISSEMINATION OF RESULTS AND PUBLICATION POLICY 27

16. ARCHIVING 28

17. STUDY REPORT 28

18. LITERATURE REFERENCES 28

19. APPENDIX ..... ERREUR ! SIGNET NON DEFINI.

4. LIST OF ABBREVIATIONS AND DEFINITIONS

%BW	percent of body weight (relative value)
ACTH	adrenocorticotropin
AE	adverse event
AGP	ambulatory glucose profile
ASMM	appendicular skeletal muscle mass
ASMMI	appendicular skeletal muscle mass index
BIA	bioelectrical impedance analysis
BMI	body mass index
BIVA	bioelectrical impedance vector analysis
CAP	controlled attenuation parameter
CGM	continuous glucose monitoring
CPK	creatine-phosphokinases
CRC	clinical research coordinator
CRF	case report form
CRP	C-Reactive Protein
CT	computed tomography
DPO	data protection officer
DXA	dual x-ray absorptiometry
EC	ethics committee
EDC	electronic data capture
EDIN	Endocrinology, Diabetes, and Nutrition, UCLouvain
EGID	European Genomic Institute for Diabetes
ELISA	enzyme-linked immunosorbent assay
ESPEN/EASO	European Society for Parenteral and Enteral Nutrition / European Society for the Study of Obesity
EWGSOP2	European Working Group on Sarcopenia in Older People 2
FDR	False discovery rate
FFM	fat-free mass
FM	fat mass
GH	growth hormone
HbA1c	glycated haemoglobin A1c
HGS	handgrip strength
HOMA	Homeostasis Model Assessment
HU	Hounsfield units
IGF-I	insulin-like growth factor I
IPAQ	International Physical Activity Questionnaire
LSM	liver stiffness measurement
MSTN	myostatin
MRI	magnetic resonance imaging
PI	principal investigator
RNA-Seq	RNA-sequencing
R	resistance
RT-qPCR	Real-Time quantitative Polymerase Chain Reaction
SAE	severe adverse event
SMM	skeletal muscle mass
T1D	type 1 diabetes
T2D	type 2 diabetes
TAR	time above range
TBR	time below range
TIR	time in range
TSH	thyrotropin
TSIs	thyroid-stimulating immunoglobulins
Xc	reactance

5. ETHICS

- *This protocol, any protocol amendments, informed consent form and other relevant documents (eg. recruitment advertisements) will be submitted to the Ethics Committee (EC) for formal approval to conduct the study. The decision of the EC concerning the conduct of the study will be made in writing to the sponsor. All correspondence with the Ethics Committee will be retained in the Investigator File.*
- *The study will be conducted in accordance with legal and regulatory requirements (Belgian law of 7 May 2004, Belgian law for Patient rights 22 August 2002, Private life GDPR 2018), as well as the Guidelines for Good Clinical Practice (International Conference on Harmonization 1996), and the last version of Declaration of Helsinki (World Medical Association).*
- *All subjects for this study will be provided a consent form describing this study and providing sufficient information for subjects to make an informed decision about their participation in this study. This consent form will be submitted with the protocol for review and approval by the EC. The formal consent of a subject, using the EC-approved consent form, will be obtained before that subject is submitted to any study procedure. This consent form must be signed by the subject or legally acceptable surrogate, and the investigator-designated research professional obtaining the consent. The written informed consent document should be prepared in the language of the potential patient population.*
- *The identity of the participant will remain kept confidential according to the General Data Protection Regulation of 27 April 2016 (in application on 25 May 2018), to the Belgian law of 30 July 2018 on the protection of natural persons with regard to the processing of personal data and the Belgian patient's right law (22 August 2002). Personal data will be coded. Subjects will not be identified by name or in any other recognizable way in any of the records, results or publications related to the experiment.*

6. OBJECTIVES

Objectives should be simple (not complex), specific (not vague), and stated in advance (not after the research is done). After statement of the primary objective, secondary objectives may be mentioned.

6.1. Primary

- To evalute the prevalence of sarcopenia / sarcopenic obesity in individuals with metabolic and / or endocrine diseases.
- To identify risk factors for sarcopenia / sarcopenic obesity in individuals with metabolic and / or endocrine diseases.
- To evaluate the changes in the prevalence of sarcopenia / sarcopenic obesity in individuals with metabolic and/or endocrine diseases after standard-of-care treatment for their condition.
- To evaluate the one-, five- and ten-year prognosis of sarcopenia / sarcopenic obesity in individuals with metabolic and / or endocrine diseases.
- To test whether plasma myostatin (MSTN) – a circulating myokine - is a biomarker for sarcopenia / sarcopenic obesity in individuals with metabolic and / or endocrine diseases.

6.2. Secondary

- To identify myokines potentially contributing to sarcopenia / sarcopenic obesity in individuals with metabolic and / or endocrine diseases in a targeted and untargeted fashion.
- To test whether these myokines are biomarkers of sarcopenia / sarcopenic obesity in individuals with metabolic and / or endocrine diseases.
- To test whether these myokines are drivers of muscle atrophy *in vitro*.

7. ENDPOINTS

7.1. Primary

- The diagnostic criteria from the EWGSOP2 and ESPEN/EASO consensuses will be used to determine the prevalence of sarcopenia / sarcopenic obesity, respectively.
- Logistic and linear regression analyses will be performed to identify risk factors for sarcopenia / sarcopenic obesity.
- The incidence of events considered as complications of sarcopenia / sarcopenic obesity - such as falls, hospitalizations, cardiovascular events, and death - will be analyzed to assess the one-, five-, and ten-year prognosis in affected individuals.
- Logistic and linear regression analyses will be performed to test whether plasma MSTN, as measured by ELISA, is a biomarker for sarcopenia / sarcopenic obesity and their components (i.e. low muscle mass and strength).

7.2. Secondary

Targeted study

- Myokines known to regulate muscle mass will be measured at the muscle level using RT-qPCR and at the circulating level using ELISA or multiplex immunoassays.

Untargeted study

- RNA-sequencing (RNA-Seq) will be performed on muscle samples to identify myokines whose expression differs between individuals with and without sarcopenia / sarcopenic obesity.
- These myokines, which potentially contribute to sarcopenia / sarcopenic obesity, will be measured in plasma using ELISA.

Associations of myokines with sarcopenia / sarcopenic obesity

- Logistic and linear regression analyses will be performed to test the association between these myokines and sarcopenia / sarcopenic obesity, as well as their components (i.e. low muscle mass and strength). These analyses should enable us to identify myokines as potential new biomarkers or drivers of sarcopenia / sarcopenic obesity in individuals with metabolic and / or endocrine diseases.

In vitro study

- The ability of these myokines to induce muscle atrophy will be tested on human muscle cells *in vitro*.

8. Background Information and Scientific Rationale

Sarcopenia is a progressive, generalized skeletal muscle disorder involving accelerated loss of muscle mass and function (Cruz-Jentoft, 2019; Donini, 2022). It primarily affects older adults (primary sarcopenia) because the natural aging process is associated with a loss of muscle mass and function (Cruz-Jentoft, 2019; Donini, 2022). However, sarcopenia can also occur at any age (secondary sarcopenia) when metabolic, cancerous, inflammatory, neurological, or immobilization conditions accelerate the loss of muscle mass and function (Cruz-Jentoft, 2019; Donini, 2019). Regardless of its cause, sarcopenia is associated with increased morbidity, functional decline, falls, frailty, and mortality. Sarcopenic obesity is an emerging and distinct phenotype of sarcopenia in individuals with excess adiposity (Donini, 2022; Wei, 2023). The prognosis for sarcopenic obesity appears to be worse than for obesity or sarcopenia alone.

Metabolic and endocrine diseases include a broad spectrum of conditions. On one end, they comprise common disorders such as obesity and type 2 diabetes (T2D). They also include less prevalent conditions like insulin-deficient diabetes—such as type 1 diabetes (T1D) and diabetes secondary to total pancreatectomy—as well as Graves' disease. Finally, they encompass rare endocrine disorders, including Cushing's syndrome and acromegaly (American Diabetes Association Professional Practice Committee, 2025; Fleseriu, 2021; Kahaly, 2025). Certain metabolic disorders, such as T2D and obesity, are recognized as "accelerators" of skeletal muscle mass and function decline, thereby contributing to the development of sarcopenia across all age groups (Donini, 2022). The pathophysiological mechanisms involved include impaired myogenesis, alterations in muscle fiber composition, anabolic resistance, chronic low-grade inflammation within muscle tissue, and ectopic fat infiltration (myosteatosis) (Espino-Gonzalez, 2024). Despite these associations, the prevalence and risk factors of sarcopenic obesity—a distinct clinical phenotype of sarcopenia—remain insufficiently characterized in individuals with T2D. Furthermore, there is a paucity of data regarding the prevalence, determinants, and clinical outcomes of sarcopenia and sarcopenic obesity in other metabolic (e.g., T1D) and endocrine disorders (e.g., Cushing's syndrome, Graves' disease, and acromegaly). These conditions are nevertheless associated with impaired secretion or action of hormones involved in muscle mass regulation, such as insulin, glucocorticoids, thyroid hormones and growth hormone.

