Zonulin in serum as a biomarker fails to identify the IBS, functional dyspepsia and non-coeliac wheat sensitivity

We read with interest the recent work by Stevens *et al*[1] showing the association between zonulin, fatty acid binding protein 2 (FABP2) and lipopolysaccharide (LPS) plasma levels and increased gut permeability in asymptomatic gastroenterology patients with depression and anxiety disorders, when compared with controls. Zonulin is a regulator of

intestinal permeability and a marker of intestinal barrier impairment,[2] which is reported in the irritable bowel syndrome (IBS) and functional dyspepsia (FD),[3] both of which are associated with anxiety and depression.[4 5] The tight junctional protein zonulin (ZO-1) is significantly reduced in these patients possibly driving a low-grade immune response.[3 6]

The utility of serum zonulin as a biomarker is controversial. One study observed higher serum zonulin levels in coeliac disease (CD) (mean 0.033 ng/mg total proteins), non-coeliac gluten sensitivity (NCGS) (mean 0.030 ng/mg total proteins) and IBS-diarrhoea (mean 0.012 ng/mg total proteins) patients, versus healthy controls,[7] but the Malmö Diet and Cancer cardiovascular cohort showed no association between serum zonulin and reported GI symptoms or IBS and FD.[8] We aimed to examine serum zonulin as a biomarker for IBS and FD.

Adult participants were recruited from two sources[9]; (1) a population-based study (n=242; mean age 62.1 (SD=11.9) years; 45.9% female) and (2) a two-site hospital-based study (n=102; mean age 45.0 (SD=16.2) years; 67.4% female). In the population-based study, IBS (n=40) and FD (n=42) were diagnosed according to modified Rome III criteria, while CD (n=6), gluten intolerance (n=6) and NCGS (n=44) had self-reported doctor diagnoses. Healthy controls (n=182) did not meet Rome III criteria. Participants in the hospital-based study were diagnosed with IBS (n=47) and/or FD (n=5). Controls included an organic GI gastrointestinal (GI) disease group (n=26), including clinician-diagnosed CD (n=5), IBD (inflammatory bowel disease) (n=6),gastro-oesophageal reflux disease (GORD) (n=5), other oesophageal diseases (n=4), lactose intolerance (n=1), hepatic cyst (n=1), *Helicobacter pylori* (n=1), collagenous colitis (n=1), ulcer (n=1) and cholecystectomy complication (n=1). Serum zonulin concentrations were determined by ELISA. All contrasts are between mutually exclusive groups and differential overlap between conditions results in small differences in participant numbers across contrasts.

Our findings showed no significant differences in serum zonulin concentrations (pg/mg) in the healthy control population cohort (mean=3755.1, SD=2566.4) versus IBS (mean=3999.8, SD=2463.7, p=0.5) (figure 1A) or FD (mean=3707.2, SD=2613.1, p=0.9) (figure 1B). Similarly, serum zonulin levels did not discriminate organic disease (mean=3407.2, SD=2441.4) from IBS (mean=3577.9, SD=2533.2, p=0.7) (figure 1C) or FD (mean=3015.6, SD=1513, p=0.9) (figure 1D). In a subset of the population sample, we further explored the value of zonulin in differentiating self-reported physician diagnoses of CD (mean=3601.4, SD=2874.6) versus no CD (mean=3796.4, SD=2517.9, p=0.9), gluten intolerance (mean=4012.1, SD=2603.2) versus no gluten intolerance (mean=3783.8, SD=2524.6, p=0.8) and self-reported GI symptoms with wheat ingestion (mean=3508.0, SD=2257.3) versus no GI symptoms after eating wheat (mean=3845.2, SD=2596.1, p=0.7). There were no significant differences in zonulin levels between overlap IBS/FD and healthy controls and organic disease or between any of the IBS and FD subgroups (table 1).

