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Original article

Inconsistent effects of gluten on obesity: is there a role for the haptoglobin isoforms?

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SUMMARY

Background and aims: There is no clear evidence about the effects of gluten intake on obesity. It is known that gluten's effects on gut permeability are mediated by zonulin, a protein identified as pre-haptoglobin 2, a physiological regulator of the intestinal barrier. We investigated the obesogenic and inflammatory effects of gluten and its association with the haptoglobin genotype.
Methods: This was a single blinded, crossover study, including 40 overweight or obesity women free of celiac disease. Participants adopted a gluten-free diet (GFD) for 8 weeks and consumed a gluten-free muffin (GF-M) or a gluten-containing muffin (GLU-M, 24 g gluten) for 4 weeks, switching muffin type during the subsequent 4 weeks. During a follow-up period of 4 weeks we evaluated the usual diet (UD). Food diaries were collected to estimate the macronutrient intake and dietary inflammatory index (DII®). Bodyweight and composition, resting energy expenditure (REE), and cytokines were assessed. Haptoglobin alleles (Hp1 and Hp2) were genotyped to characterize zonulin expression.
Results: Energy and macronutrient intakes were similar during both periods, except for protein intake, which was higher during GLU-M. DII scores indicated a more inflammatory profile during the GF-M and GLU-M periods compared to UD. No differences were observed in body composition or REE between interventions when the Hp genotype was not considered. Nonetheless, those carrying the Hp2-2 genotype (overexpressing zonulin) presented lower REE and higher levels of IL6 and IL1beta only during gluten intake (GLU-M and UD) compared to age- and body mass index-matched Hp1-1 carrier. These results suggest an obesogenic and inflammatory action of gluten only in those overexpressing zonulin (Hp2-2).
Conclusion: These results highlight the importance of zonulin as the mediator of gluten obesogenic and inflammatory effects. Our data suggest that in the presence of gluten, zonulin release is associated with a reduction of REE and an increase of inflammatory markers that are not seen in zonulin low producers.
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Introduction

Gluten effects and the benefits of a gluten-free diet (GFD) have been the focus of much discussion during recent decades [1,2]. The popularity of GFD has grown recently, with claims that it promotes weight loss and health. However, there is no clear scientific evidence on the obesogenic and pro-inflammatory effects of gluten in humans. Experimental studies in mice have demonstrated increased weight gain, adiposity, inflammation, and insulin resistance when they were fed on a gluten-containing diet [3]. Moreover, gluten intake reduced the energy expenditure and the thermogenic capacity of adipose tissue in obese mice fed a high-fat diet [4].

Gluten is a complex protein composed of glutenins and prolamins, which are named secalin in rye, avenin in oat, hordein in barley, and gliadin in wheat [5]. Gliadin has been extensively studied because of its cytotoxic, immunomodulatory, and gut-permeating activities [6]. It has been demonstrated that undigested gliadin peptides bind to the chemokine receptor CXCR3 in the intestine and trigger the release of zonulin [7,8]. Zonulin, identified as pre-haptoglobin (Hp) 2, is a protein that reversibly modulates the epithelial tight junction [9]. Upon gluten stimulus, zonulin is release and causes small intestine tight junctions to disassemble and increases intestinal permeability [6]. It has been suggested that this mechanism occurs in individuals with celiac disease and also, to a lesser extent, in non-celiac persons [10].

Hp is a plasma protein composed of α- and β-polypeptide chains. The β-chain is constant, while the α-chain exists in two isoforms (α-1 and α-2). The presence of one or both of the α-chains results in the three human HP genotypes: HP1-1 homozygote, HP2-1 heterozygote, and HP2-2 homozygote [6].

In this context, the aim of this blinded-controlled trial was to identify the possible obesogenic and pro-inflammatory effects of gluten intake in non-celiac, with overweight and obesity in a blinded-controlled trial. We also aimed to establish an association between gluten intake and the HP genotype, representing the expression of zonulin (pre-HP2).

Subjects and methods

Study design and participants

This was a crossover, placebo-controlled, simple masked study conducted over 12 weeks. We included 40 healthy premenopausal women, aged from 18 to 50 years, with overweight or obesity (body mass index (BMI) range from 25.0 to 35.4 kg/m^2). We excluded diagnosis or suspicion of celiac disease and other gluten disorders, as well as chronic diseases, such as diabetes mellitus, systemic hypertension, and chronic renal failure (Supplementary Fig. S1).

Subjects were prescribed a GFD for 8 weeks. A trained registered dietitian provided guidance and instruction on how to implement a GFD maintaining the overall food intake as close as possible to the habitual. The eight experimental weeks were divided into two 4-week periods when they received either a gluten-free muffin (GF-M) or a gluten-containing muffin (GLU-M), followed immediately by the alternate muffin. Twenty women were assigned to start receiving the GLU-M and 20 to start receiving the GF-M for 4 weeks, before switching muffins during the subsequent 4 weeks. To ensure homogeneity of the sample, we paired age and baseline BMI of the participants that initiate the study in each intervention (GLU-M or GF-M).

We prepared both muffins in the experimental kitchen of the Nutrition Department of the Federal University of Minas Gerais (Brazil). These muffins were designed to present similar sensorial aspects and nutritional composition (Supplementary Table S1). Each GLU-muffin contained 12 g of vital gluten (Vital Wheat Gluten, Granolab Granotec, Brazil), while the GF muffins contained corn and corn flour. Participants were instructed to eat two muffins per day (total of 24 g gluten/day during GLU-M period) and were blinded to the type of muffin. After the 8-week experiment, we followed up the subjects for a further 4 weeks to assess the usual diet (UD). In this period, participants received instructions to return to their usual gluten-containing diet, while maintaining their food diary (Fig. 1).

Food intake analysis and the dietary inflammatory index (DII®)

We analysed GFD compliance and food intake of the participants by a food diary recording all foods and beverages consumed each day during the 12 experimental weeks. We randomly selected two weekdays and one weekend day of each experimental week (GLU-M, GF-M, and UD) to estimate total energy, carbohydrate, protein, fat, and fibre using the software Avanutri 4.0 (Brazil), as well as to calculate the DII®.