Sarcopenia often remains underdiagnosed, as its diagnosis requires direct measurement of muscle mass, which is not easily performed in clinical practice. Direct measurement of muscle mass relies on imaging modalities that are expensive and/or not readily available for routine clinical use. Examples include dual-energy X-ray absorptiometry (DXA), computed tomography (CT) scan, and magnetic resonance imaging (MRI) (Mijnarends, 2012; Buckinx, 2018). Bioelectrical impedance (BIA), on the other hand, can easily provide an indirect estimate of muscle mass using prediction equations (Janssens, 2000; Sergi, 2015). Identifying easily obtainable biomarkers of muscle mass and function from a blood sample would facilitate screening for sarcopenia and sarcopenic obesity.

Skeletal muscle is an endocrine tissue that secretes bioactive molecules, including proteins, exosomes, metabolites, and microRNAs (Barlow, 2018; Eckardt, 2014). The proteins released by skeletal muscle are called myokines (Febbraio, 2005). Over 600 myokines have been identified in conditioned media from human muscle cells (Florin, 2020). Myokines can exert autocrine, paracrine, or endocrine effects, enabling them to regulate metabolism in various tissues. Previous work from our team suggests that some myokines, such as MSTN, are positively associated with muscle mass in individuals with cancer cachexia or obesity (Loumaye, 2015; Orioli, 2024). We also found that MSTN is inversely associated with sarcopenic obesity after bariatric surgery (Orioli, 2024). Together with the data of others, our data suggest that MSTN may serve as a biomarker of muscle mass, sarcopenia, and sarcopenic obesity in diverse human conditions (Alexopoulos, 2021; Orioli, 2024; Loumaye, 2015; Milan, 2004). Thus, circulating myokines hold potential as biomarkers for monitoring sarcopenia in humans.

However, the role of myokines in regulating muscle mass in humans is poorly understood (Orioli, 2025). Metabolic and endocrine diseases are associated with altered expression and secretion of specific myokines. These alterations may contribute to the development of sarcopenia in humans. For example, MSTN seems to play a role in muscle atrophy in T1D and Cushing's syndrome (Nunan, 2025; Ma, 2003).

Other myokines have been identified as potential drivers of myopathy/muscle atrophy related to endocrine diseases, such as serpina3n in Cushing's syndrome (Gueugneau, 2018) and irisin in T1D, Cushing's syndrome and Graves' disease (Jiang, 2021; Guarnotta, 2020, Yalcin, 2016). Thus, myokines hold potential as drivers of sarcopenia in humans.

The first aim of this study is to evaluate the prevalence of sarcopenia / sarcopenic obesity in individuals with metabolic and/or endocrine diseases using current diagnostic criteria for both conditions. The second aim is to identify the risk factors and to evaluate the prognosis of sarcopenia / sarcopenic obesity in these individuals. The third aim is to evaluate the changes in the prevalence of sarcopenia / sarcopenic obesity in individuals with metabolic and/or endocrine diseases after standard-of-care treatment for their condition. In the translational part of the study, we will identify myokines as potential new biomarkers or drivers of sarcopenia / sarcopenic obesity or their components (i.e. low muscle mass and strength), using targeted and untargeted analyses. Lastly, we will test the ability of candidate myokines to induce muscle atrophy in human muscle cells *in vitro*.

9. INVESTIGATIONAL PLAN

9.1. Design

a. Study design

This is a prospective, interventional, single-center, non-treatment, non-randomized study.

This study does not directly benefit participants. However, participants may receive management recommendations according to the standard-of-care if sarcopenia / sarcopenic obesity is identified as a result of participating in the study.

There will be one study group for each evaluated metabolic or endocrine disease (see inclusion criteria). The groups will include individuals with and without sarcopenia / sarcopenic obesity. Participants will undergo an evaluation at baseline and, if willing, at one-, five-, and ten-year intervals.

In cases where a standard-of-care treatment is provided for the management of the metabolic or endocrine disease, participants will also be evaluated three months after the treatment, if willing. Standard-of-care treatments can include: weight-loss intervention such as bariatric surgery or anti-obesity medications (semaglutide, tirzepatide) in individuals with obesity (American Diabetes Association Professional Practice Committee, 2025), adrenalectomy or hypophysectomy in individuals with Cushing's syndrome (Reincke, 2023), dedicated medications or hypophysectomy in individuals with acromegaly (Fleseriu, 2021) and dedicated medications in individuals with Grave's disease (Kahaly, 2018). In addition, individuals undergoing standard-of-care total pancreatectomy, which always leads to insulin - dependent diabetes, will also be assessed before and three months after the surgery. These standard-of-care treatments are anticipated to influence muscle mass and strength, potentially altering the prevalence of sarcopenia / sarcopenic obesity.

Because some of the evaluated diseases are rare endocrine diseases (e.g. Cushing's syndrome), enrollment will occur over the next 20 years. Participation to the study is expected to last 10 years, in participants willing to undergo the longitudinal assessment at one-, five-, and ten-year intervals. If the participants take part only in the first visit, this will allow us to determine the prevalence of sarcopenia. The longitudinal assessment will allow us to evaluate its incidence and complications.

b. Methods for collecting data for assessment of study objectives

Clinical evaluation

Participants will undergo a clinical evaluation after fasting overnight. Their body mass index (BMI) will be calculated using their weight in kilograms and their height in centimeters (weight/height*height). BMI is expressed in kilograms per square meters (kg/m^2) and is used to define obesity (BMI ≥ 30.0 kg/m^2) and overweight (BMI ≥ 25.0 kg/m^2). BMI is also used to select individuals for weight loss interventions (BMI ≥ 27.0 kg/m^2), including anti-obesity medications and bariatric surgery, as well as to monitor their progress over time. Abdominal and hip circumferences will be measured in centimeters. Abdominal circumference is measured halfway between the lower edge of the last rib and the upper edge of the

iliac crest. Hip circumference is measured at the level of the greater trochanter. An abdominal circumference greater than 94 cm in men and 80 cm in women indicates central, or android, obesity. Central obesity is also defined as an abdominal-to-hip circumference ratio greater than 1 in men and greater than 0.85 in women. All these parameters will be measured at screening, inclusion, baseline and the other established time points according to the study group (Table 1). The history and occurrence of complications, such as falls, hospitalizations, cardiovascular events, and death, will be recorded (when applicable) at enrollment and at the established time points. Sarcopenia and sarcopenic obesity are known risk factors for such adverse events (Wei, 2023). The risk for sarcopenia and the level of physical activity will be assessed used the SARC-F questionnaire and the IPAQ-court, respectively.

Body composition

Body composition will be assessed using bioelectrical impedance analysis (BIA). BIA measures the resistance (R) of the human body to an electrical current at 50 kHz. This measurement will be obtained using a Tanita BC-418MA (III) device. After fasting for at least two hours, the subject will stand barefoot on the device's conductive surfaces (lower-limb electrodes) and grasp its conductive handles (upper-limb electrodes), keeping her/his arms as far away from her/his body as possible. The device will then measure R and use this value in an equation to calculate fat mass (FM) and fat-free mass (FFM) in kilograms, as well as the relative value (% of body weight, %BW). FM and FFM will also be reported as indexes (FMI and FFMI, kg/m^2). Two measurements will be taken and both will be recorded.

Additionally, body composition will be assessed using the BIA101 device (Akern) and the manufacturer's provided software. The subject will lie down on a non-conductive surface, such as a bed or stretcher, without a pillow, with their arms and legs spread 40° apart. After degreasing the skin with alcohol, electrodes will be applied to the right hand (wrist: styloid line and back of the hand: space between the second and third metacarpals) and right foot (ankle: malleolar line and back of the foot: space between the second and third metatarsals). The interval between the electrodes should be greater than 5 cm. The device measures the R and reactance (Xc) of the human body when an electrical current of 50 kHz is passed through it. Whole-body FFM and FM will be obtained using the built-in equations and reported as absolute values (kg), relative values (%BW), and indexes (kg/m^2). Three measurements will be taken and all will be recorded. This technique does not use any equations or assumptions to estimate muscle mass (the main component of FFM). R and Xc measurements will be used in the appropriate equations to estimate muscle mass (see below). Body composition will be measured at baseline and the other established time points according to the study group (Table 1).

