

## Article

# Dietary behavior and risk of orthorexia in patients with celiac disease

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**Abstract:** Evidence points to a link between gluten-free diet or celiac disease and eating disorders, but not with orthorexia. This study is the first to examine adults with celiac disease in terms of the prevalence of risk of orthorexia. The study included 130 adults diagnosed with celiac disease. The standardized ORTHO-15 questionnaire was used to assess the risk of orthorexia. Cronbach's alpha test was used to determine the reliability of the ORTHO-15 questionnaire. Eating habits of the subjects were assessed using a questionnaire. The effect of celiac disease on diet was assessed on a 5-point scale. A risk of orthorexia was found in 69% of subjects with celiac disease. A statistically significant ( $p < 0.005$ ) positive correlation was observed between age and ORTHO-15 test scores ( $\rho = 0.37$ ). In the group with orthorexia risk, meals were more often self-prepared (93.3%) compared to those without orthorexia (80%) ( $p = 0.023$ ). For 80% of those at risk for orthorexia versus 20% without risk, mood was a factor influencing dietary choices ( $p = 0.001$ ). The study observed a strong association between celiac disease and the presence of orthorexia risk. The numerous risk factors for orthorexia suggest the need for holistic care, including nutritional and psychological support among individuals with celiac disease.

**Keywords:** celiac disease; orthorexia; eating disorders; ORTHO-15; nutrition

## 1. Introduction

Celiac disease is an autoimmune disease characterized by malabsorption resulting from chronic inflammation of the small intestinal mucosa caused by gluten ingested in the diet, [1] The prevalence of celiac disease is approximately 1% of the population in Europe and the USA, but an increasing trend is observed in many countries [2]. The basic therapy in patients diagnosed with celiac disease is strict adherence to a gluten-free diet throughout life [3].

Orthorexia is an eating disorder characterized by an obsession with eating only healthy foods, which consequently leads to significant dietary restrictions and, in extreme cases, to abandon food consumption altogether [4]. The average prevalence rate of orthorexia is 6.9% for the general population and 35-57.8% for high-risk groups [5].

The diagnostic criteria for orthorexia have not yet been standardized [6]. The most commonly used tool to assess the risk of orthorexia is the ORTHO-15 questionnaire developed by Donini et al. [7].

While people with celiac disease must exclude gluten from foods for health reasons, people with orthorexia voluntarily pursue healthy eating through a restrictive diet, focusing on food preparation, and analyzing the sources of the foods they eat [8]. People with orthorexia exhibit obsessive behaviors related to maintaining and improving their physical health and well-being [9]. Excessive focusing on elimination, initially of small groups of products, especially unhealthy, i.e. fried, grilled, rich in simple sugars, and later of whole groups of products, leads in consequence to deficiencies of calories, nutrients, vitamins and minerals [10, 11]. In addition, orthorexia can lead to real difficulties

both physical and psychological, such as gastrointestinal problems, gastritis, anemia, osteopenia, social isolation, feelings of guilt and lack of self-acceptance after consuming unadvisable food [12].

There are no studies in the literature on the association of celiac disease with orthorexia, but there are reports of an association between celiac disease and the occurrence of eating disorders [13]. Passannanti et al. [14] found that celiac disease itself, rather than gastrointestinal symptoms or psychological factors, may contribute to pathological eating behaviors in adults with celiac disease. It has been observed that eating disorders may be more common in young women with celiac disease than in men, both healthy and with celiac disease. The increased frequency of eating disorders may be related to the obsessive consumption of some types of food [15], as for example intake of added sugar has been shown to be higher in people with celiac disease than in healthy individuals [16].

With the increasing trend of celiac disease, there may be an increasing risk of orthorexia, which is still a newly recognized disorder [15]. The purpose of this study was to evaluate the eating habits of individuals with celiac disease to assess the risk of orthorexia and to fill gaps in knowledge on this topic.

