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Alterations to the mechanical response of the gastrointestinal tract induced by functional gastrointestinal disorders and the feasibility of developing an ultrasonic diagnostic system

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Abstract

Functional gastrointestinal disorders (FGIDs) affect millions of people of all ages, regardless of race or gender, but little information related to FGIDs is available and no diagnostic methods exist. Therefore, the aim of this study was to investigate the alterations to gastrointestinal tracts induced by FGIDs, and to identify the feasibility of developing an ultrasonic diagnostic system to detect these alterations. Palpation/percussion examinations and in vitro micro-indentation tests were performed on 40 participants with or without FGIDs and 20 Sprague–Dawley rats with or without simulated FGIDs to identify any alterations to the mechanical response of the gastrointestinal tract. A finite difference analysis was performed for three simple models with or without assumed FGIDs to identify the feasibility of developing an ultrasonic diagnostic system. The results obtained from the palpation/percussion examinations and in vitro micro-indentation tests showed that a gastrointestinal tract with an FGID became more rigid than a healthy tract ($p < 0.05$). The results obtained from the finite difference analysis showed that an ultrasonic diagnostic system for FGIDs effectively detects alterations to the rigidity of the gastrointestinal tract, indicating that the development of an ultrasonic diagnostic system is reasonable and will aid in the diagnosis and treatment of FGIDs.

Keywords: Functional gastrointestinal disorders; Palpation and percussion examination; In vitro micro-indentation test; Finite different analysis; Feasibility of developing an ultrasonic diagnostic system

1. Introduction

Functional gastrointestinal disorders (FGIDs) affect millions of people of all ages, regardless of race or gender [1]. Approximately 60-70% of the modern population has at least one syndrome induced by an FGID [2-4], with irritable bowel syndrome (IBS) and dyspepsia being the most common [1-5]. IBS alone affects 10-15% or more of adults. Drossman et al. [2] reported that 69% of the surveyed population had at least one of 20 syndromes induced by FGIDs; the symptoms were attributable to four major anatomic regions: the esophageal (42%), gastroduodenal (26%), bowel (44%), and anorectal (26%) regions, with considerable overlap. Talley et al. [3] found that 60% of the total population had four or more gastrointestinal symptoms induced by FGIDs, and considerable overlap of IBS with dyspepsia occurred. The major abdominal syndromes induced by FGIDs were dyspepsia, IBS, reflux, painless constipation, painless diarrhea, and bloating. Thompson et al. [4] showed that at least one FGID occurred in 61.7% of 1149 participants (65.6% for females versus 57.6% for males); the most prevalent were functional bowel disorders at 41.6%, followed by esophageal disorders at 28.9%.

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FGIDs exhibit very heterogeneous conditions and are primarily referred to as abnormalities induced by an alerted physiological function rather than an identifiable structural or biochemical cause [1, 6-8]. Thus, the cause of occurrence for FGIDs has not been clearly elucidated. Recently, psychological causes as risk factors for the development of FGIDs have been implicated [9-16]. Several clinic-based studies [9-11, 14, 15] reported that patients with dyspepsia induced by FGIDs had more life stress and psychological distress than healthy controls; the studies identified these psychological causes as risk factors for the development of FGIDs. Other clinic-based studies [10, 12, 16] also reported that patients with IBS induced by FGIDs had a higher prevalence of psychological distress, major depression, somatization, hypochondriasis, and anxiety than healthy controls. However, much still remains unknown about the causes of the development of FGIDs because these studies were based on limited data and the exact nature of the association remains to be ascertained. More recently, Feinle et al. [17] and Niec et al. [18] advocated that food ingestion can variably precipitate gastrointestinal symptoms, including dyspepsia and IBS. Talley et al. [8], Cremonini et al. [19], Delgado-Aros et al. [20], Svedberg et al. [21], and Locke et al. [22] reported an association between an increased body mass index (BMI) or obesity and gastrointestinal symptoms induced by FGIDs. However, little information is available about this association between obesity and gastrointestinal symptoms, resulting in a poor understanding of the alterations on the gastrointestinal tract induced by FGIDs.