The DII® was developed to measure the inflammatory potential of diet and is computed using a scoring algorithm relating 45 foods and dietary constituents to six inflammatory biomarkers (IL1β, IL4, IL6, IL10, TNF, and C-reactive protein), which was developed by Shivvapa et al., in 2014 [11]. In the current study, 22 items were used to generate an individual DII® score for each experimental period (GLU-M, GF-M, and UD). A positive DII® score represents overall dietary patterns with a pro-inflammatory potential, whereas a negative DII® score represents an anti-inflammatory pattern.

Anthropometrics and resting energy expenditure (REE) analysis

We evaluated body weight, body composition, and waist circumference in four moments during our experimental period: Initial, fourth, eighth, and 12th weeks (Fig. 1). The participants were instructed to undergo an overnight fast of at least 8 h and absent from physical activity and alcohol consumption for at least 24 h. Bodyweight and height were measured using a scale (Tanita BF−680 W) and wall stadiometer (Compact 2.1 cm Mod 210 Wiso), respectively. Waist circumference was measured twice using a flexible measuring tape at the point of the umbilicus after exhalation.

Body composition was evaluated using a tetrapolar bioelectrical impedance device (BIA 450 Byodinamics), strictly following the manufacturer's instructions. Readings of resistance and reactance values were used to calculate body fat percentage (BF%), fat-free mass, and body water.

Resting energy expenditure (REE) was measured by indirect calorimetry (MetaCheck Metabolic Rate Analysis System, KORR). Subjects rested in the supine position for at least 20 min before the test. Volumes of O_2 and CO_2 (VO_2 and VCO_2, respectively) were measured for 10 min and used to calculate the REE.

Serum cytokine concentration

After fasting, blood samples were collected and centrifuged for 10 min at 3000 rpm for serum extraction. Serum was stored at −80 °C for subsequent analysis of TNF, IL1β, and IL6 concentrations. Cytokine determinations were performed using ELISA kits according to the manufacturer's instructions (R&D Systems: TNF-α, #DY210; IL 1β #DY201; and IL6, #DY206).

Haptoglobin (Hp) genotyping

DNA isolation was performed by silica-based spin-column (#69504, Qiagen, USA) according to the manufacturer's instructions. Haptoglobin genotyping was performed using specific primers (Fw:

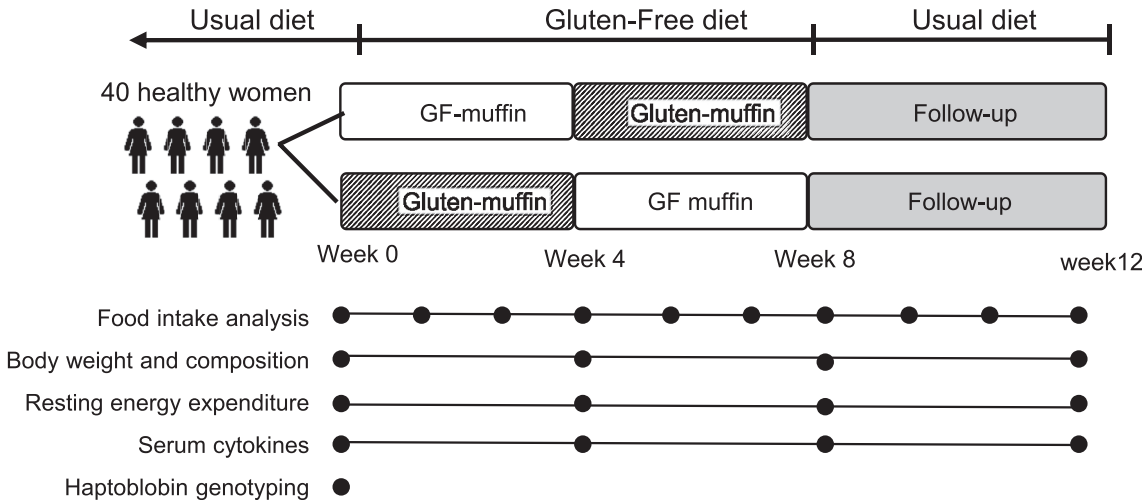

Fig. 1. Study design and participants. Forty women with BMI >25.0 kg/m^2 were prescribed a GFD for eight weeks. The experimental weeks were divided into two four week-period when they received either a gluten-free muffin (GF-M) or a gluten-containing muffin (GLU-M) followed immediately by the alternate muffin. The haptoglobin genotype was determined through an oral swab. The food diaries were collected from the beginning (W0) to the end of the follow-up period (W12). Haptoglobin genotype only once (W0). Anthropometric data, resting energy expenditure, and serum cytokines were measure at the beginning, 4th and 8th weeks, and four weeks after the end of muffin intake (12th week).

TTTCTGGCTGCTAAGTGG; Rev: AATGTCTTTCGCTGTTGC) amplified by a high-fidelity PCR system, as described by Freire et al. (2019) [12].

Study approval

The study protocol was approved by the Research Ethics Committee (CAAE #49480215.0.000.5149) of the Federal University of Minas Gerais (Brazil) and the Institutional Review Board (#2016P002748) of the Massachusetts General Hospital (Boston, US). All protocols were performed following relevant guidelines and regulations. All subjects provided a written informed consent form and were instructed on the ethical and legal procedures.

The clinical trial design, interventions, and outcomes have been reported and previously registered on ClinicalTrials.gov (Identifier 9480215.0.0000.5149).

Statistical analysis

Statistical analyses were performed using the software GraphPad Prism 5.0. The normal distribution of the data set was evaluated using the Shapiro Wilk test. Data are presented as mean ± standard deviation (SD). Parametric and nonparametric variables were analyzed by Student's t-test and Wilcoxon test, respectively. Paired tests were applied to compare the same subject in different moments. Unpaired tests were applied to independent subjects. Pearson test was applied to evaluate the correlation. A significance level of 5% (0.05) was adopted.

Results

Baseline characteristic of study subjects

We enrolled 40 healthy, non-celiac women with overweight or obesity, with average (±standard deviation) age of 31.6 ± 7.7 years and BMI 28.6 ± 3.0 kg/m^2. Both initial groups of participants presented a similar age, body weight, anthropometrics, and cytokine levels at baseline (Supplementary Table S2). Subjects denied intake of any medication, supplement, or tea that could influence energy expenditure measurement.