Transient elastography (FibroScan®) will be used to non-invasively assess liver stiffness (Liver stiffness measurement (LSM), expressed in kilopascals (kPa), higher stiffness values are associated with more advanced fibrosis and cirrhosis) and estimate hepatic steatosis (Controlled Attenuation Parameter (CAP), expressed in decibels per meter (dB/m), higher CAP values indicate greater liver fat content). This technique provides validated surrogate markers of liver fibrosis (LSM) and fat accumulation (CAP), which are common comorbidities in individuals with metabolic diseases such as obesity and diabetes. The examination is non-invasive, painless, and does not involve radiation exposure. It is performed using an ultrasound-based probe placed on the skin overlying the liver while the participant lies in a supine position with the right arm elevated. The device emits low-frequency vibrations that generate shear waves in the liver tissue, allowing the measurement of liver stiffness within a few minutes. The procedure typically lasts 10 minutes, does not require sedation or recovery time, and is well tolerated by participants. It poses minimal risk and discomfort, making it particularly suitable for clinical research settings. Transient elastography is already a SOC procedure in the hepatology field.

Muscle mass

Skeletal muscle mass (SMM) and appendicular skeletal muscle mass (ASMM) will be calculated using R and Xc measurements with the appropriate prediction equations (Janssen, 2000; Sergi, 2015). Additionally, muscle mass will be assessed using a CT scan in a subgroup of willing participants. For each participant, a transverse CT image from the third lumbar vertebra (L3) will be analyzed, and the tissue area will be estimated using Hounsfield unit (HU) thresholds and quantified by Slice-O-Matic software (version 6, Tomovision), as described previously (Loumaye, 2015). The most common and accepted HU ranges are −29 to +150 HU for muscle tissue and −190 to −30 HU for adipose tissue. The cross-sectional areas of muscle and adipose tissues will be normalized for height and expressed as

muscle and fat indexes (cm^2/m^2). All CT images will be analyzed by a single trained radiologist (Prof. E. Danse, Service d'Imagerie Médicale, Cliniques Universitaires Saint-Luc, Brussels). Previous data from our team showed an intra-observer coefficient of variation of 2.2% (Loumaye, 2015). The radiation from a CT scan is very low (65.6mGy*cm), as reported previously at our center (Nachit, 2021), allowing the use of CT scan in this study. Muscle mass will be assessed at baseline and at the other established timepoints according to the study group (Table 1).

Muscle function and quality

Muscle strength will serve as the primary indicator of muscle function (Cruz-Jentoft, 2019; Donini, 2022). Upper limb muscle strength will be assessed using a Jamar hand dynamometer to measure hand grip strength (HGS). Patients should sit with their shoulders pressed against their bodies. Three force measurements will be taken on the non-dominant side. These three measurements should be taken within 30 seconds. The best measurement will be recorded. Lower limb muscle strength will be assessed using the five-repetition sit-to-stand test. The participant will be asked to stand up and sit down five consecutive times from a chair. The time required to complete these five repetitions will be measured using a stopwatch. Physical performance is defined as an objectively measured whole-body function related to locomotion (Cruz-Jentoft, 2019). Physical performance can be variously measured by gait speed, the Short Physical Performance Battery, and the Timed-Up and Go test. In this study, we will measure gait speed as it is considered a quick, safe and highly reliable test for sarcopenia, and it is widely used in practice (Cruz-Jentoft, 2019). We will perform the 4-m usual walking speed test, with speed measured either manually with a stopwatch or instrumentally with an electronic device to measure gait timing. For simplicity, a single cut-off speed ≤0.8 m/s is advised by EWGSOP2 as an indicator of severe sarcopenia. Gait speed has been shown to predict adverse outcomes related to sarcopenia.

Skeletal muscle quality will be evaluated based on skeletal muscle density, which is determined by the mean muscle radiation attenuation for the entire muscle area at L3. This measurement is taken using a CT scan in HU. This parameter is inversely related to muscle fat content and linked to reduced muscle strength and performance (Loumaye, 2015). The generally accepted lower limit of normal mean attenuation for muscle is 30 HU, defined as two standard deviations below the mean attenuation value of the muscles of young and healthy individuals (Aubrey, 2014). Attenuation of skeletal muscle suggest abnormal ectopic lipid deposition called myosteatosis. Muscle strength and quality will be assessed at baseline and at the other established timepoints according to the study group (Table 1).

Definition of sarcopenia and sarcopenic obesity

Sarcopenia is defined as having low muscle mass and strength (Cruz-Jentoft, 2019). In this context, muscle mass is expressed as the ASSM index (ASMMI, kg/m^2). Low muscle mass and low muscle strength will be defined according to the EWGSOP2 criteria (Table 2). Subjects with sarcopenia will be compared to those without sarcopenia in subsequent analyses. A single cut-off speed ≤0.8 m/s is advised by EWGSOP2 as an indicator of severe sarcopenia.

Sarcopenic obesity is defined as the coexistence of excess adiposity and sarcopenia (Donini, 2022). In this context, muscle mass is expressed as relative SMM (%BW). Low muscle mass, low muscle strength, and excess adiposity will be defined according to the ESPEN/EASO criteria (Table 2). Subjects with sarcopenic obesity will be compared to those without sarcopenic obesity in subsequent analyses.

For participants undergoing a CT scan, the cut-offs for low muscularity are those proposed by Fearon et al. (2011).

Table 2. Diagnostic criteria for sarcopenia and sarcopenic obesity		
Parameter	**Sarcopenia (EWGSOP2)**	**Sarcopenic obesity* (ESPEN/EASO)**
Low muscle mass	ASMMI < 5.5 kg/m^2 in women < 7.0 kg/m^2 in men	Relative SMM <26.7 %BW in women < 37.7 %BW in men
Low muscle strength	HGS (upper limbs) < 16 kg in women < 27 kg in men Five-repetition sit-to-stand test (lower limbs) ≤ 17 seconds	
Excess adiposity	/	Relative FM 20-39 ans >39%BW in women, >26%BW in men 40-59 ans >41% in women, >29% in men 60-79 ans >43% in women, >31% in men

* Applicable if BMI ≥ 30.0kg/m^2 and or abdominal circumference ≥ 80 cm in women and ≥90 cm in men. Abbreviations: %BW: percent body weight ; ASMMI: appendicular skeletal muscle mass; EWGSOP2, European Working Group on Sarcopenia in Older People 2; ESPEN/EASO, European Society for Parenteral and Enteral Nutrition/European Association for the study of Obesity ; FM: fat mass; HGS: handgrip strength.

Blood samples

Blood samples will be taken after an overnight fast. The following parameters will be measured as part of the standard of care: C-reactive protein (CRP), albumin, lipid profile, glycated hemoglobin A1c (HbA1c), and creatine phosphokinase (CPK). In addition to these analyses, a HOMA (homeostasis model assessment) test will be performed (Levy, 1998). The HOMA test measures insulin sensitivity and beta cell function. This test is performed on an empty stomach after discontinuing antidiabetic medication for three days, except for individuals with T1D, for whom insulin cannot be discontinued. Blood glucose and insulin levels will be measured three times (at 0, 5, and 10 minutes). The average blood glucose and insulin levels are then entered into the HOMA2 calculator (https://www.rdm.ox.ac.uk/about/our-facilities-and-units/DTU/software/homa). The HOMA2 model uses a mathematical algorithm to estimate insulin sensitivity (HOMA2-S%) and beta cell function (HOMA2-B%). Normal values for insulin sensitivity and beta cell function are 1 and 100%, respectively. There are known relationships between myokines and glucose homeostasis (Orioli, 2025), justifying the use of the HOMA test in this study. Biological parameters will be analyzed using routine methods (Cliniques Universitaires Saint-Luc). Blood samples will be taken at baseline and at the other established timepoints according to the study group (Table 1).

Continuous glucose monitoring data

Continuous glucose monitoring (CGM) helps people with diabetes (including T1D, T2D and diabetes after total pancreatectomy) assess their blood glucose levels (American Diabetes Association Professional Practice Committee, 2025). Participants wearing CGM will have their glucose data recorded, including time in range (TIR), time below range (TBR), and time above range (TAR). This data is collected from ambulatory glucose profiles (AGP) and .csv files downloaded from the cloud (https://www.libreview.com/ or https://clarity.dexcom.eu/). Participants for whom data is available on Libreview or Clarity have given their consent for their data to be stored in the cloud in accordance with GDPR regulations as part of their diabetes care prior to the study (standard of care). Both CGM data and HbA1c levels evaluate glucose control. Emerging data suggests that there may be an association between parameters of glucose control and sarcopenia in individuals with T1D. No data are available regarding T2D and diabetes after total pancreatectomy. CGM data will be collected at baseline and at the other established timepoints according to the study group (Table 1).