## 2. Materials and Methods

### 2.1. Study group

The study was conducted in 2018-2019 among 130 people with celiac disease, aged 18-64, affiliated with online celiac disease social media groups. The sample size was calculated based on the prevalence of celiac disease in the Polish population which is estimated to be 1%, with confidence interval 95% and margin of error 1.71%. Inclusion criteria were age over 18 years and diagnosed celiac disease. The method of diagnosis in subjects was the presence of one of the anti-tissue transglutaminase (tTG), anti-smooth muscle endomysial (EmA) antibodies and a small bowel biopsy result of Marsh's scale classification  $\geq 3a$ . The largest group of patients were those who had been ill for several years ( $n=77$ ), followed by newly diagnosed patients ( $n=32$ ) and patients ill for more than several years ( $n=21$ ). Demographic information was collected that included age, gender, education, work situation. The health history included methods of diagnosis, duration of celiac disease and presence of comorbidities. Nutritional status was assessed with anthropometric measurements to determine BMI for which the 2019 WHO standards were adopted [17]. Eating behavior was assessed with a questionnaire developed for this study.

The study was approved by the Bioethics Committee.

### 2.2. Eating habits

Dietary habits were examined using a dietary survey questionnaire. The frequency of eating meals per day, the difficulty of eating meals out, the way of preparing gluten-free meals, paying attention to the calorific value of food and the composition of food products, as well as the influence of celiac disease on the way of eating were analyzed on a 5-point intensity scale (1 - does not influence at all or to a small extent, 5 - influences very much).

### 2.3. Questionnaire ORTHO-15

The validated Polish version of the ORTHO-15 self-report questionnaire was used in this study [18, 19] based on the original questionnaire ORTHO-15 by Donini et al. [20]. In the ORTHO-15 test each question is answered by the subject on a four-point Likert scale: always, often, rarely, never, which are assigned 1, 2, 3, 4 points respectively. A cut-off point defined by Donini et al. [20] at 40 points was used. Scoring lower indicates a propensity for orthorexia. The results obtained with the ORTHO-15 questionnaire were interpreted using the reference recommendations of its authors by calculating the total score assigned to individual responses. The sum of points obtained by the respondent (theoretical range: 15-60) constitutes the so-called orthorexia risk index (ORI).

#### 2.4. Statistical analysis

The results obtained were statistically analyzed using Statistica 13.3 StatSoft program. Results with a significance level of  $p < 0.05$  were considered significant.

Chi-square test of concordance, Spearman's correlation and alpha-Cronbach's coefficient for reliability analysis of psychological tests were used for data analysis and intergroup comparisons.

The ORTHO-15 questionnaire was subjected to reliability analysis to confirm the consistency of the test. The reliability analysis of the ORTHO-15 questionnaire included 15 questions and responses to the questions provided by the research participants. The reliability of the questionnaire was analyzed by examining the statistical properties of the test items and the relationship of the test items to the overall test score. An alpha coefficient between 0.60 and 0.90 was taken as a satisfactory level of consistency of the questionnaire as recommended [21,22].

### 3. Results

#### 3.1. Characteristics of the study participants.

Table 1. shows the general characteristics of the study participants. The study group was predominantly female ( $n=123$ , approximately 95%). The median age was 34 years, and the median BMI was 21.4 kg/m<sup>2</sup>. The majority of subjects had tertiary education (78.5%). Permanent employment declared 75% of respondents. Normal body weight was observed in the majority of the group, 73.2 %. The main methods of diagnosing people with celiac disease were small bowel biopsy and Marsh scale scores  $\geq 3a$  (in 75.4% of subjects), as well as tissue transglutaminase (tTG) antibody testing (in 54.6% of subjects). The largest group of people, about 60%, were patients for several years (3-10 years). The least numerous group were patients for more than several years, 16%. The most common comorbidities were: lactose intolerance (in 18% of individuals) and Hashimoto's disease (in 16% of individuals). Strict adherence to a gluten-free diet was declared by 82% of respondents. In the subjective assessment of their health status, as many as 86% of the subjects rated that their health was better after following a gluten-free diet.

