Constrictive and Hypertrophic Strictures in Ileal Crohn's Disease

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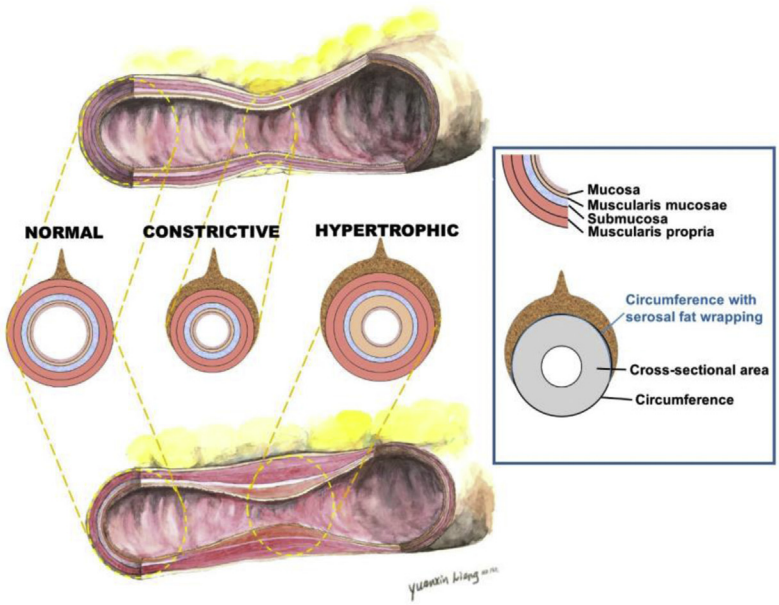

Clinical Gastroenterology and Hepatology

BACKGROUND & AIMS: **Strictures in Crohn's disease (CD) are classically attributed to fibromuscular hypertrophy of the intestinal wall. We have identified and characterized CD-related ileal strictures that result instead from mural constriction (ie, reduced external circumference).**

METHODS: **Twenty-four strictures and internal controls from 17 adults with obstructive CD were analyzed by cross-sectional morphometry.**

RESULTS: **The stricture-to-control circumference ratios (CRs) ranged from 0.53 to 1.7. Six strictures with CR ≥1.0, designated hypertrophic, had concentrically thickened walls, mean 3-fold increases in**

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Abbreviations used in this paper: AR, stricture-to-internal control cross sectional area ratio; CD, Crohn's disease; CR, stricture-to-internal control circumference ratio; MM, muscularis mucosae; SMA, smooth muscle actin; SR, sirius red.

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cross-sectional area and stainable fibromucular tissue, and high transmural inflammation scores. In contrast, 18 strictures with CR <1.0, designated constrictive, had thin, pliant walls, cross-sectional areas and stainable fibromuscular tissue comparable with control values, and low transmural inflammation scores. Eight mildly constrictive strictures also showed mild fibromuscular mural expansion that fell short of statistical significance. Twelve of 18 constrictive strictures (67%) occurred multiply (2–4 strictures per specimen) in contrast with hypertrophic strictures, all of which occurred singly ($P = .01$). Constriction correlated quantitatively with circumferential serosal fat wrapping ($P = .003$) and was associated with myenteric lymphocytic plexitis ($P = .02$). Disease duration was shortest among subjects with constrictive strictures and correlated with increasing circumference (CR ≤0.8, 6.3 ± 6.2 years; CR >0.8, 8.7 ± 6.4 years; and CR ≥1.00, 13.7 ± 5.0 years, respectively; $P = .03$).

CONCLUSIONS: **Constrictive ileal strictures in CD differ pathologically and clinically from hypertrophic strictures, featuring little or no fibromuscular mural expansion, frequent multiplicity, and earlier onset. Mesenteric fat wrapping and myenteric plexitis may contribute to their pathogenesis. Pathologic manifestations of constriction and hypertrophy can coexist, suggesting that stricture heterogeneity may be shaped in part by the dynamics of constrictive and hypertrophic processes.**

Keywords: Contrictive; Crohn's Disease; Strictures.