Muscle biopsies

Muscle biopsies will be performed percutaneously in the vastus lateralis of the quadriceps femoris using BARD Monopty and TruGuide disposable coaxial biopsy needles, as previously described (Orioli, 2023). Participants must not be taking antiplatelet agents (including aspirin) or anticoagulants. The skin will be disinfected with either isobetadine or chlorhexidine. The biopsy will be taken under local anesthesia (1 mL of 2% lidocaine hydrochloride). The sample will be taken from the vastus lateralis of the quadriceps, 25 cm from the tibial tuberosity and 5 cm from the midline of the thigh. Biopsies are

performed after an overnight fast of at least 6 hours. Participants must not have received any anti-diabetic treatment during the 12 hours prior to the muscle biopsy, except for those T1D, for whom insulin cannot be discontinued. An occlusive dressing is applied for 72 hours to minimize the risk of infection and bruising. According to the literature and our experience, the incidence of side effects (pain, erythema, and bruising) is very low (less than 1.5%) after percutaneous muscle biopsy (Neves, 2011; Loumaye, 2015; Orioli, 2023). Participants are monitored for 15 minutes to prevent vagal or panic reactions. Based on our experience, 20–40 mg of muscle tissue should be collected after one to three samples are taken using the TruGuide Disposable Coaxial Biopsy Needle. The samples will be immediately cleaned of blood and fat. Then, the samples will be immersed in liquid nitrogen for RTq-PCR analysis and stored at -80°C. Muscle samples will be collected at baseline and at the other established timepoints according to the study group (Table 1).

Identification and quantification of myokines in muscle biopsies

Total RNA will be isolated from muscle samples using TRIzol reagent and chloroform, as previously described (Orioli, 2023). In the targeted study, the expression of myokines known to regulate muscle mass (e.g., MSTN, irisin) will be quantified in muscle samples using conventional RT-qPCR. For the untargeted study, RNA-seq will be performed in collaboration with Dr. Amélie Bonnefond of the European Genomic Institute for Diabetes (EGID) in Lille, France, as previously described (Orioli, 2023). Putative myokines (i.e., predicted secreted proteins) will be identified using databases that predict signal peptides, as previously described (Orioli, 2023). The expression levels of the putative myokines will be confirmed by performing conventional RT-qPCR on muscle RNA, as RT-qPCR is more specific than RNA-seq (Deshpande, 2023; Wang, 2009; Reimand et al., 2019). This should limit the number of false positive results. RNA isolation and RT-qPCR will be performed in collaboration with Prof. JC Jonas (EDIN, UCLouvain, Brussels, Belgium). By comparing the expression of myokines in individuals with sarcopenia / sarcopenic obesity to those without, we should be able to identify myokines that may be potential drivers or biomarkers of these conditions. Similarly, by comparing myokine expression in individuals before and after standard-of-care treatment for their metabolic or endocrine condition (e.g. weight loss intervention), we can identify myokines that may drive or serve as biomarkers for changes in muscle mass and strength in this context. This approach is consistent with our previous findings in individuals undergoing bariatric surgery which allowed us to identify several candidate myokines related to changes in FFM and muscle mass (Orioli, 2023; Orioli, 2024).

Quantification of myokines in plasma

Blood samples will also be used to measure levels of circulating myokines. These measurements will be performed using commercially available enzyme-linked immunosorbent assay (ELISA) kits, multiplex immunoassays, or Western blots. In the targeted study, myokines known to regulate muscle mass will be measured in plasma (Orioli, 2025). In the untargeted study, myokines identified by RNA-seq whose expression levels are confirmed by RT-qPCR will be measured in plasma. These analyses will be performed in collaboration with Prof. JC Jonas (EDIN, UCLouvain, Brussels, Belgium). By comparing myokine levels in individuals with sarcopenia / sarcopenic obesity to those without, we should be able to identify myokines that may drive or indicate these conditions. Similarly, by comparing circulating myokine levels in individuals before and after standard-of-care treatment for their metabolic or endocrine condition (e.g. weight loss intervention), we can identify myokines that may drive or serve as biomarkers for changes in muscle mass and strength in this context. This approach is consistent with our previous findings in individuals undergoing bariatric surgery which allowed us to identify several candidate myokines related to changes in FFM and muscle mass (Orioli, 2023; Orioli, 2024). The quantification of myokine levels in muscle and blood samples are complementary as there may be discrepancies between both measurements (Orioli, 2025).

Effects of myokines on muscle mass *in vitro*

Skeletal muscle-derived cells (SkMDCs, Cook MyoSite, Pittsburgh, PA, USA) will be treated with candidate myokines previously identified as potential drivers of sarcopenia to determine their capacity to induce muscle atrophy *in vitro*. Primary myoblasts from healthy donors will be expanded and differentiated into myotubes as described (Loumaye, 2023). Briefly, SkMDCs will be cultured in DMEM with GlutaMAX™, 20% FBS, 0.5% Ultroser G, 1% antibiotic-antimycotic, and 1% non-essential amino acids at 37 °C, 5% CO_2. Cells will be used before the 13th population doubling. Upon reaching 70–80% confluence, growth medium will be replaced with fusion medium (DMEM + GlutaMAX™, 2% horse

serum, 1% FBS, antibiotics, and non-essential amino acids). Myotube differentiation will be confirmed by multinucleated morphology and expression of sarcomeric proteins (α-actinin, myosin heavy chains), CPK, and myogenic markers (MyoD, myogenin). After 4–5 days of differentiation, cells will be treated with increasing concentrations of myokines. Cell growth and surface area will be compared to control-treated cells. Alternatively, muscle cells will be exposed to plasma from sarcopenic versus non-sarcopenic individuals to test whether circulating factors induce atrophy. To explore causality, additional conditions will involve co-incubation with neutralizing antibodies (e.g., anti-MSTN antibody, Hittel, 2009). Post-treatment, RNA and protein will be extracted and stored at –80 °C for RT-qPCR and Western blot to assess expression of atrophy-related markers. These experiments are expected to identify certain myokines as novel drivers of muscle atrophy, potentially contributing to sarcopenia in vivo.

Statistics

For continuous data, differences in cross-sectional data between individuals with and without sarcopenia /sarcopenic obesity will be assessed using a Student's t-test or a Wilcoxon test, depending on the data's distribution. Longitudinal differences will be assessed using a paired samples t-test or a Mann-Whitney test, depending on the data's distribution. Cross-sectional differences for discrete data will be assessed using a chi-square test, and longitudinal differences will be assessed using a McNemar test. We will investigate associations between myokines and muscle parameters (muscle mass and strength) using univariate and multivariate linear regressions. Associations between myokines and sarcopenia / sarcopenic obesity will be investigated using univariate and multivariate logistic regressions. Due to the exploratory nature of the study and the inclusion of individuals with rare diseases (e.g., Cushing's syndrome), the sample size is not pre-specified. For RNA-seq data, we will apply a correction for multiple comparisons to limit the false discovery rate (FDR) according to Benjamini-Hochberg to less than 0.10. Unless otherwise stated, statistical significance will be set at < 0.05. Statistical analysis will be performed in IBM SPSS Statistics 31 or R version 4.5.1.

9.2. Description of population

9.2.1. Patient population studied

- Participants will be adults (18 years or older, men and women) who are being followed by diabetologists/endocrinologists for metabolic and / or endocrine diseases in our center.
- Metabolic and endocrine diseases include obesity, diabetes, Cushing's syndrome, Grave's disease, and acromegaly.
    - Obesity is defined as a BMI ≥ 30.0kg/m^2 (American Diabetes Association Professional Practice Committee, 2025). Individuals with obesity may have normal blood glucose, prediabetes or T2D according to the Steering committee of the ADA criteria (American Diabetes Association Professional Practice Committee, 2025). Weight loss interventions may include bariatric surgery and / or anti-obesity medications such as semaglutide and tirzepatide (American Diabetes Association Professional Practice Committee, 2025).
    - T2D is defined as hyperglycemia due to a non-autoimmune progressive loss of adequate β-cell insulin secretion, frequently on the background of insulin resistance and metabolic syndrome (American Diabetes Association Professional Practice Committee, 2025). Anti-GAD65, anti-IA2 and/or anti-ZnT8 are negative in T2D.
    - T1D is defined as hyperglycemia with one or more antibodies against beta cells (anti-GAD65, anti-IA2 and/or anti-ZnT8) (American Diabetes Association Professional Practice Committee, 2025) or well-established history of T1D according to the medical record.
    - Insulin-dependent diabetes results from total pancreatectomy and is considered a secondary type of diabetes (American Diabetes Association Professional Practice Committee, 2025). Pancreatectomy will be performed as a standard-of-care procedure as determined by the treating physician/surgeon for the underlying condition (ex. chronic pancreatitis). These individuals are evaluated for diabetes before and after total pancreatectomy as part of the standard-of-care and are therefore followed by diabetologists in our center.