Dietary intake was similar during the GLU-M and GF-M periods

All participants were compliant with the prescribed GFD and reported the correct intake of two muffins per day during both interventions, as documented by the food diaries. Throughout the UD follow up (w8 to w12), the participants returned to their usual gluten-containing diet, as confirmed by their food diaries.

Analysis of food diaries showed a similar intake of carbohydrates and fat (Fig. 2A), as well as energy (Fig. 2B), and dietary fibre (Fig. 2C) during the GLU-M, GF-M, and UD periods. As expected, protein intake (g/kg) was higher during the GLU-M period (1.1 ± 0.25 g/kg) when compared to the GF-M period (0.95 ± 0.20 g/kg; p = 0.01); this was due to gluten addition to the muffin (Fig. 2A). Nonetheless, the Dietary Inflammatory Index (DII®) revealed no difference during GLU and GF interventions, suggesting similar patterns during both interventions. Therefore, we observed a lower DII® score during the UD period, suggesting a less inflammatory potential when UD was reintroduced (Fig. 2D).

We observed that GLU-M or GF-M interventions led to a discrete body weight loss (GLU-M: −0, 46 (±0, 95); GF-M: −0, 45 (±0,92); p = 0.94) when compared to baseline. In the GLU-M group, 71.4% of the participants lost weight, while in the GF-M group, 61.9% lost weight. Therefore, a similar reduction was seen in both interventions (Fig. 3A and B).

Analysis of body composition by bioimpedance revealed no differences in body fat and fat-free mass changes for GLU-M or GF-M periods (Fig. 3C and D). Indeed, indirect calorimetry analysis showed no difference in REE changes (Fig. 3 E).

Finally, we evaluated variations in the pro-inflammatory profile. The changes in cytokine concentrations (IL6, TNF, and IL1β) were not statistically significant when comparing the GLU-M and GF-M interventions (Fig. 3F-H).

Gluten intake was associated with higher proinflammatory cytokine secretion and decrease of REE in Hp2 subjects

Out of 40 participants, we were able to genotype 34 subjects for haptoglobin (Hp). In 6 participants, we were not able to properly collect sufficient material for genotyping. Among them, we observed that seven (20.6%) presented two alleles of Hp1 and seven

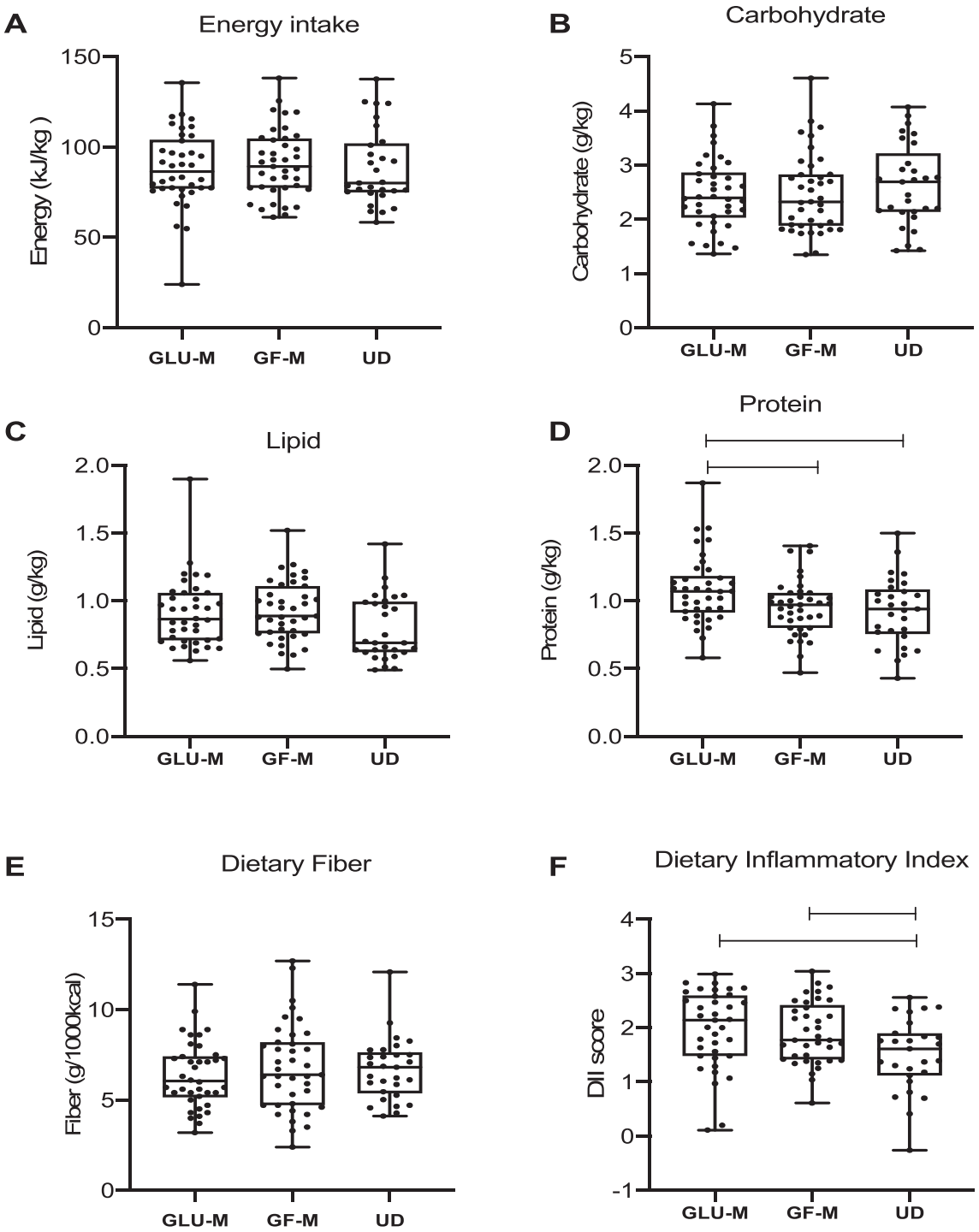

Fig. 2. Macronutrient intake and Dietary inflammatory Index evaluations. A. Energy, macronutrient (B. Carbohydrate, C. Protein, D. lipid, E. Fiber) intakes, and E). Dietary Inflammatory Index in women with overweight or obesity in a gluten-free diet receiving gluten-containing muffins (GLU-M) or gluten-free muffins (GF-M) for four weeks and during the usual diet (UD). Dots represent individual data; boxes represent the first and third quartile, and median, vertical lines represent min and max values. * Statistically different ($P < 0.05$) using paired ANOVA test and Tukey post-test. For macronutrient analysis GLU-M = 38 GF-M = 39; UD = 29; for DII n = 37 in all periods. Changes in body weight, body composition, REE, and inflammatory profile were similar during both interventions: GLU-M or GF-M.