Therefore, the aims of this study were to investigate the alterations on the gastrointestinal tract induced by FGIDs and to identify the feasibility of developing an ultrasonic diagnostic system to detect these alterations. The study was based on the hypothesis that the mechanical response of the gastrointestinal tract of a patient with an FGID changes. This hypothesis was established based on the fact that psychological distress and obesity associated with the development of FGIDs may result in changes in the mechanical responses of the gastrointestinal tract. This endeavor may be valuable by identifying for the first time mechanical alterations of the gastrointestinal tract induced by FGIDs, and by demonstrating the feasibility of developing an ultrasonic diagnostic system for FGIDs.

2. Materials and methods

2.1 Participant and specimen preparation

Participants: Following approval from our institutional review board, 40 female human subjects were randomly placed into two groups, with 20 normal subjects (age: 23.8 ± 1.6years, weight: 55.3 ± 3.6kg, height: 160.0 ± 3.6cm, and waist: 73.6 ± 2.0cm) assigned to the control group and 20 subjects with symptoms related to FGIDs (age: 42.2 ± 8.6years, weight: 62.3 ± 3.6kg, height: 156.0 ± 2.5cm, and waist: 73.8 ± 2.6cm) assigned to the patient group. The participants maintained an empty stomach for 24 h before the palpation/percussion examinations to ensure that their stomachs were flattened parallel to the abdomen surface to minimize distortions caused by ingesta inside the stomach.

Specimens: Twenty specific pathogen-free Sprague–Dawley rats (6 weeks old, approximate weight 253g) were housed in individually ventilated cages under vivarium conditions (temperature: 22 ± 2°C, humidity: 50 ± 10%, with a 12-h light–dark cycle). All rats were randomized into two groups: a normal diet group (ND, n = 10) and overeating group (OE, n = 10). The OE group was created to simulate FGIDs based on an association between obesity and gastrointestinal symptoms induced by FGIDs [8, 19-21]. Expanded pellets (Superfeed Co., Gangwon, Korea) were administered to the ND group at a rate of 11 g/day and to the OE group at a rate of 22 g/day for 12 weeks. The rats in both groups were killed by CO_2 asphyxiation after 12 weeks, and their gastrointestinal tracts were immediately harvested under sterile conditions. All specimens were dissected from the greater curvature of the glandular region for *in vitro* micro-indentation. The dimensions of the specimens were 10mm (width) × 10mm (length) × 1mm (height). All procedures for specimen preparation were in accordance with the approved NIH Guide for Care and Use of Laboratory Animals under a protocol approved by the Yonsei University School of Animal Care and Ethics Committee.

2.2 Palpation/percussion examinations and in vitro micro-indentation tests

2.2.1 Palpation/percussion examinations

The palpation examinations were performed by an experienced physician to identify alterations in the rigidity of gastrointestinal tracts induced by FGIDs, which were perceptible while palpating the abdomi-

nal region overlaying the gastrointestinal tract. The alterations were then identified qualitatively by comparing the sensuously determined rigidity of the control group with that of the patient group.

Percussion examinations were conducted by the same physician who performed the palpation examinations to identify the degree of rigidity alteration in the gastrointestinal tract induced by FGIDs through analyzing the characteristics (pattern, attenuation ratio, and frequency change) identified from percussion sounds. The percussion examinations were performed on the same abdominal regions that were examined in the palpation examinations. A schematic diagram that describes the characteristics of the percussion sounds is shown in Fig. 1. First, the reflective sound signals generated by a plexor were detected with a stethoscope (3M Littman Classic II; 3M Corp., Minneapolis, MN, USA). Then the detected reflective sound signals were converted to voltage signals by a microphone (CMIC-6050; SG Co., Ltd., Seoul, Korea). The voltage signals were amplified and filtered by a customized amplifier and band-pass filter with cutoff frequencies of 30 to 55Hz. Finally, the amplified and filtered signals were transmitted to a digital oscilloscope (LeCroy LC574AM; LeCroy Corp., Santa Clara, CA, USA) and analyzed.