**Table 1.** General characteristics of the study group ( $n=130$ ).

Parameter	Value
Number of women (n)	123
Age (y)	
(median/Q1-Q3)	34 (28-39)
(Average/age range)	34.55 $\pm$ 9.0
BMI (kg/m <sup>2</sup> )	
(Average/range)	21.63 (14.1-29.75)
(median/Q1-Q3)	21.4 (19.4 - 23.7)
Education (%)	
Tertiary	78.5
Secondary	17.7
Middle	3.0
Primary	0.8
Occupation (%)	
Pension/retirement	1.5%
odd job	2.3%
unemployed	5.4%
parental leave	7.7%

students	7.7%
Permanent employment	75.4%
Nutritional Status (W%/M%)	
Underweight (moderate thinness (16.0-16.99)	6/0%
Underweight (mild thinness) (17.0-18.49)	11/0%
Normal body weight (18.5-24.99)	73/57%
Overweight (25.0-29.99)	10/43%
Methods of diagnosis in people with celiac disease (multiple response question) (%)	
small bowel biopsy and Marsh <u>score</u> $\geq$ 3a	75.4%
antibodies against tissue transglutaminase (tTG)	54.6%
anti-smooth muscle endomysial antibodies (EmA)	26.9%
genetic testing	21%
Duration of celiac disease (n/%)	
newly diagnosed (1-2 years)	32 (24.6%)
diagnosed 3-10 years ago	77 (59.2%)
diagnosed more than 10 years ago	21 (16.2%)
Comorbidities (%)	
Lactose intolerance	18%
Hashimoto's disease	16%
hypothyroidism	12%
Food allergy	12%
Duhring's disease	4.6%
The most common symptoms reported before treatment of celiac patients	
Bloating	52%
Chronic diarrhea	49%
Anemia	42%
Low body weight	23%
Adherence to gluten-free diet (%)	
Full	82%
Health self-assessment on a gluten-free diet (%)	
Better	86.20%
No change	9.2%
Worse	3.1%
No answer	1.5%

### 3.2. Reliability assessment of the ORTHO-15 questionnaire

Raw Cronbach's  $\alpha$  coefficient in this study was 0.69 and the standardized coefficient was  $\alpha = 0.68$ . Table 2. shows the mean scores, the correlations of each question with the overall score, as well as the values of the raw and standardized Cronbach's  $\alpha$  coefficients, after the removal of a question from the questionnaire, if any. The highest correlation coefficients were recorded for the influence of mood on eating behavior ( $\rho=0.60$ ), worries about eating for the past 3 months ( $\rho=0.53$ ) and for more than 3 hours a day ( $\rho=0.53$ ), and the belief of increased self-esteem due to eating only healthy foods ( $\rho=0.51$ ).

**Table 2.** Reliability analysis of individual questions of the ORTHO-15 questionnaire.

Questions from ORTHO-15 test	Score (mean ± SD)	Spearman's rho	Cronbach's alpha coefficient, raw
1. When eating, do you pay attention to the calories of the food?	2.95 ± 0.90	0.34	0.66
2. When you go in a food shop do you feel confused?	3.38 ± 0.88	0.33	0.67
3. In the last 3 months, did the thought of food worry you?	2.60 ± 1.00	0.53	0.63
4. Are your eating choices conditioned by your worry about your health status?	1.65 ± 0.61	0.12	0.69
5. Is taste of food more important than the quality when you evaluate food?	2.52 ± 0.76	0.10	0.69
6. Are you willing to spend more money to have healthier food?	2.02 ± 0.65	0.11	0.69
7. Does the thought about food worry you for more than three hours a day?	3.35 ± 0.87	0.53	0.64
8. Do you allow yourself any eating transgressions?	3.02 ± 0.82	-0.04	0.71
9. Do you think your mood affects your eating behavior?	2.33 ± 0.80	0.60	0.63
10. Do you think that the conviction to eat only healthy food increases self-esteem?	2.98 ± 0.92	0.51	0.64
11. Do you think that eating healthy food changes your life-style (frequency of eating out, friends...)?	2.41 ± 0.93	0.36	0.66
12. Do you think that consuming healthy food may improve your appearance?	2.12 ± 0.87	0.42	0.65
13. Do you feel guilty when transgressing?	2.52 ± 1.13	0.26	0.68
14. Do you think that on the market there is also unhealthy food?	1.46 ± 0.71	0.07	0.69
15. At present, are you alone when having meals?	2.70 ± 0.82	0.08	0.70