- Cushing's syndrome is a rare endocrinopathy defined as a prolonged increase in plasma cortisol levels that is not due to a physiological etiology (Reincke, 2023). Individuals with endogenous Cushing's syndrome due to either an adrenocorticotropin (ACTH)-secreting pituitary tumor (i.e. Cushing's disease) or an adrenal adenoma (ACTH-independent CS) will be included. These tumors are most often benign tumors. Standard-of-care treatment may include dedicated medications, adrenalectomy or hypophysectomy.
- Graves' disease represents the leading cause of hyperthyroidism and is classified as an organ-specific autoimmune disorder. It is driven by the production of thyroid-stimulating immunoglobulins (TSIs), which aberrantly activate the thyrotropin (TSH) receptor on thyroid follicular cells, resulting in unregulated synthesis and release of thyroid hormones. Common clinical features include diffuse thyroid enlargement (goiter), Graves' orbitopathy, thyroid-associated myopathy, and less frequently, pretibial dermopathy (Davies, 2020).
- Acromegaly is a rare endocrine disorder characterized by chronic excess secretion of growth hormone (GH), most commonly due to a GH-secreting pituitary adenoma. Elevated GH levels lead to increased production of insulin-like growth factor 1 (IGF-1), resulting in progressive somatic disfigurement (e.g., enlargement of hands, feet, and facial features) and systemic complications, including insulin resistance, type 2 diabetes, hypertension, cancer and cardiovascular disease (Fleseriu, 2021).

- There will be one study group for each metabolic or endocrine disease (see below). In each of these groups, individuals with sarcopenia / sarcopenic obesity will be compared to those without.
  - Individuals with obesity (group 1)
  - Individuals with T2D (group 2)
  - Individuals with T1D (group 3)
  - Individuals with insulin-dependent diabetes after total pancreatectomy (group 4)
  - Individuals with Cushing's syndrome (group 5)
  - Individuals with Grave's disease (group 6)
  - Individuals with acromegaly (group 7)
- Participants should be able to give informed consent.
- The average number of participants included should be 150.

9.2.2. Inclusion criteria

Subjects must meet all the inclusion criteria in order to be eligible to participate in the study. The inclusion criteria include:

- Age ≥ 18 years
- At least one of the following metabolic or endocrine disease: obesity, T1D, T2D, insulin-dependent diabetes after total pancreatectomy, Cushing's syndrome, Grave's disease, or acromegaly.
- Able to give informed consent in French.
- To avoid fluctuations in body weight and hydration status related to the initial hyperglycemic crisis (e.g. ketoacidosis), the study will include individuals with T1D who have had the condition for ≥ one year.

9.2.3. Exclusion criteria

Subjects meeting any of the exclusion criteria at baseline will be excluded from study participation. Exclusion criteria include:

- Major depression
- Hemorrhagic risk due to anticoagulants/antiplatelet agents (contraindication to muscle biopsy only)
- Known myopathy from inflammatory or neuromuscular origin

- Systemic steroid use (> 1 mg/kg hydrocortisone) 3 months before the study and / or Cushing's Syndrome due to exogenous steroid use
- Hyperthyroidism of other causes than Grave's disease
- Psychological, family, sociological or geographical conditions that could potentially limit compliance with the study protocol
- Participation in clinical drug studies
- Major change in anti-diabetic treatment during the month prior to the study
- Change in body weight (> 5 %) 3 months before the study
- History of cancer within the five years prior to the study or Cushing's syndrome due to ACTH-secreting tumor (most often malignant tumors)
- Hyperglycemic crisis (e.g. ketoacidosis, hyperosmolar state) 3 months before inclusion
- Severe peripheral arterial disease (contraindication to muscle biopsy only)

9.2.4. Subject eligibility screening

Screen failures are subjects who consent to participate in the study but do not meet one or more criteria required for participation in the study during the screening procedures. Screen failures will be reassessed after hyperglycemic crisis (> 3 months). A minimal set of screen failure information will be kept to ensure transparent reporting of screen failure subjects.

9.2.5. Withdrawal

Subjects are free to withdraw from participation in the study at any time.

An investigator may withdraw a subject from the study for the following reasons:

- Significant study intervention non-compliance;
- If any clinical adverse event (AE), laboratory abnormality, or other medical condition (e.g. cancer) or situation occurs such that continued participation in the study would not be in the best interest of the subject;
- If the subject meets an exclusion criterion (either newly developed or not previously recognized) that precludes further study participation;
- Other: need for anticoagulants/antiplatelet agents associated with hemorrhagic risk (contraindication for muscle biopsy only), need for systemic steroid use (> 1 month), participants entering a clinical drug study, early interruption of weight loss intervention (<3 months)

In all cases, the reason why subjects are withdrawn must be recorded in detail in the electronic Case Report Form (eCRF) and in the subject's medical records. The gathered subject data should be taken into account in the analysis of the study data.

A subject will be considered lost to follow-up if he or she fails to return for one year scheduled visits and/or is unable to be contacted by the study site staff.

The following actions must be taken if a subject fails to return for a required study visit:

- The site will attempt to contact the subject and reschedule the missed visit within 3 months and counsel the subject on the importance of maintaining the assigned visit schedule and ascertain if the subject wishes to and/or should continue in the study;
- Before a subject is deemed lost to follow-up, the investigator or designee will make every effort to regain contact with the subject (i.e. three telephone calls and a certified letter to the subject's last known mailing address or local equivalent methods). These contact attempts should be documented in the subject's medical record or study file;

- Should the subject continue to be unreachable, he or she will be considered to have withdrawn from the study with a primary reason of lost to follow-up.

9.2.6. Patient follow-up after study participation

Participation in the study will not affect the standard-of-care follow-up of the participants. Participants with sarcopenia or sarcopenic obesity may be given advice about physical activity. However, no specific recommendations does exist in this regard.

9.3. Strategies for participant recruitment

9.3.1. Recruitment process

- Participants will be identified among individuals with metabolic and endocrine diseases who are followed by diabetologists/endocrinologists at our center.
- Potential participants will receive a paper from their treating physician in the clinic. The first step in the recruitment process is for the treating physician to provide information about the study.
- The identification of potential participants involves accessing identifiable information from medical records, which the treating physician can access during a medical visit.
- The potential participants will be approached by the Principal Investigator (PI) or Co-Investigators (Co-PIs), and Clinical Research Coordinator (CRC). The PI or co-PI will obtain informed consent. The PI and co-PIs are diabetologists/endocrinologists and may be the treating physician (with prior relationships with some potential participants) or colleagues of the treating physician (without prior relationships with potential participants). The CRC is a nurse with no prior relationship with potential participants.

9.3.2. Informed consent process

Information related to the study is provided to patients by the investigator during the consultation, according to the requirements pertaining to consent covered by ICH-GCP (R3).
There are also informed they could withdraw their consent at any time during the study without any consequence. This point is written in the informed consent form.
Patients receive the patient information and consent form and have time to think about their participation to the study (one month). They have the opportunity to ask questions to the investigator (by email, phone or in consultation).
Patients come back after the reflection period. The investigator makes sure they have understood the information. They sign and date the informed consent form simultaneously with the investigator.
Patients receive a copy of the signed informed consent form.
If patients refuse the study, they will receive the standard treatment.

Special requirements :

- participants with temporary or definitive disabilities to give consent (intensive care/emergency unit, cognitive disorders, participants deprived of their rights): not applicable.
- emergency situations where an informed consent cannot be obtained prior the inclusion of the participant: not applicable.
- participants unable to sign or read the inform consent form (because of a health issue): not applicable.
- potential participants who do not speak the national language: not applicable. The inform consent will be given in French only. Only French-speaking patients will be included in the study.
- Minors: not applicable.

10. STUDY INTERVENTIONS AND PROCEDURES

The study will be conducted as follows:

Inclusion – Visit 0 (Screening/inclusion), scheduled up to 3 months prior to Visit 1 (Baseline): This 30-minute visit will take place at Cliniques Universitaires Saint-Luc.

The following procedures will be carried out:

- Study description
- Verification of inclusion and exclusion criteria
- Signing of the informed consent form
- Review of concomitant medications, medical history, and lifestyle habits (smoking, alcohol use)
- Physical examination (waist and hip circumference, weight, height, blood pressure)
- Study recommendations in willing participants: 1) fasting for at least 6 hours before blood and muscle samples; 2) avoiding intensive physical activity the day before and the day of muscle sampling; 3) discontinuing anti-diabetic medications 3 days prior to sampling (except in cases of T1D).

Baseline – Visit 1, scheduled 1 month +/- 1 week before standard-of-care treatment initiation for the metabolic or endocrine disease (can be combined with Visit 0 if no treatment is planned): This 45-minute visit will be conducted at Cliniques Universitaires Saint-Luc.

The following procedures will be performed:

- Review of concomitant medications and medical history
- Physical examination (waist and hip circumference, weight, height, blood pressure)
- Body composition measurement by BIA (fat mass %, lean mass %)
- CGM data collection (if applicable)
- Fasting blood sample (assessing liver, kidney, thyroid hormones, inflammation markers, circulating lipids, glucose, insulin, HbA1c, cortisol, and IGF-I)
- Fasting blood sample (myokines)*
- Fasting muscle biopsy of the thigh under local anesthesia*
- Abdominal CT scan (maximum 15 minutes) to assess abdominal fat and muscle levels; this procedure is non-invasive and completely painless.*
- Assessment of AE (any new or change in AE)

*In willing participants.