two alleles of Hp2 (20.6%), while the remaining 20 ones (58.8%) presented as heterozygous (Hp2-1). The Hp allele 1 frequency was 0.5 and similar of that seen in Hispanic and African-American populations [13,14]. The genotype frequency was of 19.2% (Hp1-1); 52.8% (Hp2-1) and 28.0% (Hp2-2) that is similar to the frequency found a previous study with a Brazilian population [15].

Interestingly, we observed significant differences when our data were stratified by the Hp genotype. Although no differences were observed for BMI (Table 1), we found a significantly lower REE in Hp2-2 subjects during GLU-M intervention or UD follow up (gluten-containing diet) when compared to age, and BMI matched Hp1-1 participants. Similar results were observed for IL1β and IL6

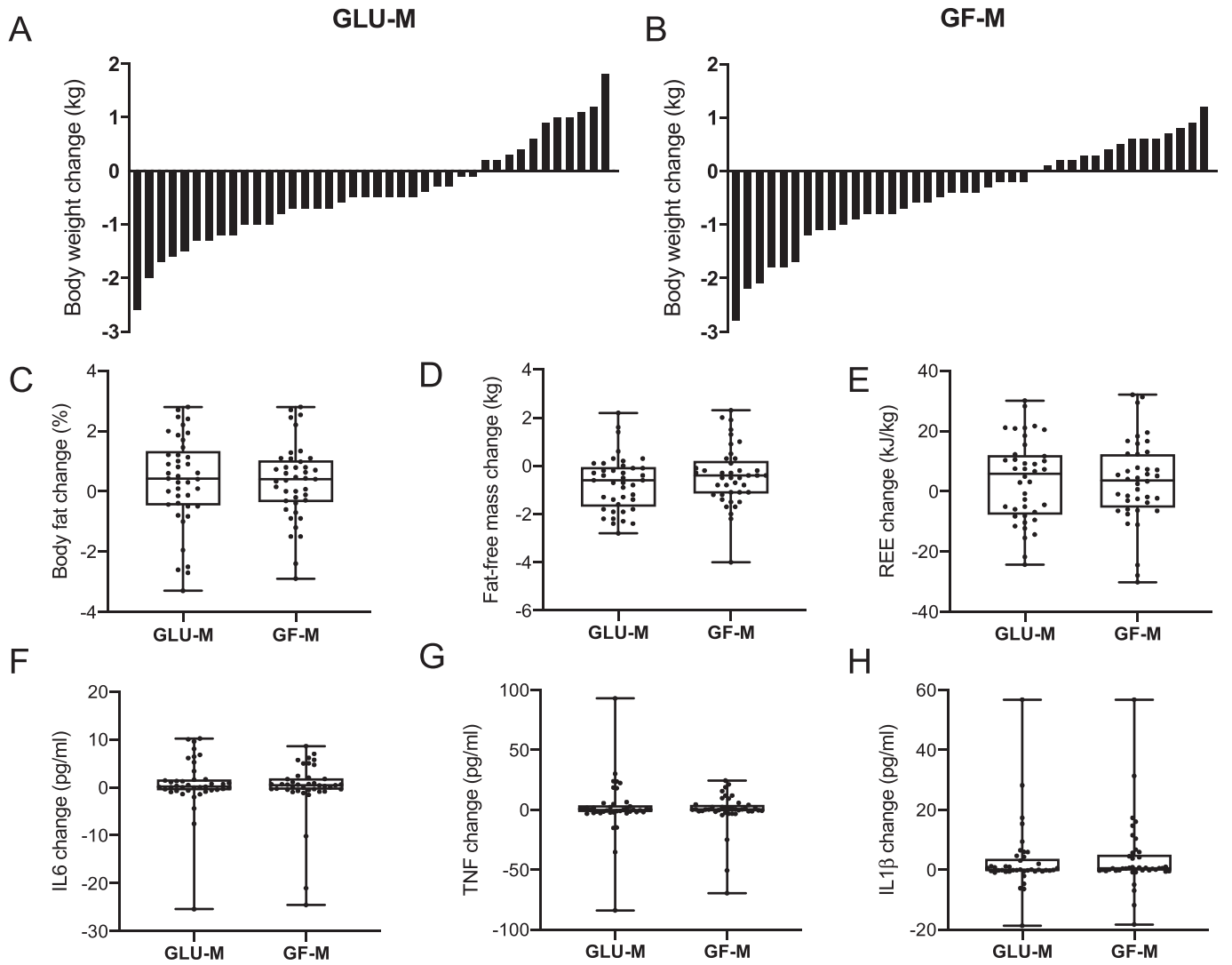

Fig. 3. Changes of body weight according to the muffin period (A and B), fat mass (C), fat-free mass (D), resting energy expenditure (E) and blood IL6 (F), TNF (G) and IL1β (H) concentrations in non-celiac women with overweight or obesity. Participants were kept in a gluten-free diet receiving gluten-containing muffins (GLU-M) or gluten-free muffins (GF-M) for four weeks. A and B: each bar represents an individual participant. C–H: Dots represent individual data, boxes represent the first and third quartile, and median, vertical lines represent min and max values. * Statistically different ($P < 0.05$) using the paired t Student test. N = 40.

variations: compared to Hp1-1, Hp2-2 individuals presented an increase of these pro-inflammatory cytokines during GLU-M intervention. No differences in TNF changes were observed in any period. Finally, we found a positive association between the number of Hp2 allele and IL1β and IL6 variations as well as a negative association between the number of Hp2 alleles and REE only during the periods with gluten intake (GLU-M and usual diet) (Table 2).