2.2.2 In vitro micro-indentation test

In vitro micro-indentation tests were performed on all harvested specimens from the 20 specific pathogen-free Sprague–Dawley rats by using an Instron micro-test system (5848 series; Instron, Norwood, MA, USA). A constant load corresponding to a 0.001 s^{-1} strain rate was applied continuously to the specimens. The *in vitro* micro-indentation tests were conducted three times per specimen to identify and reduce the effects of intra-specimen errors. The stiffness of the gastrointestinal tracts was calculated from the load-displacement data obtained from the *in vitro* micro-indentation tests. Three material phases were used to represent the nonlinearity of the specimens. The effective Young's modulus for the first material phase was calculated to rigorously identify the change in material behavior during the initial phase. Young's modulus can be written as

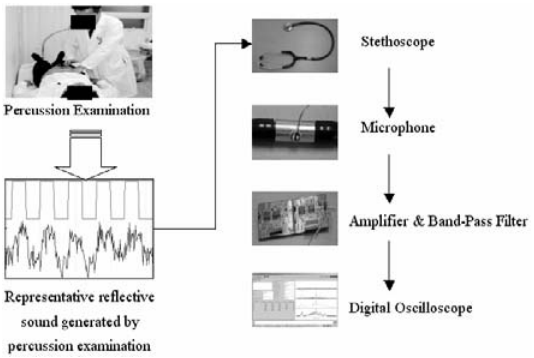


Fig. 1. Schematic diagram for analyzing the reflective sounds generated from the percussion examinations.

$$E = \frac{1-\nu^2}{2a\kappa(\nu, a/h)} \cdot \frac{P}{w}, \tag{1}$$

where ν is the Poisson ratio, P is the applied force, α is the radius of the indenter, h is the tissue thickness, w is the indented depth, and κ is a scaling factor. The scaling factor κ provides a theoretical correction for the finite thickness of the elastic layer, and it depends on both the aspect ratio a / h and Poisson ratio. We used a value of 0.49 for Poisson ratio by assuming that the soft tissue was nearly an incompressible material. This is a rigorous mathematical solution to the elastic indentation problem of a thin elastic layer bonded to a rigid half-space with a rigid, frictionless cylindrical plane-ended indenter [23].

2.3 Development of finite difference models

A finite difference model for normal gastrointestinal conditions was created (see Fig. 2(a)). The model consisted of three soft-tissue layers making up the overall gastrointestinal tract: the skin, fat, and smooth muscle, going from top to bottom across the model. The material properties required for the analysis are summarized in Table 1. The density, ρ, and longitudinal bulk acoustic wave velocity, VL, for each soft-tissue were determined from the literature [24]. Based on the determined ρ and VL, the first and second Lame constants, λ and μ, related to the elastic modulus and Poisson ratio and commonly used in finite element analyses, were computed from

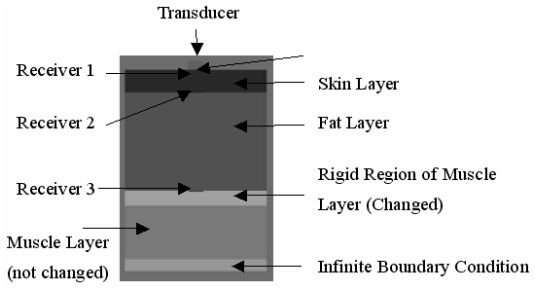
$$\lambda = \rho \times (VL^2 - 2 \times VT^2) \tag{2}$$

$$\mu = \rho \times VT^2 . \tag{3}$$

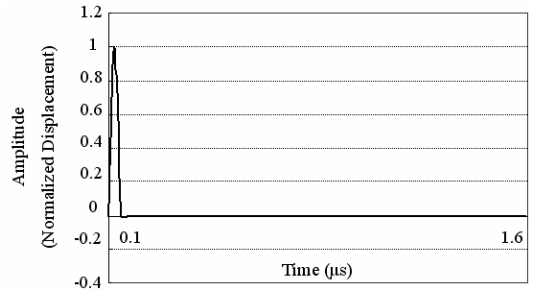
Here, μ was set to zero to correspond to a Poisson ratio of 0.5, which represents the incompressibility of soft-tissue materials [25-27].

