

# Research Protocol

## Assessment of Nutritional Status in Celiac Disease Using Muscle Ultrasound

This protocol describes the study objective, scope, methodology, materials and methods, sample size, and power analysis.

### Project Management

| Title and Name                    | Project Role           | Responsibility           | Signature |
|-----------------------------------|------------------------|--------------------------|-----------|
| MD Fatma Demirbaş Ar,             | Principal Investigator | Responsible investigator |           |
| MD. Atakan Comba,                 | Researcher             | Research activities      |           |
| Serhan Eren, MD, Specialist       | Researcher             | Research activities      |           |
| Asst. Prof. Gurbet Yanar Ateş, MD | Researcher             | Research activities      |           |

### Scientific Research Summary

**Project title in Turkish:** Çölyak hastalığında beslenme durumunun kas ultrasonu ile değerlendirilmesi

**Project title in English:** Assessment of Nutritional Status in Celiac Disease Using Muscle Ultrasound

### Background and Objective

Celiac disease is an autoimmune disorder with a familial predisposition that develops in genetically susceptible individuals following exposure to gluten in cereals and cereal products. It causes characteristic small-intestinal lesions and malabsorption and improves clinically with a gluten-free diet. Beyond the gastrointestinal tract, skeletal muscle health may be adversely affected by malabsorption and malnutrition. Sarcopenia, characterized by reductions in muscle mass and muscle strength and most commonly recognized in older adults, may also occur in patients with celiac disease at an early age because of chronic inflammation, impaired protein absorption, and reduced nutrient intake. The European Society for Paediatric Gastroenterology, Hepatology and Nutrition recommends regular assessment of growth and nutritional status in children with celiac disease.

Although magnetic resonance imaging and computed tomography can provide reference measurements of muscle mass, their clinical use is limited by cost, accessibility, lack of portability, and, in the case of computed tomography, exposure to ionizing radiation. Ultrasonography has emerged as a promising method for assessing muscle thickness and identifying a risk of low muscle mass. It is non-invasive, inexpensive, widely available, and radiation-free. Its use may increase awareness of impaired muscle health, facilitate earlier nutritional intervention, and potentially improve clinical and laboratory outcomes in celiac disease.

This study will assess nutritional status by performing muscle measurements during routine ultrasonographic examinations in pediatric patients diagnosed with celiac disease at the pediatric gastroenterology units of Balıkesir Atatürk City Hospital and Hitit University. The study will also evaluate the ability of ultrasound-derived muscle measurements to identify reduced muscle mass and the risk of sarcopenia in these patients.

## Methods Summary

This observational prospective cohort study will include children followed with a diagnosis of celiac disease at the pediatric gastroenterology units of Balıkesir Atatürk City Hospital and Hitit University, together with children without chronic disease who undergo ultrasonography as part of routine clinical evaluation for symptoms such as abdominal pain, vomiting, or diarrhea. Demographic and clinical characteristics will be recorded for participants who meet the eligibility criteria.

Anthropometric measures, including age, sex, height, body weight, and body mass index, will be evaluated. Available clinical and laboratory parameters, particularly those reflecting nutritional status, will also be examined.

Muscle mass will be evaluated using ultrasonography. Muscle thickness and the muscle measurements specified in the protocol will be obtained from predefined anatomical sites during routine ultrasonographic examination, using a standardized technique. Ultrasound-derived muscle measurements will be compared with anthropometric characteristics and indicators of nutritional status, and their relationships with clinical and laboratory variables will be analyzed. Appropriate statistical methods will be used to investigate the potential value of muscle ultrasonography for assessing reduced muscle mass and the risk of sarcopenia in children with celiac disease.

## Expected Results

A significant association is expected between nutritional status and ultrasound-derived indicators of muscle thickness or muscle mass in children with celiac disease. Muscle measurements are anticipated to be lower in participants with nutritional insufficiency or a greater risk of active disease and malabsorption. The study will evaluate whether ultrasound-derived muscle measurements constitute a practical method for detecting reduced muscle mass and the risk of sarcopenia in celiac disease. It will also provide evidence regarding whether ultrasonography is reproducible, safe, and readily integrated into pediatric clinical practice.

**Keywords:** celiac disease, muscle ultrasound, pediatrics, sarcopenia

# Main Elements of the Research Project

## Topic and Rationale

Celiac disease is a chronic autoimmune disorder that develops after gluten exposure in genetically susceptible individuals and is characterized by small-intestinal mucosal injury and consequent malabsorption. Its clinical manifestations are not limited to the gastrointestinal system.

Malabsorption, inadequate energy and protein intake, micronutrient deficiencies, and chronic inflammation may adversely affect growth, development, body composition, and muscle health. Reduced muscle mass and muscle function may occur particularly in children with delayed diagnosis or nutritional insufficiency.

Sarcopenia is primarily characterized by reduced muscle mass and muscle function. In childhood, however, it must be evaluated in the context of ongoing growth and development. Chronic inflammation, intestinal malabsorption, inadequate nutrient intake, and associated protein-energy malnutrition may impair muscle mass and function in celiac disease. Early assessment of nutritional status and muscle health is therefore important for identifying patients who require nutritional support.