These data suggested that upon gluten intake, individuals carrying Hp-2 allele, which are higher zonulin producers, present lower REE and increase pro-inflammatory cytokines (IL6 and IL1β) compared to Hp-1carriers.

Discussion

The majority of the general population adopt the GFD with the intent to lose weight or develop a healthier lifestyle [16,17]. Nonetheless, they are moved by the information of non-controlled reports or individual opinions. So, this study was designed to answer some open questions about the risk and benefit of such diets and to observe the possible interferences of gluten on body composition, metabolism, and inflammation. Here we did not find any advantage in adopting a gluten-free diet for four weeks considering changes in body weight or body composition as well as diet quality improvement.

A large-scale database from National Health and Nutrition Examination Survey (NHANES) 2009–2014 showed that the BMI of those participants who identified themselves as GFD followers was lower than the general population [18]. Nonetheless, this reduction in BMI should be carefully interpreted since it can be due not only (or explicitly) to gluten exclusion but also to other actions intended to improve life quality, such as better food choices and more physical activity.

A study comparing the effects of low-gluten (2 g/day) or high-gluten (18 g/day) diets on intestinal microbiome found a modest but significant weight loss (0.8 kg) after low-gluten diet period [19]. Results showed that most of the differences between diets were

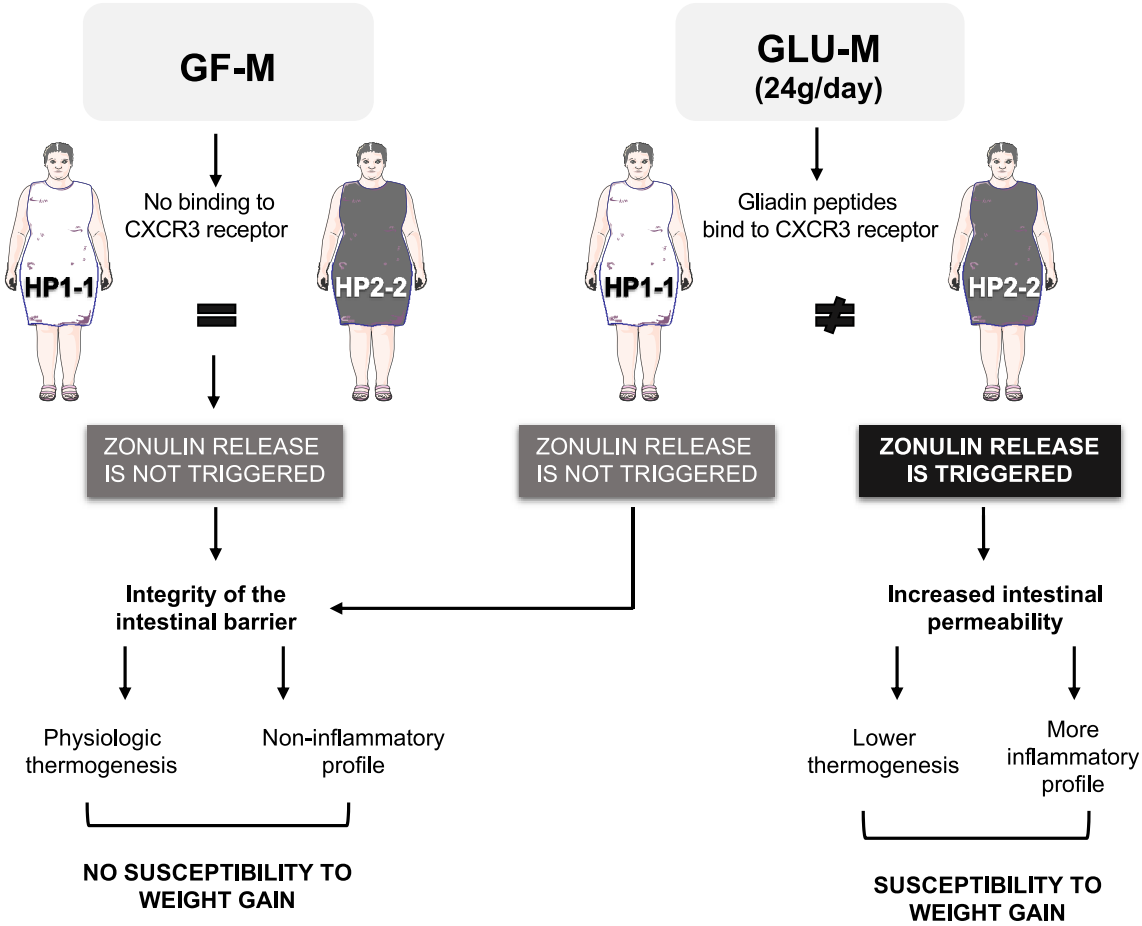

Fig. 4. The hypothesis of the gluten effect associated with the haptoglobin genotype. The impact of gluten on body weight and inflammatory markers depends on the expression of zonulin. During a gluten-free diet, zonulin release is not triggered by Hp2 allele, and resting metabolism or inflammatory cytokines are similar in HP1-1 and HP2-2 genotypes. In a gluten-containing diet, those carrying Hp2 allele will present a higher release of zonulin that will increase inflammation and reduce REE compared to low producer Hp1-1.

Table 1
Comparison of anthropometric characteristics and energy expenditure of participants carrying HP1-2 or HP2-2 genotyping after four weeks in a gluten-free diet receiving gluten-free or gluten-containing (24 g/day) muffins.