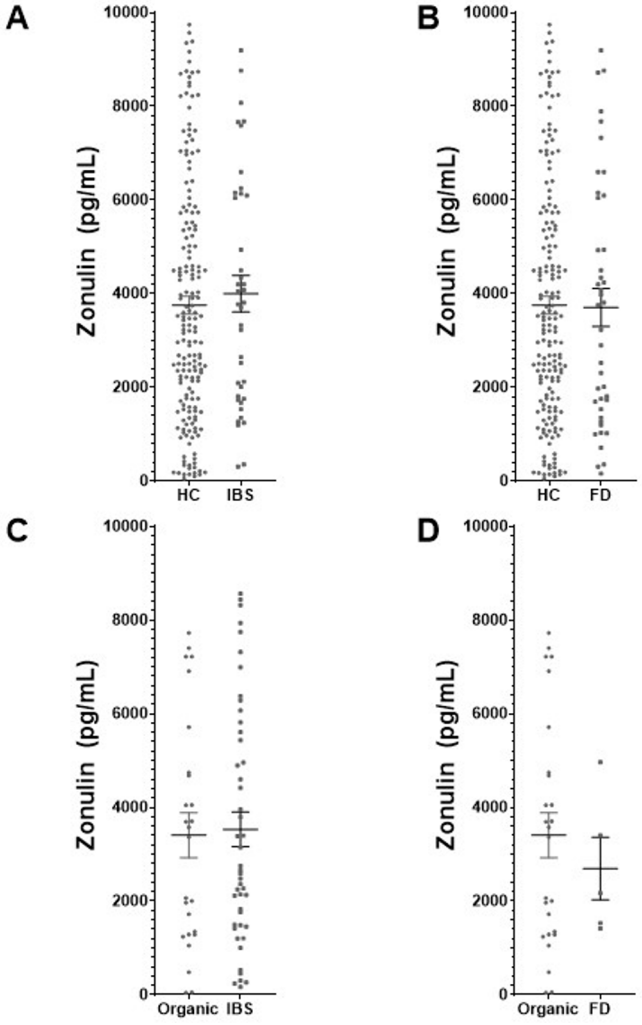

Figure 1 (A) Zonulin dot plot for IBS versus healthy controls (HC) in the population. (B) Zonulin dot plot for FD versus HC in the population. (C) Zonulin dot plot for IBS versus organic GI disease in the clinic. (D) Zonulin dot plot for FD versus organic GI disease in the clinic. Error bars denote mean±SEM. FD, functional dyspepsia.

These findings do not support the hypothesis that serum zonulin discriminates FGIDs from controls. This is consistent with Ohlsson *et al*, although further validation is required. One possible explanation may lie in the specificity of the ELISA in serum. Recent studies suggest a high level of cross-reactivity between zonulin and the serum protein properdin, which may impede the assay sensitivity.[10] While we did observe a significantly higher mean value of zonulin in IBS and FD versus CD, the low sample size of CD in our study warrants caution when interpreting these findings. Gliadin has been shown to drive zonulin secretion in vitro[2] and gluten avoidance may explain the low zonulin levels detected in this group.

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Table 1 Mean (SD) of zonulin serum levels in population and clinical samples

Zonulin (pg/mg)			
Population sample			
	FGID mean (SD)	**Healthy control mean (SD)**	**P value**
IBS			
IBS (n=40) versus healthy control (n=182)	3999.8 (2463.7)	3755.1 (2566.4)	0.5
FD			
FD (n=42) versus healthy control (n=182)	3707.2 (2613.1)	3755.1 (2566.4)	0.9
Overlap IBS/FD			
Overlap (n=25) versus healthy control (n=182)	3818.5 (2587.7)	3755.1 (2566.4)	0.9
FGID subgroups			
IBS (n=15) versus FD (n=17)	4301.8 (2296.2)	3543.5 (2721.3)	0.3
IBS-D (n=14) versus IBS-C (n=7)	4342.9 (1878.7)	4887.3 (2833.4)	0.9
FD-PDS (n=21) versus FD- EPS (n=6)	3999.0 (2811.3)	3375.6 (2001.6)	0.7
Clinic sample			
	FGID mean (SD)	**Organic diagnosis mean (SD)**	**P value**
IBS-D			
IBS-D (n=18) versus organic (n=26)	3050.6 (2522.6)	3407.2 (2441.4)	0.6
IBS-D (n=18) versus coeliac (n=5)	3050.6 (2522.6)	1027.3 (567.8)	0.05
IBS-D (n=18) versus IBD (n=6)	3050.6 (2522.6)	5006.2 (2223.4)	0.07
IBS			
IBS (n=46) versus organic overall (n=26)	3577.9 (2533.2)	3407.2 (2441.4)	0.7
IBS (n=46) versus coeliac (n=5)	3577.9 (2533.2)	1027.3 (567.8)	0.01
IBS (n=46) versus IBD (n=6)	3577.9 (2533.2)	5006.2 (2223.4)	0.2
FD			
FD (n=4) versus organic (n=26)	3015.6 (1513)	3407.2 (2441.4)	>0.9
FD (n=4) versus coeliac (n=5)	3015.6 (1513)	1027.3 (567.8)	0.01
FD (n=4) versus IBD (n=6)	3015.6 (1513)	5006.2 (2223.4)	0.2

EPS, epigastric pain syndrome; FD, functional dyspepsia; FGID, functional gastrointestinal disorders; IBS-C, irritable bowel syndrome - constipation predominant; IBS-D, irritable bowel syndrome - diarrhoea predominant; PDS, postprandial distress syndrome.