Intestinal strictures occur in over one-half of patients with Crohn's disease (CD), imposing high risks of obstructive complications, multiple surgeries, and impaired quality of life.[1] Despite recent advances in inflammatory bowel disease therapeutics, stricturing in CD remains unpredictable, unpreventable, and medically refractory, posing a high-priority therapeutic challenge.[2,3]

Recent consensus reports defining the pathologic[4] and cross-sectional imaging features[5] of naïve small bowel strictures have identified luminal narrowing, mural hypertrophy, and prestenotic dilatation as cardinal features. Functionally, resistance to intestinal transit is multifactorial, combining diminished luminal cross-sectional area, reduced compliance of the thickened intestinal wall, and impaired peristalsis.[1,6,7] Pathologically, major contributors to mural hypertorphy include fibromuscular expansion of the muscularis mucosae (MM) layer resulting from dysregulated myofibroblast and smooth muscle proliferation,[7,8] lymphatic remodeling, and inflammatory cell infiltration.[7–12] Recognition of these pathologic changes provides guidance for investigation into stricture pathogenesis and the identification of potential therapeutic targets.[13,14]

We describe here a subset of ileal strictures in CD that result from constriction (ie, reduced external circumference), rather than mural hypertrophy. To our knowledge, constrictive stricturing has not been featured in recent reviews and consensus papers[4,15] or in the classical pathology literature.

Methods

Case Identification

Specimens were identified prospectively among consecutive surgeries performed at The Mount Sinai Hospital during a 5-month interval. The inclusion criteria were patient age ≥17 years; a verified preoperative clinical diagnosis of obstructive ileal CD; surgery performed for relief of obstructive symptoms; and pathologic features characteristic of CD including the presence of 1 or more strictures. Strictures were defined grossly as segments with (1) luminal narrowing relative to macroscopically normal segments in the same specimen and (2) prestenotic dilatation. Preoperative cross-sectional computerized tomography or magnetic resonance enterography had been performed within the preceding 6 months in most cases, but was not required for inclusion. We did not account for strictures other than those identified in the resection specimens.

Exclusion criteria were: (1) the presence of any complicating features that might distort the stricture microanatomy, such as an adjacent anastomosis, strictureplasty site, fistula, abscess, or dense fibrous adhesions and (2) the absence of macroscopically normal segments to serve as internal morphometric controls.

Patient Characteristics

The admission diagnoses of CD were confirmed clinically by the respective clinical teams and verified retrospectively by 2 of the authors (J.F.C. and J.E), who reviewed the patients' electronic medical records. The following data were extracted: demographics, ethnicity, age at diagnosis of CD, family history of inflammatory bowel disease, duration from diagnosis of CD to surgery, stricture location, revised Montreal classification,[16] smoking history, body morphometric index, and perioperative medical therapies.

Pathology and Digital Morphometry

Each specimen was examined by a specialist gastrointestinal pathologist to confirm the diagnosis of

CD and identify strictures. After overnight formalin fixation, the strictures, one or more cross-sections of normal control segments, and selected diseased non-strictured segments were serially cross-sectioned in intervals of approximately 0.5 cm. Comparison of pre- and post-fixation cross sections by morphometry showed <4% mean reduction in circumference. Routine laboratory processing yielded 5-micron thick microscopic sections, which were stained with hematoxylin and eosin, sirius red (SR), anti-smooth muscle actin (SMA, clone 1A4, Agilent Technologies, Inc, Santa Clara, CA), and anti-desmin (clone DE-R-11, Roche Holding AG, Basil, Switzerland).

The slides were digitally scanned at 20-fold magnification with a Pannoramic 250 instrument (Pelkin-Elmer, Waltham, MA), and the images were analyzed with Halo software (v.1.4, Indica Labs, Albuquerque, NM). Quantitation of collagen and smooth muscle was performed in stained regions of interest after spectral filtering as described previously.[7]

The following measurements were determined (see graphical abstract): circumference along the muscular-mesenteric interface, mural cross-sectional area (excluding the mucosa), stricture-to-control circumference ratio (CR), stricture-to-control cross-sectional area ratio (AR), cross-sectional area of each mural layer, and proportion of the circumference covered by serosal fat. Strictures were classified according to CR based on their minimum circumference.