Follow-up – Visit 2, scheduled 3 months +/- 1 week after standard-of-care treatment initiation for the metabolic or endocrine disease: This 45-minute visit will be conducted at Cliniques Universitaires Saint-Luc and follow the same procedures as Visit 1.

Follow-up – Visit 3, scheduled 12 months +/- 3 months after Visit 1 (Baseline): This 45-minute visit will be conducted at Cliniques Universitaires Saint-Luc and follow the same procedures as Visit 2.

Follow-up – Visit 4, scheduled 60 months +/- 3 months after Visit 1 (Baseline): This 45-minute visit will be conducted at Cliniques Universitaires Saint-Luc and follow the same procedures as Visit 3.

End of Study – Visit 5, scheduled 120 months +/- 3 months after Visit 1 (Baseline): This 45-minute visit will be conducted at Cliniques Universitaires Saint-Luc and follow the same procedures as Visit 4.

See the course of the study in the flowchart.

10.1. Method of assigning participant to interventions groups

Participants will be divided into groups based on their underlying metabolic or endocrine disease. There will be no randomization.

10.2. Sample lab collection

10.2.1. Types and number of samples

- Blood samples: two 7.5 ml tubes of blood (Sarstedt EDTA S-monovette 01.1605.001 and Sarstedt serum gel S-Monovette 01.1602.001) will be collected after an overnight fast. Blood samples will be taken at baseline and at the other established timepoints according to the study group (Table 1).
- Muscle samples: one to three muscle samples (20-40mg) will be taken from the quadriceps femoris through a TruGuide Disposable Coaxial Biopsy Needle, as described above. Muscle samples will be taken at baseline and at the other established timepoints according to the study group (Table 1).

10.2.2. Timepoints of sample collection

Blood and muscle samples will be taken under standard conditions (overnight fast, 2-hour rest) at baseline and at the other established time points according to the study group (Table 1). A time window of one month will be allowed for each visit.

10.2.3. Sample handling and analysis

Blood samples will be taken at the phlebotomy center after an overnight fast in addition to the tubes collected as part of the standard-of-care. Two 7.5 ml tubes of blood (Sarstedt EDTA S-monovette 01.1605.001 and Sarstedt serum gel S-Monovette 01.1602.001) will be collected. All tubes must be filled to capacity. The tubes will be immediately placed at 4°C for 15 to 30 minutes. The samples must be processed within 30 minutes of collection. The tubes will be centrifuged at 2000g for 10 minutes at 4°C in the EDIN tabletop centrifuge to collect the plasma and serum. It is important to avoid contamination of the plasma/serum with the pellet. The plasma/serum will be aliquoted in 500 µl Eppendorf tubes with screw caps. The tubes will be labelled and stored within 30 minutes at -80°C at EDIN.

The PI or Co-PIs will take muscle samples after an overnight fast. After being taken from the quadriceps femoris, the samples will be cleaned of blood, adipose and connective tissue. Then, they will be placed into a 500 µL Eppendorf tube and immersed in liquid nitrogen. The tubes will be labelled and stored at -80°C at EDIN.

10.2.4. Sample storage and shipment

Plasma, serum and muscle samples will be stored in 500 µl Eppendorf tubes with screw caps at -80°C at EDIN lab, under the responsibility of Prof. JC Jonas. The sample storage tubes will be anonymized. Each participant will be identified by a unique study number. For RNA sequencing, muscle RNA will be sent to Dr. Amélie Bonnefond (EGID, European Genomic Institute for Diabetes, Lille, France), by an approved transporter (e.g. World Courrier) on dry ice. Each participant will be identified by a unique study number.

10.2.5. Future use of stored samples

Blood and muscle samples will be stored at -80°C at EDIN lab up to 30 years after the end of the study. Preserving these samples will allow them to be analysed using techniques that are currently under development or that are not currently available for technical or financial reasons.

10.3. Protocol Amendements

If amendments to the protocol (modifying sense or objectives or modifying the undergone constraints or the risks incurred by the subjects) turn out to be necessary, they will be submitted to the opinion of the Ethic Committee having examined the initial protocol.

10.4. Protocol Deviations

Sponsor and all investigators agree to take any reasonable actions to correct protocol deviations/violations. All deviations must be documented on a protocol deviation log by the study team that is kept available at any time for audit/inspection purposes. Under emergency circumstances, deviations from the protocol to protect the rights, safety or well-being of human subjects may proceed without prior approval of the sponsor and the EC.

Any significant deviation from study inclusion or exclusion criteria, study conduct, patient management or evaluation will be described, justified and communicated to the ethics committee, as appropriate.

11. SAFETY REPORTING

11.1. Definitions and reporting process

EVENTS	
Term	**Definition**
ADVERSE EVENT (AE)	Any untoward medical occurrence in a subject which does not necessarily have a causal relationship with the study procedures.
SERIOUS ADVERSE EVENT (SAE)	Any untoward medical occurrence in a subject that meets at least one of the following seriousness criteria: - Results in death; - Is life-threatening (immediate risk of death); - Requires inpatient hospitalisation or prolongation of existing hospitalisation; - Results in persistent or significant disability/incapacity; - Results in congenital anomaly/birth defect
RELATED UNEXPECTED SERIOUS ADVERSE EVENT	Any SAE where in the opinion of the Investigator the event was considered to be: **Related** –results from administration of any research procedures **Unexpected**–the type of event that not listed in the protocol as an expected occurrence

CAUSALITY	
Term	**Definition**
NOT RELATED	An adverse event which is not related to the study intervention.
UNLIKELY RELATED	An adverse event for which an alternative explanation is more likely and/or the relationship in time suggests that a causal relationship is unlikely.
POSSIBLY RELATED	An adverse event which might be due to the study intervention. An alternative explanation is inconclusive. The relationship in time is reasonable; therefore the causal relationship cannot be excluded.
PROBABLY RELATED	An adverse event which might be due to the study intervention. The relationship in time is suggestive. An alternative explanation is less likely.
DEFINITELY RELATED	An adverse event which is listed as related to the study intervention and cannot be reasonably explained by an alternative explanation. The relationship in time is very suggestive.

11.2. Assessing, Recording, and Analyzing Safety Parameters

11.2.1. Time Period and Frequency for Collecting AE and SAE Information

Adverse events

Collect and record on the CRF from the time the patient has signed the consent through last patient visit, all AEs/SAEs, including laboratory abnormalities, that are not known for the study intervention, that are potentially related to the study intervention and unrelated to the patient's disease or known history. Collect related events will be performed during the intervention and for a minimum of seven days.

Blood samples can cause mild pain or bruising, as observed in clinical practice. According to the literature and our experience, the incidence of side effects (pain, erythema, and bruising) after percutaneous muscle biopsy is very low (less than 1.5%) (Neves, 2011; Loumaye, 2015; Orioli, 2023). The radiation from a CT scan is very low (65.6mGy*cm) and lower than used in common diagnostic procedures, as reported previously at our center (Nachit, 2021).

Serious adverse events

All SAEs must be collected and require immediate (within 24 hours) notification from the time of signing the consent through last patient visit, during the intervention and for a minimum of seven days after. The investigator must report any SAE that occurs after these time periods and that is believed to be related to study intervention or protocol-specified procedure.

In the event that the investigator does not become aware of the occurrence of a serious adverse event immediately, the investigator is to report the event within 24 hours after learning of it and document the time of his/her first awareness of the adverse event.

11.2.2. Method of Detecting AEs and SAEs

AEs will be reported by the participant (or, when appropriate, by a caregiver, or a surrogate, or the participant's legally acceptable representative).

AEs can be spontaneously reported or elicited during open-ended questioning, examination, or evaluation of a participant. Care should be taken not to introduce bias when collecting AEs and/or SAEs. Inquiry about specific AEs should be guided by clinical judgement in the context of known AEs, when appropriate for the program or protocol.

AEs including SAEs occurring during at home period should be collected during in-clinic visits or during call with participants, and reported as early as possible (In the case of SAE, within 24 hours of learning of the event).

11.2.3. Follow-up of AEs and SAEs

Non-serious AEs should be followed to resolution or stabilization, or reported as SAEs if they become serious.

For adverse events with a causal relationship to study intervention, follow-up by the investigator is required until the event or its sequelae resolve or stabilize at a level acceptable to the investigator.

Follow-up is also required for non-serious AEs that cause interruption or discontinuation of study intervention and for those present at the end of study intervention as appropriate.

All identified non-serious AEs and/or laboratory abnormalities must be recorded and described on CRF.

Investigators are not obligated to actively seek AEs or SAEs in former study participants. However, if the investigator learns of any SAE, including a death, at any time after a participant has been discharged from the study, and he/she considers the event reasonably related to the study intervention or study participation, the investigator must promptly notify the Sponsor.