Additionally, two finite difference models were created to simulate alterations to the rigidity of a gastrointestinal tract that may be induced by FGIDs (see Fig. 2(a)). These were identified as assumed medium

and severe FGID models and used to predict alterations in the ultrasonic reflective signals (rigidity) of the gastrointestinal tract along with the degree of the FGID progress. Unfortunately, because no information was available related to the material properties of the smooth muscles in the FGID models, these properties were arbitrarily assumed (see Table 1). The densities were not altered for the FGID models, and the other parameters (i.e., boundary and loading conditions) were the same as those for the normal model.



(a) Standard two-dimensional finite difference model, its elements, and boundary conditions



(b) Source signal (loading condition) applied to the finite difference models through transducer

Fig. 2. (a) Standard two-dimensional finite difference model, its elements, and boundary conditions. Model types (normal or simulated FGID models) were determined corresponding to the characteristics of the material properties used in the rigid region of the muscle layer. (b) Source signal as a loading condition (5MHz frequency with an amplitude of 1 over 0.1μs) applied to the finite difference models through a transducer.

Table 1. Material properties of the developed finite difference models [24].

Soft-Tissue Layers		ρ (g/cm^3)	VL (m/s)	VT (m/s)	A (N/mm^2)
Skin		1000	1519	1074	2307
Fat		920	1478	1045	2010
Muscle	Normal	1040	1552	1097	2505
	Medium FGID	1040	2328	1646	5636
	Severe FGID	1040	3104	2195	10020

One infinite (absorbing) boundary condition was employed at the bottom of the finite difference models (see Fig. 2). For the loading condition, a sine pulse (source signal) with a frequency of 5MHz and a maximum amplitude of 1 produced from the trans ducer defined over the skin layer was applied once to each finite difference model over a period of 0.1 μs (see Fig. 2(b)). The sign pulse is a specification of a time-dependent displacement and corresponds to a mechanical response induced by the ultrasound signal generated from the actual transducer of the ultrasound system [25, 27]. Three receivers were defined at the top of the finite difference model and the interfaces between the soft-tissue layers to predict the displacements, which corresponded to the source signal at locations of interest in the object (see Fig. 2(a)). The displacement amplitude predicted at the receiver was inversely proportional to the amplitude of the ultrasonic reflective signal obtained by the receiver in the actual ultrasound system [25, 27].

All finite difference analyses were conducted by using a stand-alone computer software package (Wave2000 Pro^{TM}; CyberLogic Inc., Troy, MI, USA) suitable for two-dimensional ultrasonic problems related to elastic wave propagation.

2.4 Statistical analysis

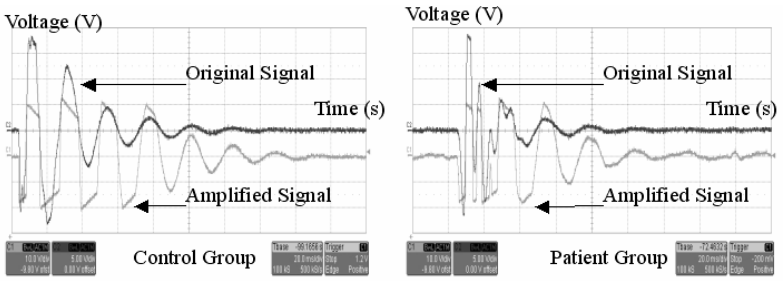
For the percussion examination results, we used a paired student's t-test to identify a significant difference between the characteristics of the percussion sounds obtained from the normal and patient groups. For the *in vitro* micro-indentation test results, we used a one-way ANOVA test with Tukey's B post hoc multiple comparisons to identify a significant difference among the three material phases for each group, followed by a paired t-test to identify a significant difference between the effective moduli for the OE and ND groups. The significance level for all statistical tests (p) was set at 0.05.