Table 3. shows the reliability analysis for the scoring with 15 questions (ORTHO-15) and excluding two questions, 8 and 15. These questions decreased the internal consistency of the ORTHO-15 questionnaire, because the raw Cronbach's alpha coefficients for these questions deviated from the sum of the standardized Cronbach's alpha coefficient values for the other questions in the questionnaire, for which it was 0.68. The raw Cronbach's alpha coefficient and thus the internal consistency of the questionnaire was higher after excluding questions 8 and 15 and was 0.71. The mean score of the ORTHO-15 questionnaire was  $37.98 \pm 5.51$  points with a range of 23-53.

**Table 3.** Reliability analysis for the ORTHO-15 questionnaire overall and after removing questions 8 and 15.

Reliability analysis	Mean± SD*	Min-Max*	Cronbach's raw $\alpha$ coefficient	Standardized Cronbach's $\alpha$ coefficient
ORTHO-15 questionnaire (15 scale items)	37.98 ± 5.51	23 – 53	0.69	0.68
ORTHO-15 questionnaire (13 scale items, excluding questions 8 and 15)	32.2 ± 5.34	18 – 46	0.71	0.70

\*SD – standard deviation; Min-Max – Minimum-Maximum

#### 3.4. Prevalence of orthorexia in celiac participants

According to the reference cut-off point (40 points) of the ORTHO-15 questionnaire, the number of adults with celiac disease showing risk of orthorexia is 69% (Table 4).

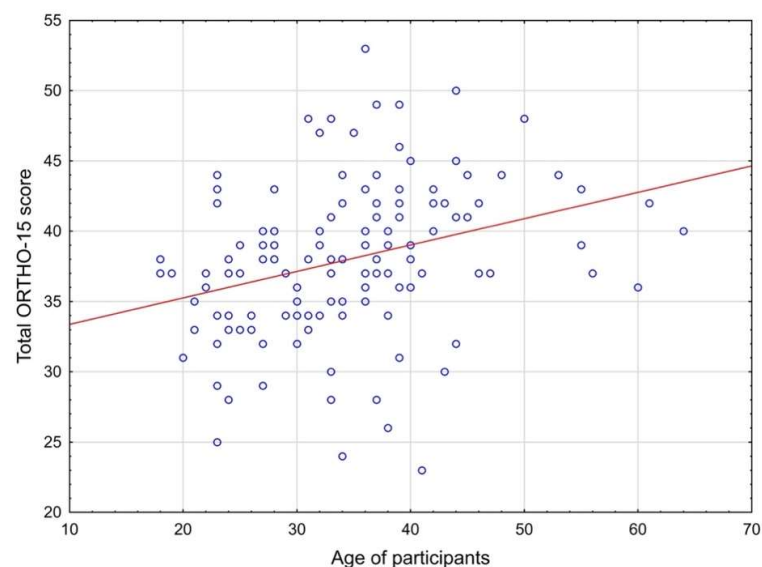
**Table 4.** Assessment of the prevalence of orthorexia risk based on ORTHO-15 test scores.

Risk of orthorexia	ORTHO-15*	
	N*	%
The entire study group	130	100
People at risk of orthorexia	90	69

\*N= number of respondents; ORTHO-15 risk assessment of orthorexia with a 40-item cut-off on the ORTHO-15 questionnaire

#### 3.5. The relationship between the age of study participants and the ORTHO-15 questionnaire total score

Relationship between age of participants and the ORTHO-15 scoring was shown in Figure 1. There was a positive correlation ( $\rho=0.37$ ,  $p=0.001$ ) found in the Spearman's correlation test. The interpretation of this result is that the higher the total ORTHO-15 score, the lower the risk of orthorexia.