Genotype	GF-M PERIOD			GLU-M PERIOD			USUAL DIET		
	HP1-1	HP2-2	p	HP1-1	HP2-2	p	HP1-1	HP2-2	p
BMI (kg/m^2)	27.58 (3.14)	27.41 (3.18)	0.746	27.41 (3.18)	27.81 (2.21)	0.801	26.76 (2.79)	27.97 (2.31)	0.987
Fat Mass (%)	34.40 (5.31)	34.66 (4.56)	0.431	34.66 (4.56)	37.81 (2.64)	0.757	34.17 (4.79)	38.00 (3.29)	0.416
Fat-Free Mass (kg)	47.34 (4.58)	46.90 (4.71)	0.849	46.90 (4.71)	48.17 (5.95)	0.670	45.83 (4.19)	8.32 (6.50)	0.564
REE (kJ/kg)	74.6 (19.2)	69.9 (12.2)	0.367	77.5 (4.7)	61.7 (9.4)	0.035	73.7 (11.1)	58.3 (6.9)	0.004
REE (kJ/FFM)	114.2 (18.1)	102.3 (11.6)	0.373	119.2 (11.6)	99.4 (14.7)	0.007	112.5 (19.3)	94.4 (13.4)	0.007

HP1-1 and HP2-2 carriers were matched by age (HP1-1: 34 + 9.93 years and HP2-2: 27.71 + 5.82 years) and initial BMI (HP1-1: 27.58 + 3.14 kg/m2 and HP2-2: 27.54 + 2.11 kg/m^2) before analysis (paired Student t test). *statistically different of HP1-1 data. GLU-M: gluten-containing muffin; GF-M: gluten-free muffin; CI: confidence interval; BMI: body mass index; REE: resting energy expenditure measured by indirect calorimetry n = 7/group.

related to carbohydrate metabolism and fiber fermentation. It suggests that qualitative changes of dietary fiber rather than the reduction of gluten intake itself were responsible for the microbiota modifications and, indirectly, by the changes in body weight during the low-gluten diet period.

When we analyzed body weight and composition, we did not observe changes in body weight and composition when comparing GLU-M or GF-M periods. However, a modest weight loss was detected in the first weeks of the intervention. It was possibly caused by the adoption of a “new diet” because weight loss was similar between those that initially received GLU-M or GF-M. Based on our results, we concluded that the adoption of GFD for a short time (4 weeks) was ineffective in reducing body weight or improving fat and fat-free body mass.

Regarding the risk and benefits of GFD, it has been described that it is associated with lower consumption of protein and fiber and a higher intake of total and saturated fat [1,20–22]. The main causes of this nutritional imbalance seem to be the intake of gluten-free commercial preparations, frequently containing more lipids and less fibre than the natural gluten-containing foods [23]. Nonetheless, we did not detect differences in macronutrient intake when comparing experimental periods and usual diet. It might be partly because our participants use gluten-free commercial preparations only occasionally, and gluten substitution was based

Table 2
Association of the number of Hp2 alleles and BMI resting energy expenditure and level of serum cytokines during the periods of gluten-free diet adoption and intake gluten-free (GF-M period) or gluten-containing (GLU-M period) muffins and during, usual diet (follow up).

	GF-M PERIOD			GLU-M PERIOD			USUAL DIET (FOLLOW UP)		
	r	CI	p	r	CI	p	r	CI	p
BMI (kg/m^2)	−0.004	−0.341 to 0.334	0.982	0.040	−0.301 to 0.373	0.820	0.120	−0.252 to 0.459	0.459
REE (kg/FFM)	−0.303	−0.581 to 0.039	0.082	**−0.494**	**−0.713 to −0.188**	**0.003**	**−0.404**	**−0.664 to −0.051**	**0.027**
IL6 (pg/mL)	−0.251	−0.551 to 0.106	0.165	0.067	−0.294 to 0.411	0.721	−0.027	−0.389 to 0.343	0.891
TNF (pg/mL)	0.304	−0.037 to 0.582	0.080	0.004	−0.345 to 0.346	0.983	−0.270	−0.569 to 0.093	0.143
IL1β (pg/mL)	0.289	−0.054 to 0.571	0.097	**0.381**	**0.042 to 0.640**	**0.029**	**0.408**	**0.055 to 0.669**	**0.025**

GLU-M: gluten-containing muffin; GF-M: gluten-free muffin; CI: confidence interval; BMI: body mass index (kg/m^2); REE: resting energy expenditure measured by indirect calorimetry. N = 40 for GF-M and GLU-M periods and 34 for Usual Diet.
Bold data denote statistically significant results ($p < 0.05$).

mainly on natural products (mostly potatoes, rice, and cassava flour).

Although macronutrient intake was kept unchanged, the exclusion of gluten-containing foods increased the inflammatory potential of diet, as suggested by the DII results. DII is an algorithm that evaluates up to 45 dietary parameters related to inflammatory blood markers [11]. Because gluten is not included in these parameters, we did not expect any differences in DII when comparing the GLU-M and GF-M periods. However, DII scores were higher in both experimental periods compared to the usual (gluten-containing) diet. Since macronutrient intake was quantitatively similar among diets, we believe that other food components (such as vitamins and minerals) caused a negative impact on DII. Indeed, many gluten-free kinds of cereal contain inferior concentrations of thiamin, riboflavin, niacin [24], iron, and folate [25] compared to wheat. All these vitamins and minerals are included in the DII calculation and could contribute to the differences in DII between usual and gluten-free diets.

It has been suggested that gluten intake induces inflammation. Our previous studies and others have reported an increase of pro-inflammatory markers in obese animals fed gluten (or gliadin) supplemented diets for 5 weeks [3,4,19]. On the other hand, Zhang et al. (2017) fed mice with a high-fat diet with or without gliadin for 23 weeks, and did not find differences in inflammatory blood markers. Nevertheless, an inflammatory phenotype was seen in the visceral adipose tissue of mice receiving gliadin. Although our DII® result indicates that GFD leads to a more inflammatory state, we did not identify differences in IL6, TNF, or IL1β blood concentrations, regardless of gluten intake. A similar result was seen in non-celiac athletes receiving gluten-free or gluten-containing diets for 7 days; there were no differences between diets regarding IL1β, IL6, IL8, IL10, IL15, and TNF blood concentrations [26,27]. It should be noted that the level of cytokines in our study presented a large intra and interindividual variation. It is possible that a larger group could reveal more consistent results.