3. Results

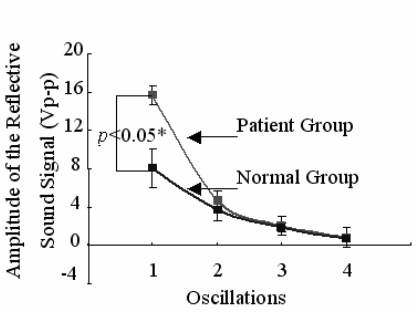
3.1 Alterations to the gastrointestinal tract induced by FGIDs

3.1.1 Results from the palpation/percussion examinations

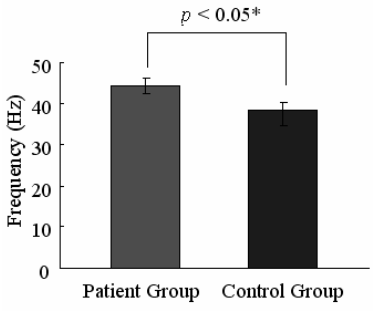
During the palpation examinations, the physician qualitatively perceived that the gastrointestinal tract in



(a) Representative reflective sound signals



(b) Average amplitude at each oscillation and its standard deviation



(c) Average frequency and its standard deviation

Fig. 3. (a) Representative reflective sound signals measured from the normal and the patient groups; (b) amplitudes at the first, second, third, and fourth oscillations computed from the reflective sound signals for the normal and the patient groups; and (c) frequency determined from the reflective sound signals measured from the normal and the patient groups. The asterisk (*) indicates a significant difference.

people with FGIDs (patient group) were harder than those in healthy people (control group).

For the percussion examinations, the results (pattern, attenuation ratio, and frequency change in the signals converted from percussion sounds) quantitatively showed that the gastrointestinal tracts of the patient group were harder than those of the control group. The reflective sound signals measured from the patient group generally exhibited distorted patterns, with a reduction in the number of oscillations compared to the control group (see Fig. 3(a)). The amplitudes of the first oscillation in the reflective sound signals measured from the patient group, 15.7 ± 3.0V p-p, increased significantly by an average of 92.7% compared to the control group, 8.1 ± 2.5V p-p ($p < 0.05$) (see Fig. 3(b)). The amplitudes of the second, third, and fourth oscillations of the reflective sound signals measured from the patient group were, however, similar to those from the control group ($p > 0.05$). The degree of attenuation in the reflective sound signals measured from the patient group was 11.0 ± 2.3V p-p between the first and second oscillations, 2.7 ± 0.9V p-p between the second and third oscillations, and 1.2 ± 0.5V p-p between the third and fourth oscillations. The degree of attenuation in the reflective sound signals measured from the control group was 4.4 ± 2.0V p-p between the first and second oscillations, 1.8 ± 1.3V p-p between the second and third oscillations, and 1.2 ± 0.4V p-p between the third and fourth oscillations. The frequencies of the reflective sound signals measured from the patient group, 43.9 ± 3.8Hz, generally increased on average by 15.4% compared to those from the control group, 38.4 ± 1.77Hz ($p < 0.05$).

3.1.2 Results from the in vitro micro-indentation tests

The average load-displacement curves for both the ND and OE groups are shown in Fig. 4. In the ND group, the stiffness values of the first, second, and third phases were 1.5±0.5, 27.9±5.9, and 406.8±44.7 N/mm, respectively. In the OE group, the stiffness values of the first, second, and third phases were 1.9±0.5, 30.5±7.0, and 454.0±143.2N/mm, respec-