The density of myenteric plexitis was quantitated manually following double immunostaining of the histologic sections for CD3 (clone 2GV60, Roche, Basel, Switzerland) and synaptophysin (clone SP11, Roche, Basel, Switzerland). It was expressed as CD3-positive lymphocytes per 1-mm^2 hotspot containing the largest number of lymphocytes in apposition to or within an enteric ganglion or nerve bundle.[17]

Inflammation scores were determined semiquantitatively by 2 methods[18,19] without knowledge of the other corresponding measurements.

Statistics

Continuous variables were expressed as means ± standard deviations. Independent *t*-tests were used to compare different stricture types and paired *t*-tests to compare strictures with controls. One-way analysis of variance was used for comparison of more than 2 means. The Pearson correlation coefficient and 95% confidence interval were used to assess the correlation between disease intervals and CR. All statistical tests were 2-tailed. $P < .05$ was the significance threshold.

Statistical analyses were performed using SPSS (IBM SPSS Statistics 20; SPSS Inc, Chicago, IL) or GraphPad Prism version 8.0.0 for Windows, GraphPad Software (San Diego, CA).

What You Need to Know

Background

Intestinal strictures in Crohn's disease are currently unpredictable, unpreventable and medically refractory, posing a high priority therapeutic challenge. Conventional stricturing is characterized by segmental fibromuscular expansion of the ileal wall.

Findings

We have identified and characterized a subset of ileal strictures in Crohn's disease that feature markedly reduced outer circumferences, increased fat wrapping and plexitis but that lack fibromuscular mural expansion.

Implications for patient care

Ileal strictures in Crohn's disease are phenotypically heterogeneous. The lack of fibromuscular expansion in some strictures may warrant consideration in designing future therapeutic trials involving anti-fibrotic and anti-inflammatory agents.

Approvals

This study was approved by the Institutional Review Board of the Icahn School of Medicine at Mount Sinai.

Results

Surgical Specimens

Seventeen resection specimens met the study criteria. Twelve specimens (71%) contained 1 stricture, and 5 specimens (29%) each contained 2 to 4 strictures, for a total of 24. In addition to the stricture characteristics required for inclusion, the resection specimens all contained non-strictured regions with typical gross and microscopic characteristics of Crohn's ileitis, such as creeping serosal fat, effaced mucosal folds, aphthous or longitudinal ulcerations, granulomas, or chronic serositis. Ten strictures that were excluded from the study due to adjacent anastomoses (n = 6) or distorting fistulas or fibrous adhesions (n = 4); all had concentrically thickened, rigid walls.

Stricture Classification

The CRs ranged from 0.53 to 1.7. Six strictures with normal or expanded circumferences (CR ≥1) were designated hypertrophic, and 18 strictures with diminished circumferences (CR <1) were designated constrictive (Figure 1A). The latter were subdivided by the group mean CR of 0.8 for the purpose of testing intragroup heterogeneity by comparing the properties of mildly and severely constrictive strictures (n = 10 and n = 8, respectively).