It has been described that two non-digested gluten peptides bind the CXCR3 receptor in the intestinal epithelium, inducing the release of zonulin [7,8,19,28]. Zonulin actives a cascade of reactions that culminates on intestinal barrier disruption, favouring intestinal permeability of nutrient and microorganism components, such as LPS and, consequently, inflammation [6,29]. To observe the interference of zonulin in the gluten effect, we intended to measure blood zonulin concentration. Nonetheless, it has been described that current commercial zonulin assays do not detect zonulin [30]. For this reason, we characterize participants as low and high producers of zonulin according to Hp genotype. Our results demonstrate a positive association between the presence of Hp2 allele and higher IL1β concentrations during gluten intake (GLU-M period and usual diet), reinforcing the pro-inflammatory effect of zonulin and its relationship with gluten intake.

The interference of GFD on thermogenesis was seen in our previous experimental study [4]. We demonstrated that gluten intake was associated with weight gain through a mechanism related to the reduction of the browning process and thermogenesis, as suggested by the lower expression of UCP-1 and BMP7. Based on these results, we expected to observe a reduction in the REE during the GLU-M period. Considering all participants (regardless of Hp genotyping), we did not find any changes in REE that could corroborate the animal findings. However, the Hp genotype analysis showed that during the GLU-M period, those carrying the Hp2-2 genotype (higher zonulin producers) presented a lower REE compared to age and BMI matched Hp1-1 carriers. When gluten was not included in the diet, REE was similar between Hp1-1 and Hp2-2 carriers. This suggests that gluten influences thermogenesis and that zonulin is a mediator of this effect. Based on our previous results showing the impact of gluten on thermogenesis, we hypothesized that, in Hp2-2 carriers, gluten was associated with downregulation of expression of proteins, such as UCP1 and BMP7, reducing the "browning" process and thermogenesis. Since macronutrient and energy intakes were similar in Hp1-1 and Hp2-2 carriers during all periods, it can be assumed that the reduction of REE in those Hp2-2 carriers during the gluten period will lead to a positive energy balance. We believe that in a long-term follow-up, this lower REE could favour weight gain if other actions, such as increased physical activity, were not implemented for susceptible (Hp2) individuals.

Our study has some limitations. First, the duration (4 weeks) of each period might have been insufficient to observe more consistent changes in body composition or metabolism, mainly because we did not restrict energy intake. Moreover, individual variations might not have allowed the observation of changes in blood cytokines related to gluten intake. Also, a more significant number of participants could be necessary to observe a more consistent association of Hp2-2 carriers and gluten effect. Besides these limitations, our results underscore the importance of Hp allele expression and zonulin production on the comprehension of gluten action in the non-celiac individuals (Fig. 4).

In conclusion, our findings highlight the importance of individual differences in zonulin secretion to understand the effects of gluten (or GFD) on body composition and inflammation. We hypothesize that the impact of gluten on body weight and inflammatory markers depend on the expression of zonulin, induced by gluten peptides. During a GFD, zonulin release is not triggered by the Hp2 allele, and resting metabolism or inflammatory cytokines are similar in HP1-1 and HP2-2 genotypes. In a gluten-containing diet, those carrying the Hp2 allele will present a higher release of zonulin that will increase inflammation and reduce REE compared to low Hp1-1 producers. Future research should now focus on the long-term consequences of such effects.

Credit statements

Silva, RB, Freire, RH; Alvarez-Leite, JI contributed for conceptualization Ideas; Silvestre, SCM; Rodrigues, E contributed for formal analysis; Silva, B; Coelho, BS; Andrade, K; Fonseca, L; Fernandes, WB; Shivappa, N: Freire, Rachel H contributed for investigation conducting; Ferreira, A; Hébert, JR; Fasano, A; Alvarez-Leite, Jacqueline contributed for resources provision of study materials, and other analysis tools and Silva, RB, Freire, RH Alvarez-Leite, JI wrote the original draft. All authors reviewed the manuscript.

Declaration of competing interest

Disclosure: Dr. James R. Hébert owns controlling interest in Connecting Health Innovations LLC (CHI), a company planning to license the right to his invention of the dietary inflammatory index (DII) from the University of South Carolina in order to develop computer and smartphone applications for patient counseling and dietary intervention in clinical settings. Dr. Nitin Shivappa is an employee of CHI.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.clnesp.2020.09.008.