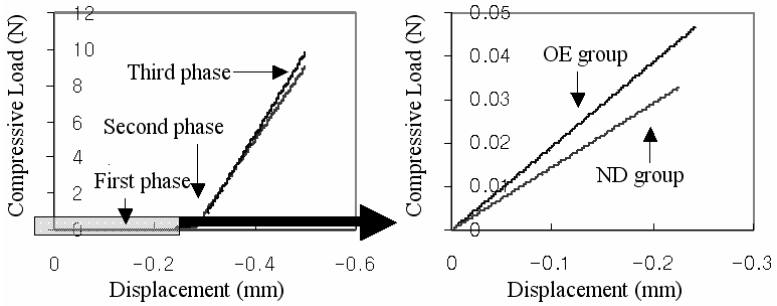
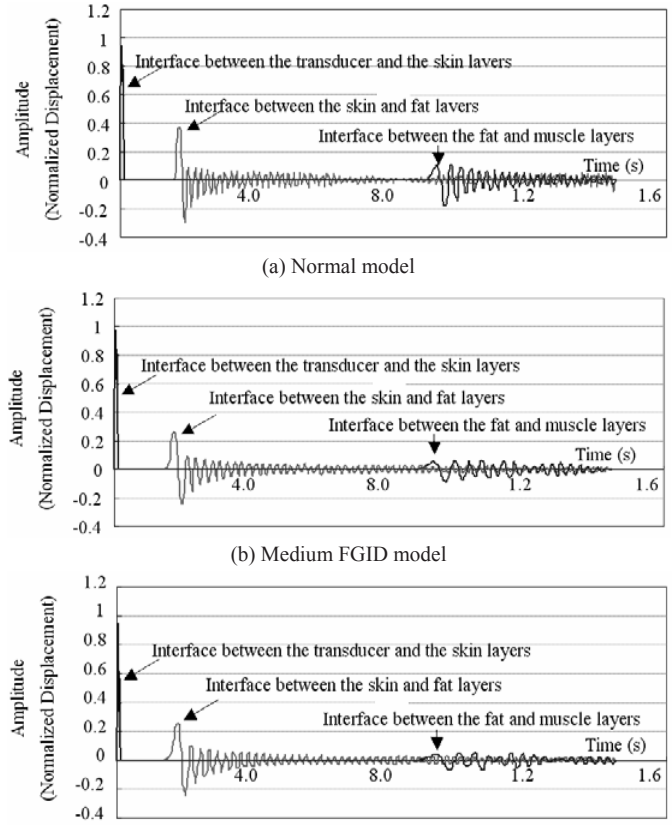


Fig. 4. Average load-displacement curves for all three material phases (left) and the emphasized first phase (right), determined from the ND and OE groups.



(a) Normal model

(b) Medium FGID model

(c) Severe FGID model

Fig. 5. Ultrasonic responses for the (a) normal, (b) medium FGID, and (c) severe FGID models predicted by the finite difference analyses.

tively. Significant differences were detected among the material phases for both groups ($p < 0.05$). When comparing the ND with the OE group, only the stiffness of the first material phase, not the second or third material phases, was significantly different ($p < 0.05$). The effective moduli in the first material phase for the ND and OE groups, which were calculated from Eq. (1), were 0.3 ± 0.1 and 0.4 ± 0.1 MPa, respectively. A significant difference was observed between the effective moduli ($p < 0.05$).

3.2 Feasibility of utilizing the ultrasonic characteristics

Ultrasonic responses for the normal, medium FGID, and severe FGID models predicted by the finite dif-

ference analyses are shown in Fig. 5. The maximum displacement amplitudes at the interface between the skin and the fat layers (Receiver 2) decreased by 29.9% (normal model), 46.9% (medium FGID model), and 47.4% (severe FGID model) relative to those between the transducer and the skin layers (Receiver 1). The alterations of the maximum displacement amplitudes induced by the change in rigidity were 17.0% between the normal model and the medium FGID model and 17.5% between the normal model and the severe FGID model. The maximum displacement amplitudes at the interface between the fat and the smooth muscle layers (Receiver 3) decreased 55.5% (normal model), 69.5% (medium FGID model), and 75.1% (severe FGID model) relative to those at the interface between the skin and the fat layers (Receiver 2). The alterations of the maximum displacement amplitudes induced by the change in rigidity were 14.0% between the normal model and the medium FGID model and 19.6% between the normal model and the severe FGID model.

4. Discussion and conclusions

We demonstrated qualitatively and quantitatively that the rigidity of the gastrointestinal tract changed with the degree of the FGID. FGIDs are heterogeneous conditions that are primarily referred to as abnormalities induced by an alteration of a physiological function rather than an identifiable structural or biochemical cause [1, 6-8]; this has resulted in few explicit descriptions regarding the causes of FGIDs and alterations due to the development of FGIDs, and few methods for diagnosing FGIDs. Therefore, this study may prove to be valuable by providing for the first time qualitative and quantitative identifications of mechanical alterations to the gastrointestinal tract induced by FGIDs. Based on this finding, it may be reasonable to develop a diagnostic system for FGIDs by using ultrasonic characteristics, which will aid in the diagnosis and treatment of FGIDs.