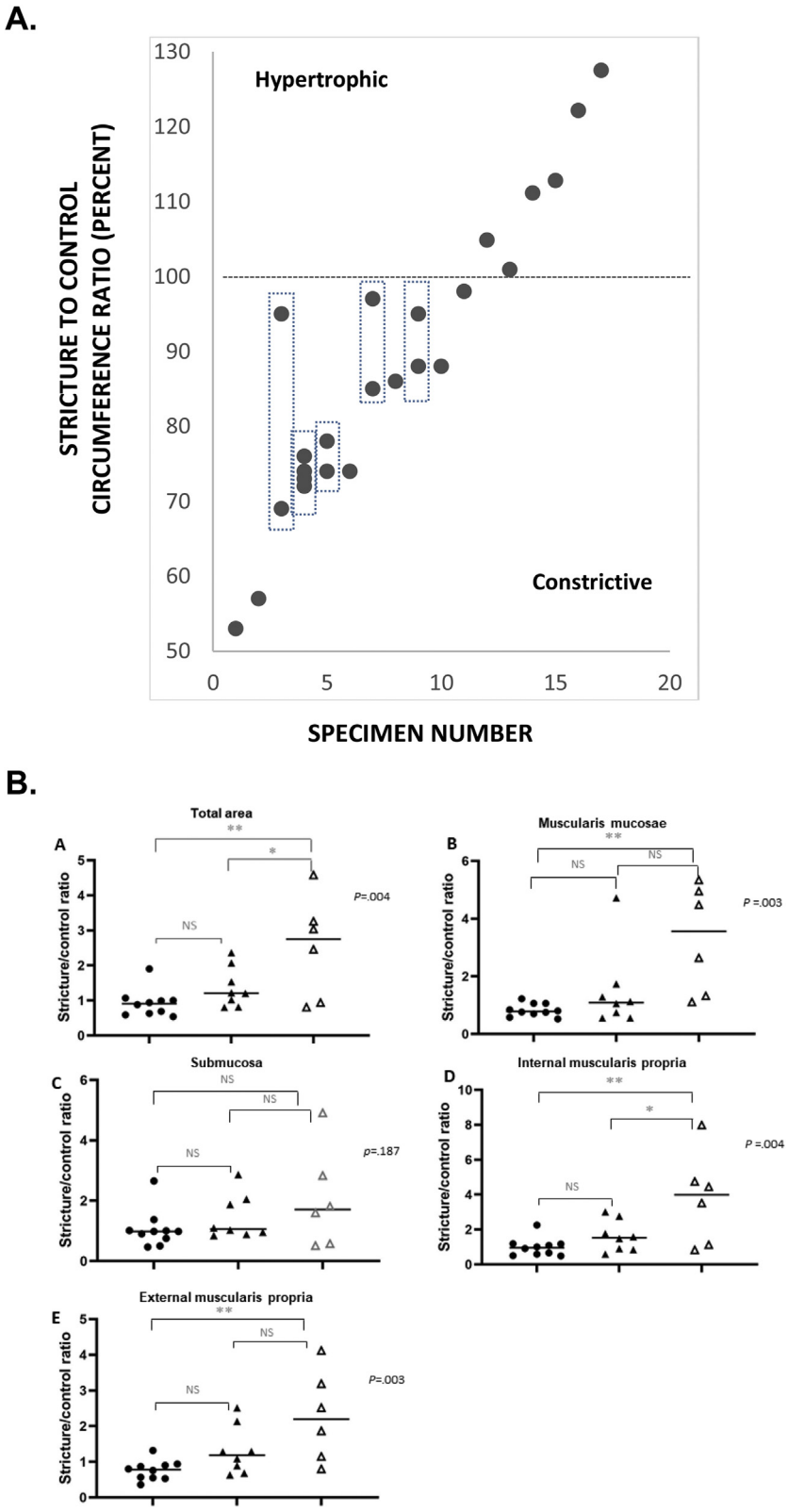

Figure 1. *(A)* Distribution of strictures in 17 resection specimens based on increasing CRs and numbered serially. Specimens with multiple strictures are enclosed in rectangles. *(B)* Cross-sectional areas of intestinal wall and anatomical layers by stricture category. *Left*, *center* and *right* groups correspond to severely constrictive (CR <0.8), mildly constrictive (CR ≥ 0.8) and hypertrophic strictures, respectively, where CR 0.8 is the group mean for constrictive strictures. *P* value calculated by 1-way analysis of variance. $^{*}P < .05$; $^{**}P < .005$.

Clinical Characteristics

The subjects' clinical characteristics are summarized in Table 1. None had undergone previous small intestinal resection. At the time of surgery, all were receiving preoperative medical therapy including steroids (n = 8), immunomodulators (n = 9), or anti-tumor necrosis factor agents (n = 12). Insufficient information was available regarding the durations of administration and dosages to permit further analysis.