**Figure 1.** The relationship between age and total scores in the ORTHO-15 test.

#### 3.6. Eating habits and behaviors and the incidence of orthorexia risk

This study found that both groups of participants, those at risk and those without the risk of orthorexia, were most likely to eat 4 meals per day, 35% and 60% participants of each group respectively (Table 5). There was no predominant category of the number of meals consumed per day in people at risk of orthorexia, but it was typical for the people without risk to consume four meals per day. In addition, those both without risk of orthorexia (80%) and at risk of orthorexia (93.3%) were more likely to choose to cook their own meals than to have a family member prepare their meals. Great difficulty in eating out was experienced by both those at risk of orthorexia 78.89% and those without risk of orthorexia 92.50%, these results were close to statistical significance. Analysis of eating behaviors showed that the group of subjects at risk for orthorexia were more likely to pay attention to the calorie content and composition of gluten-free products, but these results were not statistically significant.

**Table 5.** The differences between eating habits and behaviors and the incidence of orthorexia risk.

Eating habits and behaviors	Risk of orthorexia		$\chi^2$ test p
	Yes (N=90)	No (N=40)	
Frequency of meals per day	3 times a day 35% (n=32)	20% (n=8)	<b>p=0.043</b>
	4 times a day 37% (n=33)	60% (n=24)	
	5 times a day 28% (n=25)	20% (n=8)	
Independent preparation of gluten-free meals	93.3% (n=84)	80% (n=32)	<b>p=0.023</b>
Difficulty eating out	78.89% (n=71)	92.50% (n=37)	p=0.056
Paying attention to the caloric content of gluten-free meals	54.4% (n=49)	47.5% (n=49)	p=0.464
Paying attention to the composition of gluten-free products	87.78% (n=79)	92.5% (n=79)	p=0.422

### 3.7. Differences between the risk group of orthorexia and non-risk participants in ORTHO-15 questionnaire

Chi-square test was used to assess the relationship of individual responses to the ORTHO-15 questionnaire with the prevalence of orthorexia risk (Table 6).

People exposed to orthorexia were significantly more likely to pay attention to the caloric content of a meal (38% vs 10%), more likely to feel more confused when entering a store (26% vs 5%), and more likely to be bothered by thoughts about food for the past month (63% vs 10%) and more than 3 hours a day (22% vs 0%) than people with celiac disease not exposed to orthorexia. As many as 58% of those at risk for orthorexia often felt guilty when committing dietary transgression, while as many as 82.5% of those without risk of orthorexia have never felt guilty when they allow themselves to dietary transgression. Participants at risk orthorexia risk were willing to spend more money than those without the risk of orthorexia to buy healthier foods.



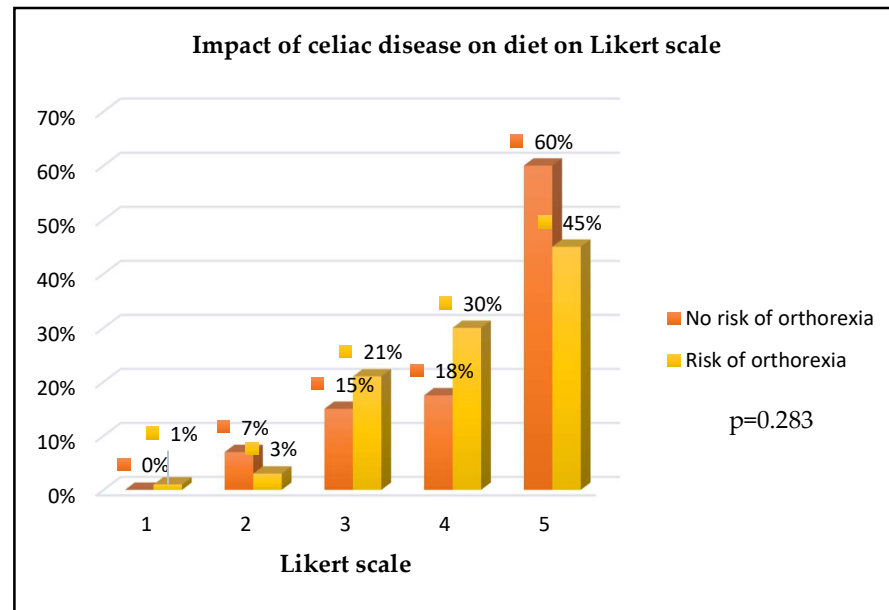
**Table 6.** Differences between the risk group of orthorexia and non-risk participants in ORTHO-15 questionnaire