The results from the percussion examinations showed that the reflective sound signals for the patient group were generally more distorted and loud (increased magnitude) with fewer oscillations than those for the control group ($p < 0.05$). These results indicate that the gastrointestinal tract may be more rigid in the patient group compared to the control group. In particular, after the first oscillation, a higher attenuation ratio was observed in the reflective sound signals measured from the patient group compared to the control group, which strongly supports the finding that the gastrointestinal tracts of the patient group were more rigid than those of the control group. The attenuation resulted because the sounds generated by a plexor on a rigid material diminish more rapidly compared to those on softer materials. In addition, the reflective sound signals measured from the patient group had higher frequencies that those of the control group, which also indicates that the gastrointestinal tracts of the patient group were more rigid than those of the control group since the sounds generated by a plexor on rigid materials propagate more rapidly to the air than those on softer materials.

The results from the *in vitro* micro-indentation tests showed that the stiffness and effective modulus in the first material phase for the OE group were generally higher than those for the ND group. This suggests that the gastrointestinal tract may be more rigid in patients with FGIDs than that in healthy subjects, supporting the findings obtained from the palpation/percussion examinations. The *in vitro* micro-indentation test results strongly support previous findings that the development of FGIDs is associated with obesity induced by overeating [8, 19-21]. These tests may therefore be valuable as a first attempt at quantifying the alteration in the mechanical response of the gastrointestinal tract with a relationship between obesity and the development of FGIDs from a mechanical point of view. However, the *in vitro* micro-indentation tests did not consider viscosity, which can influence mechanical responses such as the rigidity of the gastrointestinal tract. Thus, the *in vitro* micro-indentation test results might be underestimated or overestimated. The fact that soft tissues have a viscous mechanical behavior has been well documented and quantified in the literature over the past 20 years [28-35]; this includes ligaments [34], tendons [29, 32], articular cartilage [33, 35], and heart and skeletal muscle [28, 31]. The viscosity can arise from fluid flow in or out of the tissue, from an inherent viscosity of the solid phase, or from viscous interactions between tissue components or phases [30]. The viscosity of soft tissues should therefore be analyzed when determining their mechanical response. The *in vitro* micro-indentation tests performed in the current study, which only considered nonlinear elasticity, were only performed to determine whether the rigidity of the gastrointestinal tract increased significantly for the OE group.

The results from the finite difference analyses showed that the degree of the FGID progress was detected effectively by using ultrasonic characteristics. This finding indicates that developing a diagnostic system for FGIDs using ultrasonic characteristics may be reasonable for diagnosis and treatment. However, the first Lame constants of the smooth muscle for the FGID finite different models were arbitrarily set to be two (medium FGID model) or four (severe FGID model) times more rigid than the normal model. The densities in all the FGID models were also arbitrarily set to the same value. The first Lame constants and densities therefore did not match the actual mechanical characteristics of smooth muscle corresponding to the degree of FGID progress. The establishment of the first Lame constants was based on the hypothesis that the elastic characteristics of the smooth muscle as structural elements of the gastrointestinal tract increased with the degree of FGID progress. This hypothesis was supported by the *in vitro* micro-indentation tests. The establishment of the densities was based on the hypothesis that the structural characteristics or component ratio of the soft-tissue layers does not change in functional disorders [1, 6-8]. The finite difference analyses were performed only to investigate whether a diagnostic system using ultrasonic characteristics was reasonable by analyzing the alternation trends of the ultrasonic reflective response on smooth muscle corresponding to the degree of FGID progress.

At the conclusion, through all the results obtained from the percussion examinations, the *in vitro* micro-indentation tests, and the finite difference analyses, the current study identifies that a gastrointestinal tract with an FGID may become more rigid than a tract without an FGID. However, this finding and the resulting occurrence mechanisms require further confirmation using concepts incorporated in micro-mechanics and molecular biology. We are currently investigating this in an ongoing study, the results of which may increase the confidence levels of the results presented here. However, the current study identifies for the first time mechanical alterations to the gastrointestinal tract induced by FGIDs, and demonstrates the feasibility of developing an ultrasonic diagnostic system for FGIDs.

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