11.2.4. Regulatory Reporting Requirements for SAEs

Investigator's reporting

The investigator will report all SAEs to the sponsor within 24 hours after learning of it and document the time of his/her first awareness of the adverse event. Prompt notification by the investigator to the Sponsor of SAEs is essential so that legal obligations and ethical responsibilities toward the safety of participants are met.

The investigator uses the standard CIOMS SAE FORM (see Appendix) to submit the SAE to the sponsor. The investigator will submit any updated SAE data to the sponsor or designee within 24 hours of updated information being available.

The investigator will inform the sponsor in case of patient death, whatever the cause of the death could be, during the patient participation period within 24 hours after learning of it. The investigator will also inform the Ethics committee of patient death related to study intervention.

An investigator who receives an investigator safety report describing SAEs or other specific safety information (eg, summary or listing of SAEs) from the Sponsor will file it along with the Investigator's Brochure.

11.2.5. Laboratory Test Result Abnormalities

The following laboratory test result abnormalities should be captured on the CRF :

- Any laboratory test result that is clinically significant or meets the definition of an SAE
- Any laboratory test result abnormality that required the participant to have study intervention discontinued or interrupted
- Any laboratory test result abnormality that required the participant to receive specific corrective therapy

It is expected that, wherever possible, the clinical rather than laboratory term would be used by the reporting investigator (eg, anemia vs low hemoglobin value).

It should be noted that blood samples, muscle samples and CT scan specifically performed for the study (intervention) should not result in laboratory test result abnormalities.

12. DATA MANAGEMENT RESPONSIBILITIES

12.1. Data Quality Assurance

All study data will be handled in accordance with the law on General Data Protection Regulation (GDPR) and institutional rules [Belgian law dated on 20 July 2018 and 22 Aug. 2002].

The collection and processing of personal data from subjects enrolled in this study will be limited to those data that are necessary to fulfil the objectives of the study. These data must be collected and processed with adequate precautions to ensure confidentiality and compliance with applicable data privacy protection laws and regulations.

Appropriate technical and organizational measures to protect the personal data against unauthorized disclosures or access, accidental or unlawful destruction, or accidental loss or alteration must be put in place. Sponsor and site personnel whose responsibilities require access to personal data agree to keep the identity of subjects confidential.
The informed consent obtained from the subject includes explicit consent for the processing of personal data and for the investigator/institution to allow direct access to his or her original medical records (source data/documents) for study-related monitoring, audit, Ethics Committee review and regulatory inspection. This consent also addresses the transfer of the data to other entities, if applicable.

Privacy and confidentiality of data generated in the future on stored samples will be protected by the same standards applicable to all other clinical data. The investigator will ensure that the confidentiality of subjects' data will be preserved. On CRFs or any other documents, the subjects will not be identified by their names, but by their study number. Documents that identify the names of participants against their study number will be maintained by the investigator in strict confidence.

Monitors, auditors and other authorized agents will be granted direct access to study subject's original medical records for verification of clinical study procedures and/or data, without violating the confidentiality of the subjects, to the extent permitted by the law and regulations. In any presentations of the results of this study at meetings or in publications, the subjects' identity will remain confidential.

12.2. Statistical Analysis

For continuous data, cross-sectional differences between individuals with sarcopenia and individuals without sarcopenia will be assessed using a Student's t-test or Wilcoxon test, depending on the distribution of the data. Longitudinal differences will be assessed using a t-test for paired samples or a Mann-Whitney test, depending on the distribution of the data. For discrete data, cross-sectional differences will be assessed using a chi-square test and longitudinal differences using a McNemar test. Associations between myokines and muscle parameters (muscle mass and strength) will be investigated using univariate and multivariate linear regressions. Associations between myokines and

sarcopenia/sarcopenic obesity will be investigated using univariate and multivariate logistic regressions. The sample size is not pre-specified due to the exploratory nature of the study and the inclusion of individuals with rare diseases (e.g. Cushing's syndrome). Statistical significance will be set at < 0.05 unless otherwise stated. For RNA-Seq data, a correction for multiple comparisons will be applied to limit the FDR according to Benjamini-Hochberg (< 0.10).

12.3. Data handling and record keeping

Subjects who are included in the study will be assigned a unique study number. On all documents submitted to the sponsor, patients will only be identified by their study number.

An electronic case report form (eCRF) will be used in REDCap software. The eCRF will be completed for subjects who have signed the informed consent. This eCRF will include specific pages for inclusion and exclusion criteria, and for reporting each visit. Other specific pages will be dedicated to concomitant treatments and AEs (non-serious and serious). The investigator will review, approve and validate each completed eCRF; the investigator's signature (validation) serving as attestation of the investigator's responsibility for ensuring that all data entered on the eCRF are complete, accurate and authentic.
Subjects will be recorded in REDCap with their identification data and study number. Only participant numbers will appear in the extracted data at the end of the study.

All data will be processed according to the principles that the new European General Data Protection Regulation (GDPR) imposes, which is in force since 25 May 2018.

1. Who will responsible for the processing of personal data?
The investigator-sponsor and co-investigators as identified in the delegation log.

2. Who is Data Protection Officer for the processing?
The institutional DPO could be reached by this email address : rgpd@saintluc.uclouvain.be

3. The purpose of the processing: Scientific research

4. The legal basis of the processing: Consent, but this can be withdrawn

4. Who are potential recipients of the personal data?
All researchers involved in this clinical study or in research projects that use materials original from this clinical study. Staff involved in monitoring and ethical evaluation and people from competent authorities. Subcontracted parties that perform analysis on study-related data or materials.

5. It is possible that the personal data will be viewed by people who are in countries that do not use the same standards as the EU in terms of legal protection of data. In that case, we guarantee that the conditions of European and Belgian legislation on the protection of personal data will be respected.

6. The storage period: Study-related documents will be stored for at least 20 years, data included in the medical file for 30 years.

12.4. Case Report Form

An electronic data capture (EDC) system, i.e. REDCap, will be used for data collection. Data reported on each eCRF should be consistent with the source data. If information is not known, this must be clearly indicated on the eCRF. All missing and ambiguous data will be clarified.
The eCRFs will be developed, based on the protocol. The final eCRF design will be approved by the Coordinating Investigator.
All data entries and corrections will only be performed by study site staff, authorized by the investigator. Data will be checked and any errors or inconsistencies will be clarified. The investigator must verify that all data entries in the eCRF are accurate and correct.

REDCap is provided and maintained by Vanderbilt University; a license for use was granted to the CUSL. REDCap is a web-based system.

12.5. Data storage

The data is accessed through a web browser directly on the secure REDCap server. The server is hosted within the Cliniques universitaires Saint-Luc campus and meets hospital level security and back-up requirements.

12.6. Access to data

Direct access will be granted to authorised representatives from the Sponsor, host institution and the regulatory authorities to permit study-related monitoring, audits and inspections.
Login in REDCap is password controlled. Each user will receive a personal login name and password and will have a specific role which has predefined restrictions on what is allowed in REDCap. Any activity in the software is traced and transparent via the audit trail and log files.

13. INSURANCE

The experimentation is covered under the Belgian Law of May 7, 2004 by a no-fault insurance (type of coverage: liability insurance).

Policy holder:
Cliniques universitaires Saint-Luc
Avenue Hippocrate, 10
1200 Brussels

Issuer of the certificate of insurance:
MS Amlin Insurance SE
Boulevard du Roi Albert II, 37
1030 Brussels
N° de police : LXX00259

14. END OF STUDY

14.1. For an individual subject

The subject has completed the study if he or she has completed all of study procedures, including the last visit or the last scheduled procedure, as described in this protocol (see section “Study Specific Procedures”).

14.2. For the whole study

Overall, the end of the study is reached when the last study procedure for the last subject has occurred: last subject, last visit (LSLV).

As soon as the whole study has ended (cfr the definition above), the Investigator-sponsor shall notify the Ethics Committee in a timely manner according to the regulatory requirements (within 90 days after the end of the study, or if the study had to be terminated early, this period must be reduced to 15 days and the reasons should clearly explained).

15. DISSEMINATION OF RESULTS AND PUBLICATION POLICY

This study is registered on Clinicalstudys.gov (https://clinicalstudys.gov/) and is available to the public. Authorship will be discussed prior to publication.

The following text would be added to publication for REDCap use : *"Study data were collected and managed using REDCap electronic data capture tools hosted at Cliniques universitaires Saint-Luc. REDCap (Research Electronic Data Capture) is a secure, web-based software platform designed to support data capture for research studies, providing 1) an intuitive interface for validated data capture; 2) audit trails for tracking data manipulation and export procedures ; 3) automated export procedures for seamless data downloads to common statistical packages ; and 4) procedures for data integration and interoperability with external sources."*

16. ARCHIVING

Essential clinical study documents are kept at least 20 years after the study termination according to the Belgian legislation: RD 18 May 2006 art.24.

Source documentation are kept for 30 years, according to the Belgian legislation (Art 35 Belgian Law of 22 April 2019).

Specify who archives, where and access conditions.