ORTHO-15 questions	Risk of orthorexia		$\chi^2$ test
	Yes (N=90)	No (N=40)	p
<b>1. When eating, do you pay attention to the calories of the food?</b>			<b>p=0.001</b>
Always/ Often %	38%**	10%	
<b>2. When you go in a food shop do you feel confused?</b>			<b>p=0.006</b>
Always/ Often %	26%	5%	
<b>3. In the last 3 months, did the thought of food worry you?</b>			<b>p=0.001</b>
Always/ Often %	63%	10%	
<b>4. Are your eating choices conditioned by your worry about your health status?</b>			<b>p=0.564</b>
Always/ Often %	92%	95%	
<b>5. Is taste of food more important than the quality when you evaluate food?</b>			<b>p=0.049</b>
Always/ Often %	51%	32.5%	
<b>6. Are you willing to spend more money to have healthier food?</b>			<b>p=0.017</b>
Always/ Often %	86%	67.5%	
<b>7. Does the thought about food worry you for more than three hours a day?</b>			<b>p=0.001</b>
Always/ Often %	22%	0%	
<b>8. Do you allow yourself any eating transgressions?</b>			<b>p=0.145</b>
Always/ Often %	27%	15%	
<b>9. Do you think your mood affects your eating behavior?</b>			<b>p=0.001</b>
Always/ Often %	80%	20%	
<b>10. Do you think that the conviction to eat only healthy food increases self-esteem?</b>			<b>p=0.001</b>
Always/ Often %	42%	5%	
<b>11. Do you think that eating healthy food changes your life-style (frequency of eating out, friends...)?</b>			<b>p=0.001</b>
Always/ Often %	68%	30%	
<b>12. Do you think that consuming healthy food may improve your appearance?</b>			<b>p=0.001</b>
Always/ Often %	82%	52.5%	
<b>13. Do you feel guilty when transgressing?</b>			<b>p=0.001</b>
Always/ Often %	58%	17.5%	
<b>14. Do you think that on the market there is also unhealthy food?</b>			<b>p=0.510</b>
Always/ Often %	93%	90%	
<b>15. At present, are you alone when having meals?</b>			<b>p=0.722</b>
Always/ Often %	43%	40%	



### 3.8. Self-assessed impact of celiac disease on diet

Figure 2. shows the self-assessed impact of celiac disease on participants' diet using a five-point Likert scale according to orthorexia risk status. The percentage of subjects gradually increased as the impact of celiac disease on diet increased in both at-risk and non-at-risk celiac participants. Among those with no risk of orthorexia, as many as 78% rated the impact of celiac disease as 4-5 in the 5-point scale. Similar results were found in the group with risk of orthorexia, 75%. The results were not statistically significant ( $p=0.283$ ).



**Figure 2.** Self-assessed impact of celiac disease on the participants diet according to the orthorexia risk status

### 4. Discussion

In our study, the ORTHO-15 questionnaire was tested for reliability using Cronbach's alpha test, for which the calculated standardized reliability coefficient was within the accepted range and amounted to 0.68, while after factor analysis and exclusion of two questions reducing the consistency of the questionnaire, the coefficient was 0.71. This means that there is some similarity between the different responses of the study participants. Consistency analysis of the questions included in the ORTHO-15 questionnaire indicated that the summary results stating the presence of orthorexia risk in 69% of people with celiac disease can be considered reliable at an alpha level of 0.68 and valid for further research. With this tool we investigated the association of orthorexia with celiac disease. The study by Sutherley et al. [23] showed eating disorders in celiac disease [23], however the prevalence of orthorexia has not yet been studied in such participants. In the present study, risk of orthorexia was found among significant proportion of participants with celiac disease (69%) Brytek-Matera et al. used Düsseldorf Orthorexia Scale and the Eating Habits Questionnaire to investigate the prevalence of orthorexia. In Polish adults, the prevalence rate of orthorexia was 2.6%, while in Lebanese adults the prevalence rate was 8.4% [24]. In another study, the prevalence of orthorexia nervosa among dietetics and nutrition students in Jordan was 72% with a cut-off score of 40 and 31.8% with a cut-off score of 35 of the ORTHO-15 questionnaire [25]. Dunn et al. examined the prevalence of orthorexia among US students using ORTHO-15 questionnaire with the original cutoff score of 40 71.2% scored in the orthorexia range, 22.1% did when a cutoff score of 35 was applied [26].