The mean disease duration was lowest among subjects with severely constrictive strictures and increased

Table 1. Clinical Characteristics of Study Group

Clinical parameter		Hypertrophic n = 6	Severely constrictive[a] n = 6	Mildly constrictive[a] n = 5	*P* value
		Proportion (number)			
Gender	Male	.50 (3)	.83 (5)	.80 (4)	NS
Ethnicity	African American	0 (0)	.17 (1)	.20 (1)	NS
	Hispanic	.17 (1)	.17 (1)	0 (0)	NS
	Caucasian	.83 (5)	.67 (4)	.80 (4)	NS
Smoking history	Current	.33 (2)	0 (0)	.20 (1)	NS
	Former	0 (0)	.17 (1)	.20 (1)	NS
	Never	.67 (4)	.83 (5)	.60 (3)	NS
Family history of IBD		.33 (2)	0 (0)	.60 (3)	NS
Mean age at diagnosis ± SD, *y*		25.0 ± 9.8	36.3 ± 22.9	17.7 ± 6.9	NS
Mean disease duration ± SD, *y*		13.7 ± 5.0	6.3 ± 6.2	8.7 ± 6.4	.03
BMI, kg/m^2		22.37	22.37	20.16	NS
Disease distribution	L1	.67 (4)	1 (6)	.80 (4)	NS
	L2	.33 (2)	0 (0)	0 (0)	NS
	L3	0 (0)	0 (0)	.20 (1)	NS
Disease behavior	B1	0 (0)	0 (0)	0 (0)	NS
	B2	.67 (4)	.50 (3)	.40 (2)	NS
	B3	.33 (2)	.50 (3)	.60 (3)	NS
Perianal complications		.17 (1)	.17 (1)	.20 (1)	NS
Steroids		.67 (4)	.50 (3)	.20 (1)	NS
Immunomodulators		.50 (3)	.67 (4)	.40 (2)	NS
Anti-TNF		.83 (5)	.67 (4)	.60 (3)	NS

BMI, Body mass index; IBD, inflammatory bowel disease; NS, not significant; SD, standard deviation; TNF, tumor necrosis factor.
[a]Subjects are classified as having severely or mildly constrictive strictures based on CR values below or above the group mean of 0.8, respectively.

progressively among those with mildly constrictive (CR >0.8) and hypertrophic strictures (6.3 ± 6.2, 8.7 ± 6.4, and 13.7 ± 5.0 years, respectively; $P = .03$).

Fifteen patients (88%) had imaging studies (7 computerized tomography, 8 magnetic resonance enterography) performed within a median interval of 53 days preceding surgery (interquartile range, 16-77 days), all of which showed signs of ileal stricturing (Figure 2).

Pathologic and Histomorphometric Characteristics

In comparison with the 6 hypertrophic strictures, which were all single, 12 of 18 constrictive strictures occurred multiply ($P = .01$). There was no significant correlation between stricture category and length (constrictive: mean, 5.9 cm; range, 1.0–14.2 cm; hypertrophic: mean, 5.2 cm; range, 2.0–8.0 cm; $P = .68$). Grossly, all strictures were partially to near-completely encased in mesenteric fat. The mucosa displayed hallmarks of CD in all cases, including effacement of mucosal folds and linear or aphthous ulcers. Although the strictures were classified by morphometry for analytical purposes, they were easily distinguished on gross examination based on their mural cross sections and pliancy; hypertrophic strictures featuring classical concentric mural ("rubber hose") thickening and rigidity and constrictive strictures displaying a thin, pliant wall (Figure 3).

Microscopically, all strictures exhibited mucosal features of chronic inflammation characteristic of CD, including villous and crypt architectural distortion, pyloric gland metaplasia, or ulceration. However, hypertrophic strictures displayed exuberant lymphoid aggregates, neural hyperplasia, fibrosis, and expansion and disarray of the MM, whereas constrictive strictures generally exhibited mild inflammation including