Magnetic resonance imaging and computed tomography can be used to assess muscle mass, but they have important disadvantages, including high cost, limited availability, lack of portability, and exposure to ionizing radiation with computed tomography. Especially in children, there is a need for a safe, non-invasive, inexpensive, and readily applicable alternative. Ultrasonography can assess muscle thickness and structural characteristics, is radiation-free, and is widely available in clinical practice. These characteristics support its potential use for evaluating muscle mass and screening for sarcopenia in pediatric populations.

## Study Objective

The primary objective is to perform muscle measurements during routine ultrasonographic examinations in children with celiac disease followed at the pediatric gastroenterology units of Balıkesir Atatürk City Hospital and Hitit University and in children without chronic disease who undergo ultrasonography during routine evaluation for symptoms such as abdominal pain, vomiting, or diarrhea. The relationship between these measurements and nutritional status will be investigated. Associations between ultrasound-derived muscle measurements and anthropometric and laboratory indicators will be examined, and the clinical utility of ultrasonography for identifying possible reductions in muscle mass and the risk of sarcopenia in children with celiac disease will be evaluated.

The study is expected to contribute to earlier recognition of impaired muscle health and nutritional status in pediatric celiac disease, to evaluate muscle ultrasonography as a practical and safe assessment method, and to facilitate early identification of patients who may require nutritional support.

## References

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## Materials and Methods

Following approval by the Ethics Committee, participants will be evaluated at the pediatric gastroenterology clinics of Hitit University and Balıkesir Atatürk City Hospital. Muscle measurements obtained during routine ultrasonography will be assessed together with demographic characteristics; clinical and laboratory findings; endoscopic and histopathological findings; treatments; and clinical outcomes. Relevant information will be obtained from patient files and Hospital Information Management System records. No additional blood samples will be collected specifically for this study.

### 1 Study Design

**Study type:** Prospective cohort study

**Research hypothesis:** Children with celiac disease may develop sarcopenia associated with malnutrition.

**Study period:** Recruitment will begin within one month after Ethics Committee approval.

### 2 Participants

#### 2.1 Inclusion Criteria for the Celiac Disease Group

- Age 1-18 years.
- A diagnosis of celiac disease supported by serological findings and endoscopic biopsy findings.
- Availability of the clinical, laboratory, anthropometric, and ultrasonographic data required by the study.
- Written informed consent from a parent or legal representative for the child's participation.
- When appropriate for age and developmental level, assent from the child or adolescent.

#### 2.2 Inclusion Criteria for the Control Group

- Age 1-18 years.
- No known chronic disease.
- Ultrasonography performed as part of routine clinical evaluation for gastrointestinal symptoms such as abdominal pain, vomiting, or diarrhea.
- Availability of the data required by the study.
- Written informed consent from a parent or legal representative and, when appropriate, assent from the child.

#### 2.3 Exclusion Criteria

- Age older than 18 years.
- A known chronic systemic disease that could affect the study assessments.
- A musculoskeletal or neuromuscular disorder that could affect muscle measurements.
- Inability to obtain reliable ultrasonographic muscle measurements.
- Insufficient clinical, anthropometric, or laboratory data.
- Absence of written informed consent from the parent or legal representative, or refusal of the child to participate when assent is required.

### 3 Data Collection

#### 3.1 Demographic and Clinical Data

Age, sex, socioeconomic characteristics, parental consanguinity, a history of similar disease among siblings, and the presence of known chronic disease will be recorded. For participants with celiac

disease, age at diagnosis, time since diagnosis, adherence to the gluten-free diet, duration of follow-up, clinical manifestations, and current treatment characteristics will also be evaluated. Current clinical status and follow-up outcomes will be obtained from patient files and Hospital Information Management System records.

### **3.2 Laboratory Assessment**

Laboratory results obtained during routine follow-up of participants with celiac disease will be evaluated. These may include complete blood count, biochemical parameters, ferritin, serum iron, total iron-binding capacity, vitamin B12 and other relevant micronutrient levels, tissue transglutaminase IgA and IgG, total serum IgA, and other celiac serological markers when indicated. Existing results obtained during routine clinical care will be used, without collecting additional blood samples specifically for the study.

### **3.3 Anthropometric Measurements**

Height, body weight, and body mass index will be assessed in all participants. Percentiles and Z scores for height, weight, and body mass index will be calculated using World Health Organization reference data.

### **3.4 Ultrasonographic Measurements**

Ultrasonography will be used to assess muscle mass. Measurements will be performed during ultrasonography undertaken as part of routine clinical assessment, without any additional invasive procedure. Participants will be examined in the supine position, with the lower extremities positioned as consistently as possible and the knees extended.

Muscle thickness measurements will be performed independently by two investigators. Multiple measurements will be obtained for each muscle, and their mean will be accepted as the muscle thickness value for that muscle. To reduce the effect of anthropometric differences, a normalized muscle measurement will be calculated by dividing the sum of the muscle thickness measurements by height squared. Measurements obtained by the two investigators will be compared, and interobserver agreement will be analyzed when appropriate.

## **4 Sample Size and Power Analysis**

The computer-generated power analysis output will be appended to the protocol. To detect a difference between two independent groups, assuming a moderate effect size (Cohen's  $d = 0.60$ ), a two-sided significance level of 5% ( $\alpha = 0.05$ ), and 80% statistical power, at least 45 participants are required in each group. The planned minimum total sample size is therefore 90 participants.