We also observed a significant positive correlation between age and the total score of the ORTHO-15 questionnaire, which meant that the risk of orthorexia decreases with age, and is highest among younger individuals. Several studies have also shown that orthorexia is more common among younger than older adults [27,28]. Furthermore, with respect to celiac disease, Bongiovanni et al. [29] found that the stress of having to follow a gluten-free diet affects mainly young people and those with the disease lasting for less than four years. Changing eating habits is a long, difficult process and can contribute to stress in newly diagnosed individuals.

In the present study, women constituted a high proportion of the respondents. Gender differences in adults with celiac disease were shown in a study by West et al [30], in which the prevalence of celiac disease in women was significantly higher, especially in Finland, the Netherlands, Denmark and the UK.

Our study found that the most common digestive disorders and comorbidities were lactose intolerance, Hashimoto's disease, hypothyroidism, and the most common symptoms reported before treatment of celiac patients were bloating, chronic diarrhea, anemia, and low body weight. The results obtained are similar to the data in the literature [31]. Setty et al. [31] found that the most common symptoms in adults with celiac disease are anemia and lactose intolerance.

Our study showed high adherence to a gluten-free diet in people with celiac disease. Among the respondents, 82% followed a strict gluten-free diet and the remaining percentage admitted that despite following a gluten-free diet, they sometimes eat gluten-containing products. In comparison, in a study by Black et al, [32] as many as 96% of subjects with celiac disease strictly followed a gluten-free diet.

Adherence to a gluten-free diet is associated with better quality of life as assessed in individuals with celiac disease [33]. We found that following a gluten-free diet had positive impact on the self-assessed health status of people with celiac disease, which was better for a significant percentage of the participants. The results of our study indicate that individuals at risk for orthorexia were more likely to pay attention to the composition and caloric content of foods, snacked more often between meals, felt confused upon entering a store, were frequently troubled by thoughts about food in the past month, and their mood influenced their eating behaviors. According to our study, the conviction of eating only healthy food increases self-esteem mainly in people at risk of orthorexia. Similar observations were made about eating healthy foods that can improve appearance and change lifestyle for those at risk for orthorexia.

The study by Wolf et al. [34] demonstrated the potential negative consequences of over-sensitivity in maintaining a strict gluten-free diet in individuals with celiac disease. Adults who were particularly hypersensitive to adhering to a gluten-free diet consistently ate at celiac-friendly restaurants, asked detailed questions at dinner, and eliminated the potential for cross-contamination in their home cooking [34]. Studying a group of dietitians Tremelling et al. [35] found that the danger that orthorexia may not only be based on healthy eating or obsessive food control, but may also include an increased preoccupation with body weight and body shape among the study group.

Limitations of the study include the lack of previous research on the prevalence of orthorexia in people with celiac disease, which precludes comparisons but allows to fill knowledge gaps. In addition, our study included a small number of males among the subjects, making cross-gender comparisons impossible. The strengths of the study were the use of the standardized ORTHO-15 questionnaire, as well as the assessment of the reliability of this questionnaire, the use of an appropriate sample size of subjects, and the participation of subjects with clinically diagnosed celiac disease.

## 5. Conclusions

The study was the first to observe an association between celiac disease and orthorexia risk, finding a high percentage of people with celiac disease at risk for orthorexia. Some

of the risk factors for orthorexia among people with celiac disease include young age, feelings of guilt after eating unhealthy foods, or increased self-esteem after eating healthy foods. The study suggests the need for holistic care, including education, nutritional and psychological support for people with celiac disease. Further research should be done to explore the topic of orthorexia in celiac disease, which could contribute to uniform guidelines for the management, prevention, and treatment of orthorexia among individuals with celiac disease.

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**Institutional Review Board Statement:** The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by Ethics Committee of Medical University of Białystok (R-I-002/322/2018).

**Informed Consent Statement:** Informed consent was obtained from all subjects involved in the study.

**Data Availability Statement:** Not applicable.

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