17. STUDY REPORT

This study is expected to lead to several scientific communications throughout its duration. These will be reviewed by the principal investigator and collaborators.

18. LITERATURE REFERENCES

Alexopoulos T et al. Myostatin in combination with creatine phosphokinase or albumin may differentiate patients with cirrhosis and sarcopenia. Am J Physiol Gastrointest Liver Physiol. 2021 Nov 1;321(5):G543-G551. doi: 10.1152/ajpgi.00184.2021.

American Diabetes Association Professional Practice Committee. 7. Diabetes Technology: Standards of Care in Diabetes-2025. Diabetes Care. 2025 Jan 1;48(Supplement_1):S146-S166. doi: 10.2337/dc25-S007. Erratum in: Diabetes Care. 2025 Apr 1;48(4):666. doi: 10.2337/dc25-er04b.

Aubrey J et al. Measurement of skeletal muscle radiation attenuation and basis of its biological variation. Acta Physiol (Oxf). 2014 Mar;210(3):489-97. doi: 10.1111/apha.12224.

Barlow JP, Solomon TP. Do skeletal muscle-secreted factors influence the function of pancreatic β-cells? Am J Physiol Endocrinol Metab. 2018 Apr 1;314(4):E297-E307. doi: 10.1152/ajpendo.00353.2017.

Buckinx F et al. Pitfalls in the measurement of muscle mass: a need for a reference standard. J Cachexia Sarcopenia Muscle. 2018 Apr;9(2):269-278. doi: 10.1002/jcsm.12268.

Cruz-Jentoft AJ et al. Sarcopenia: revised European consensus on definition and diagnosis. Age Ageing. 2019 Jan 1;48(1):16-31. doi: 10.1093/ageing/afy169.

Davies TF, Andersen S, Latif R, Nagayama Y, Barbesino G, Brito M, Eckstein AK, Stagnaro-Green A, Kahaly GJ. Graves' disease. Nat Rev Dis Primers. 2020 Jul 2;6(1):52. doi: 10.1038/s41572-020-0184-y.

Deshpande D et al. RNA-seq data science: From raw data to effective interpretation. Front Genet. 2023 Mar 13;14:997383. doi: 10.3389/fgene.2023.997383.

Donini LM et al. Definition and Diagnostic Criteria for Sarcopenic Obesity: ESPEN and EASO Consensus Statement. Obes Facts. 2022;15(3):321-335. doi: 10.1159/000521241.

Eckardt K et al. Myokines in insulin resistance and type 2 diabetes. Diabetologia. 2014 Jun;57(6):1087-99. doi: 10.1007/s00125-014-3224-x.

Fearon K et al. Myopenia-a new universal term for muscle wasting. J Cachexia Sarcopenia Muscle. 2011 Mar;2(1):1-3. doi: 10.1007/s13539-011-0025-7.

Feng L, Gao Q, Hu K, Wu M, Wang Z, Chen F, et al. Prevalence and Risk Factors of Sarcopenia in Patients With Diabetes: A Meta-analysis. J Clin Endocrinol Metab. 2022;107(5):1470-83.

Fleseriu M, Biller BMK, Freda PU, Gadelha MR, Giustina A, Katznelson L, Molitch ME, Samson SL, Strasburger CJ, van der Lely AJ, Melmed S. A Pituitary Society update to acromegaly management guidelines. Pituitary. 2021 Feb;24(1):1-13. doi: 10.1007/s11102-020-01091-7.

Florin A et al. The secretome of skeletal muscle cells: A systematic review. Osteoarthr Cartil Open. 2020 Jan 2;2(1):100019. doi: 10.1016/j.ocarto.2019.100019.

Guarnotta V, Prinzi A, Pitrone M, Pizzolanti G, Giordano C. Circulating Irisin Levels as a Marker of Osteosarcopenic-Obesity in Cushing's Disease. Diabetes Metab Syndr Obes. 2020;13:1565-74.

Gueugneau M, d'Hose D, Barbe C, de Barsy M, Lause P, Maiter D, et al. Increased Serpina3n release into circulation during glucocorticoid-mediated muscle atrophy. J Cachexia Sarcopenia Muscle. 2018;9(5):929-46.

Hittel DS et al. Increased secretion and expression of myostatin in skeletal muscle from extremely obese women. Diabetes. 2009 Jan;58(1):30-8. doi: 10.2337/db08-0943.

Janssen I et al. Estimation of skeletal muscle mass by bioelectrical impedance analysis. J Appl Physiol (1985). 2000 Aug;89(2):465-71. doi: 10.1152/jappl.2000.89.2.465.

Kahaly, G., Bartalena, L., Hegedüs, L., Leenhardt, L., Poppe, K., & Pearce, S. (2018). 2018 European Thyroid Association Guideline for the Management of Graves' Hyperthyroidism. *European Thyroid Journal*, *7*(4), 167-186. Retrieved Jul 7, 2025, from https://doi.org/10.1159/000490384.

Levy JC et al. Correct homeostasis model assessment (HOMA) evaluation uses the computer program. Diabetes Care. 1998 Dec;21(12):2191-2. doi: 10.2337/diacare.21.12.2191.

Loumaye A et al. Role of Activin A and myostatin in human cancer cachexia. J Clin Endocrinol Metab. 2015 May;100(5):2030-8. doi: 10.1210/jc.2014-4318.

Loumaye A, Lause P, Zhong X, Zimmers TA, Bindels LB, Thissen JP. Activin A Causes Muscle Atrophy through MEF2C-Dependent Impaired Myogenesis. Cells. 2022 Mar 25;11(7):1119. doi: 10.3390/cells11071119.

Ma K, Mallidis C, Bhasin S, Mahabadi V, Artaza J, Gonzalez-Cadavid N, et al. Glucocorticoid-induced skeletal muscle atrophy is associated with upregulation of myostatin gene expression. Am J Physiol Endocrinol Metab. 2003;285(2):E363-71.

Mijnarends DM et al. Validity and reliability of tools to measure muscle mass, strength, and physical performance in community-dwelling older people: a systematic review. J Am Med Dir Assoc. 2013 Mar;14(3):170-8. doi: 10.1016/j.jamda.2012.10.009.

Nachit M et al. A dynamic association between myosteatosis and liver stiffness: Results from a prospective interventional study in obese patients. JHEP Rep. 2021 Jun 15;3(4):100323. doi: 10.1016/j.jhepr.2021.100323.

Neves M Jr et al. Incidence of adverse events associated with percutaneous muscular biopsy among healthy and diseased subjects. Scand J Med Sci Sports. 2012 Apr;22(2):175-8. doi: 10.1111/j.1600-0838.2010.01264.x.

Nunan E, Huff DR, Gore JL, Wright CL, Harris T, Butler L, Padgett CA, Rochowski MT, Lovern PC, Boolani A, Valdez C, Butcher JT. Targeting Myostatin as an Adjunct Treatment for the Preservation of Cardiometabolic and Skeletal Muscle Function in Type 1 Diabetes. Int J Mol Sci. 2025 May 18;26(10):4830. doi: 10.3390/ijms26104830.

Orioli L et al. 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.

Orioli L et al. Circulating myostatin as a biomarker of muscle mass and strength in individuals with cancer or obesity. Clin Nutr. 2024 Jul;43(7):1800-1808. doi: 10.1016/j.clnu.2024.05.046.

Orioli L et al. Myokines as potential mediators of changes in glucose homeostasis and muscle mass after bariatric surgery. Front Endocrinol (Lausanne). 2025 Mar 18;16:1554617. doi: 10.3389/fendo.2025.1554617.

Reincke M, Fleseriu M. Cushing Syndrome: A Review. JAMA. 2023 Jul 11;330(2):170-181. doi: 10.1001/jama.2023.11305.

Reimand J et al. Pathway enrichment analysis and visualization of omics data using g:Profiler, GSEA, Cytoscape and EnrichmentMap. Nat Protoc. 2019 Feb;14(2):482-517. doi: 10.1038/s41596-018-0103-9.

Sergi G et al. Assessing appendicular skeletal muscle mass with bioelectrical impedance analysis in free-living Caucasian older adults. Clin Nutr. 2015 Aug;34(4):667-73. doi: 10.1016/j.clnu.2014.07.010.

Wang Z et al. RNA-Seq: a revolutionary tool for transcriptomics. Nat Rev Genet. 2009 Jan;10(1):57-63. doi: 10.1038/nrg2484. PMID: 19015660; PMCID: PMC2949280.

Wei S et al. Sarcopenic obesity: epidemiology, pathophysiology, cardiovascular disease, mortality, and management. Front Endocrinol (Lausanne). 2023 Jun 30;14:1185221. doi: 10.3389/fendo.2023.1185221.

Yalcin MM, Akturk M, Tohma Y, Cerit ET, Altinova AE, Arslan E, et al. Irisin and Myostatin Levels in Patients with Graves' Disease. Arch Med Res. 2016;47(6):471-5.