References

[1] Gaesser GA, Angadi SS. Navigating the gluten-free boom. J Am Acad Physician Assistants 2015;28:1–7. https://doi.org/10.1097/01.JAA.0000469434.67572.a4.
[2] Dennis M, Lee AR, McCarthy T. Nutritional considerations of the gluten-free diet. Gastroenterol Clin N Am 2019;48:53–72. https://doi.org/10.1016/j.gtc.2018.09.002.
[3] Soares FLP, de Oliveira Matoso R, Teixeira LG, Menezes Z, Pereira SS, Alves AC, et al. Gluten-free diet reduces adiposity, inflammation and insulin resistance associated with the induction of PPAR-alpha and PPAR-gamma expression. J Nutr Biochem 2013;24:1105–11. https://doi.org/10.1016/j.jnutbio.2012.08.009.
[4] Freire RH, Fernandes LR, Silva RB, Coelho BSL, de Araújo LPT, Ribeiro LS, et al. Wheat gluten intake increases weight gain and adiposity associated with reduced thermogenesis and energy expenditure in an animal model of obesity. Int J Obes 2016;40:479–86. https://doi.org/10.1038/ijo.2015.204.
[5] Biesiekierski JR. What is gluten? J Gastroenterol Hepatol 2017;32:78–81. https://doi.org/10.1111/jgh.13703.
[6] Fasano A. Zonulin and its regulation of intestinal barrier function: the biological door to inflammation, autoimmunity, and cancer. Physiol Rev 2011;91:151–75. https://doi.org/10.1152/physrev.00003.2008.
[7] Drago S, El Asmar R, Di Pierro M, Grazia Clemente M, Sapone ATA, Thakar M, et al. Gliadin, zonulin and gut permeability: effects on celiac and non-celiac intestinal mucosa and intestinal cell lines. Scand J Gastroenterol 2006;41:408–19. https://doi.org/10.1080/00365520500235334.
[8] Lammers KM, Lu R, Brownley J, Lu B, Gerard C, Thomas K, et al. Gliadin induces an increase in intestinal permeability and zonulin release by binding to the chemokine receptor CXCR3. Gastroenterology 2008;135:194–204. https://doi.org/10.1053/j.gastro.2008.03.023.
[9] Tripathi A, Lammers KM, Goldblum S, Shea-Donohue T, Netzel-Arnett S, Buzza MS, et al. Identification of human zonulin, a physiological modulator of tight junctions, as prehaptoglobin-2. Proc Natl Acad Sci 2009;106:16799–804. https://doi.org/10.1073/pnas.0906773106.
[10] Hollon J, Puppa EL, Greenwald B, Goldberg E, Guerrerio A, Fasano A. Effect of gliadin on permeability of intestinal biopsy explants from celiac disease patients and patients with Non-Celiac gluten sensitivity. Nutrients 2015;7:1565–76. https://doi.org/10.3390/nu7031565.
[11] Shivappa N, Steck SE, Hurley TG, Hussey JRHJ. Dietary inflammatory index. Publ Health Nutr 2014;17:1689–96. https://doi.org/10.1017/S1368980013002115. Designing.
[12] Freire R, Ingano L, Serena G, Cetinbas M, Anselmo A, Sapone A, et al. Human gut derived-organoids provide model to study gluten response and effects of microbiota-derived molecules in celiac disease. Sci Rep 2019;9:1–15. https://doi.org/10.1038/s41598-019-43426-w.
[13] Carter K, Worwood M. Haptoglobin: a review of the major allele frequencies worldwide and their association with diseases. Int J Lab Hematol 2007;29:92–110. https://doi.org/10.1111/j.1751-553X.2007.00898.x.
[14] Koch W, Latz W, Eichinger M, Roguin A, Levy AP, Schömig A, et al. Genotyping of the common haptoglobin Hp 1/2 polymorphism based on PCR. Clin Chem 2002. https://doi.org/10.1093/clinchem/48.9.1377.
[15] Santos MNN, Bezerra MAC, Domingues BLTB, Zaccariotto TR, Oliveira DM, Costa FF, et al. Haptoglobin genotypes in sickle-cell disease. Genet Test Mol Biomarkers 2011;15:709–13. https://doi.org/10.1089/gtmb.2010.0235.
[16] Norwood R, Cruwys T, Chachay VS, Sheffield J. The psychological characteristics of people consuming vegetarian, vegan, paleo, gluten free and weight loss dietary patterns. Obes Sci Pract 2019. https://doi.org/10.1002/osp4.325.
[17] Pearlman M, Casey L. Who should Be gluten-free? A review for the general practitioner. Med Clin 2019. https://doi.org/10.1016/j.mcna.2018.08.011.
[18] Kim H, Demyen MF, Mathew J, Kothari N, Feurdean M, Ahlawat SK. Obesity, metabolic syndrome, and cardiovascular risk in gluten-free followers without celiac disease in the United States: results from the national health and nutrition examination survey 2009–2014. Dig Dis Sci 2017. https://doi.org/10.1007/s10620-017-4583-1.
[19] Zhang L, Andersen D, Roager HM, Bahl MI, Hansen CHF, Danneskiold-Samsøe NB, et al. Effects of gliadin consumption on the intestinal microbiota and metabolic homeostasis in mice fed a high-fat diet. Sci Rep 2017;7:44613. https://doi.org/10.1038/srep44613.
[20] Gaesser GA, Angadi SS. Gluten-free diet: imprudent dietary advice for the general population? J Acad Nutr Diet 2012;112:1330–3. https://doi.org/10.1016/j.jand.2012.06.009.
[21] Subhan FB, Chan CB. Review of dietary practices of the 21st century: facts and fallacies. Can J Diabetes 2016. https://doi.org/10.1016/j.jcjd.2016.05.005.
[22] Vici G, Belli L, Biondi M, Polzonetti V. Gluten free diet and nutrient deficiencies: a review. Clin Nutr 2016;35:1236–41. https://doi.org/10.1016/j.clnu.2016.05.002.
[23] Simon E. Nutritional differences between a gluten-free diet and a diet containing equivalent products with gluten. 2014. p. 182–7. https://doi.org/10.1007/s11130-014-0410-4.
[24] Thompson T. Thiamin, riboflavin, and niacin contents of the gluten-free diet: is there cause for concern? J Am Diet Assoc 1999. https://doi.org/10.1016/S0002-8223(99)00205-9.
[25] Thompson T, Dennis M, Higgins LA, Lee AR, Sharrett MK. Gluten-free diet survey: are Americans with coeliac disease consuming recommended amounts of fibre, iron, calcium and grain foods? J Hum Nutr Diet 2005. https://doi.org/10.1111/j.1365-277X.2005.00607.x.
[26] Lis D, Fell J, Shing C, Stellingwerff T. Athletes and gluten-free diets: exploring the popularly, experiences and beliefs of this diet in non-coeliac athletes. J Sci Med Sport 2013;16:e66–7. https://doi.org/10.1016/j.jsams.2013.10.158.
[27] Lis D, Stellingwerff T, Kitic CM, Ahuja KDK, Fell J. No effects of a short-term gluten-free diet on performance in nonceliac athletes. Med Sci Sports Exerc 2015;47:2563–70. https://doi.org/10.1249/MSS.0000000000000699.
[28] Clemente MG, De Virgiliis S, Kang JS, Macatagney R, Musu MP, Di Pierro MR, et al. Early effects of gliadin on enterocyte intracellular signalling involved in intestinal barrier function. Gut 2003;52:218–23. https://doi.org/10.1136/gut.52.2.218.
[29] Fasano A, Not T, Wang W, Uzzau S, Berti I, Tommasini A, et al. Zonulin , a newly discovered modulator of intestinal permeability , and its expression in coeliac disease. The Lancet 2000;355:1518–9. Detection of human herpes virus 6 DNA in fetal Hydrops.
[30] Ajamian M, Steer D, Rosella G, Gibson PR. Serum zonulin as a marker of intestinal mucosal barrier function: may not be what it seems. PloS One 2019;14:1–14. https://doi.org/10.1371/journal.pone.0210728.