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Acknowledgements Zonulin levels were measured in the laboratory of Professor Giovanni Barbara, MD.

Contributors NJT and GJH: Study concept and design; analysis and interpretation of data; drafting of the manuscript; critical revision of the manuscript for important intellectual content; study supervision. MMW: Study concept and design; analysis and interpretation of data; drafting of the manuscript; critical revision of the manuscript for important intellectual content. NAK: Study concept and design; acquisition of data; analysis and interpretation of data; critical revision of the manuscript for important intellectual content. GB and MDEP: Acquisition of data; analysis and interpretation of data; critical revision of the manuscript for important intellectual content. AS: Acquisition of data and critical revision of the manuscript for important intellectual content. MJ: Study concept and design; analysis and interpretation of data; critical revision of the manuscript for important intellectual content; statistical analysis. SK: Study concept and design; critical revision of the manuscript for important intellectual content; statistical analysis.

Funding This study was supported by an investigator-initiated grant by the Commonwealth Diagnostic Laboratories.

Competing interests NJT: Dr Talley reports personal fees from Allergans PLC (GI Development Programs), personal fees from Viscera Labs (IBS), personal fees from IM Health Sciences (FD), personal fees from Napo Pharmaceutical (IBS), personal fees from Outpost Medicine (IBS), from Progenity Inc San Diego (capsule SIBO), from Allakos (gastric eosinophilic disease), personal fees from Samsung Bioepis (IBD), personal fees from Synergy (IBS), personal fees from Takeda (gastroparesis), personal fees from Theravance (gastroparesis), grants and personal fees from Viscera USA (IBS), grants from Commonwealth Diagnostics (International) Inc (IBS), non-financial support from HVN National Science Challenge NZ (IBS), grants and personal fees from GI therapies (constipation), personal fees from Cadila Pharmaceuticals (CME), personal fees from Planet Innovation (Gas capsule), personal fees from Danone (Probiotic), personal fees from Pfizer (IBS), from Dr. Reddy's Laboratories (Webinar), personal fees from Arlyx (IBS), personal fees from Sanofi (Probiotic), outside the submitted work; In addition, Dr Talley has a patent Biomarkers of IBS licensed, a patent Licensing Questionnaires Talley Bowel Disease Questionnaires licensed to Mayo/Talley, a patent Nestec European Patent licensed, a patent Singapore Provisional Patent "Microbiota Modulation Of BDNF Tissue Repair Pathway" issued, and a patent Nepean Dyspepsia Index licensed to Talley copyright and Committees: Australian Medical Council (AMC) [Council Member]; Australian Telehealth Integration Programme; MBS Review Taskforce; NHMRC Principal Committee (Research Committee) Asia Pacific Association of Medical Journal Editors. Boards: GESA Board Member, Sax Institute, Committees of the Presidents of Medical Colleges. Community group: Advisory Board, IFFGD (International Foundation for Functional GI Disorders). Miscellaneous: Avant Foundation (judging of research grants). Editorial: Medical Journal of Australia (Editor in Chief), Up to Date (Section Editor), Precision and Future Medicine, Sungkyunkwan University School of Medicine, South Korea. GJH: Unrestricted educational support from Bayer and the Falk Foundation. Research support was provided via the Princess Alexandra Hospital, Brisbane, by GI Therapies, Takeda Development Center Asia, Eli Lilly Australia, F Hoffmann-La Roche, MedImmune, Celgene, Celgene International II Sarl, Gilead Sciences, Quintiles, Vital Food Processors, Datapharm Australia, Commonwealth Laboratories, Prometheus Laboratories, FALK GmbH & Co KG, Nestle and Mylan. Patent holder: A biopsy device to take aseptic biopsies (US 20150320407 A1). MJ: Consultancies with GI Therapies (abdominal stimulation in constipation) and SFI (prokinetics). NAK: None to disclose. MMW: Grant/research support: Prometheus Laboratories

(IBS Diagnostic) and Commonwealth Diagnostics International (biomarkers for FGIDs). GB: None to disclose. MDEP: None to disclose. AS: None to disclose. SK: Grant/research support: Cancer Institute NSW (Career Development Fellowship), National Health and Medical Research Council (Project Grant APP1128487), Commonwealth Diagnostics International (biomarkersfor FGIDs) and Syntrix Biosystems (contract research—drug delivery).

Patient consent for publication Not required.

Provenance and peer review Not commissioned; internally peer reviewed.

To cite Talley NJ, Holtmann GJ, Jones M, *et al*. *Gut* 2020;**69**:1719–1722.

Received 7 March 2019
Revised 13 August 2019
Accepted 31 August 2019
Published Online First 28 September 2019

Gut 2020;**69**:1719–1722. doi:10.1136/gutjnl-2019-318664

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