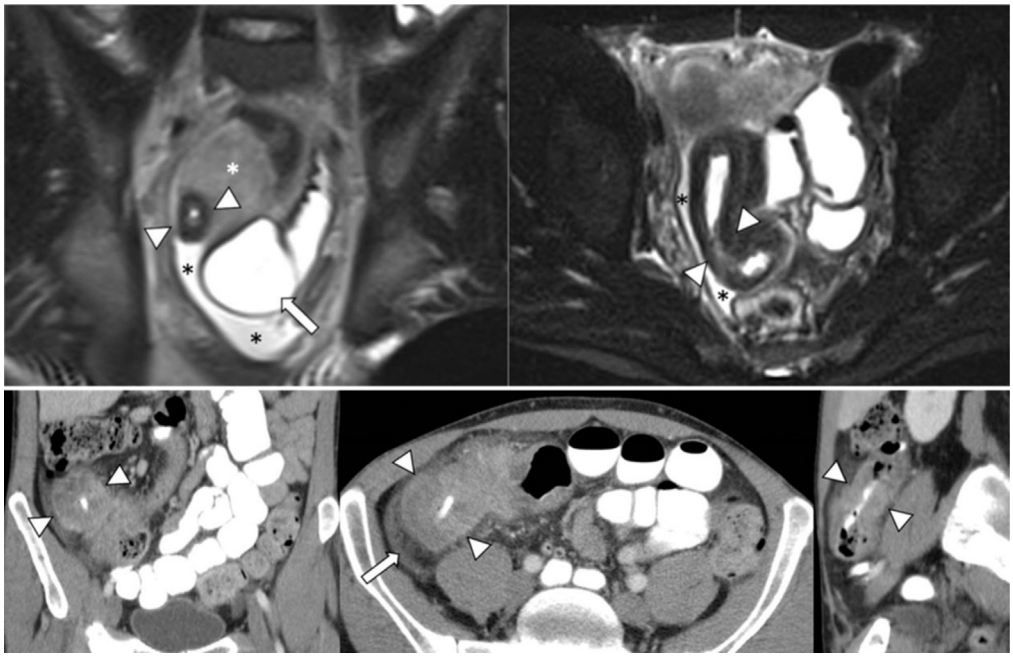

Figure 2. *(A, B)* Coronal T2-weighted magnetic resonance image (*left*) and axial fat saturated T2-weighted image (*right*) of an 18-year old patient with constrictive small intestinal stricture (*arrowheads*) in the right lower quadrant. Inflammatory changes in the adjacent mesenteric fat (*white asterisk*), pelvic free fluid (*black asterisk*), and a dilated small bowel loop (*arrow*) are noted. *(C, D, E)* Coronal (*left*), axial (*middle*), and sagittal (*right*) contrast-enhanced computed tomography images of a 33-year old male patient with a hypertrophic small bowel stricture (*arrowheads*) in the right lower quadrant. The intestinal wall is markedly thickened, and inflammatory changes are seen in the surrounding mesenteric fat (*arrow*).

scattered lymphoid aggregates, no fibrosis, and, at most, slight splaying of the MM fibers. Quantitiatively, the reduced inflammation in constrictive strictures was reflected in significantly lower inflammation scores by 2 methods ($P = .0007$ and $P = .007$, respectively[18,19] (Supplementary Table 1).

Cross-sectional area measurements of severely constrictive strictures showed no mural expansion, either overall or of their individual muscle layers (mean AR values: total wall, 0.94 ± 0.36; MM, 1.04 ± 0.69; internal layer of muscularis propria, 1.06 ± 0.52; external layer of muscularis propria, 0.88 ± 0.44). By contrast, the cross-sectional areas of hypertrophic strictures were significantly increased (mean AR values: total wall, 2.96 ± 0.92; $P < .0001$; MM, 3.31 ± 1.71; $P = .02$; internal layer of muscularis propria, 3.78 ± 2.41; $P = .002$; external layer of muscularis propria, 2.28 ± 1.15; $P = .006$), whereas those of mildly constrictive strictures assumed intermediate values (Figure 1*B*, Supplementary Table 2). Severely constrictive strictures showed no increases in fibrosis or smooth muscle content compared with normal controls. Mildly constrictive strictures showed small increases that did not achieve statistical significance, whereas hypertrophic strictures showed significant increases in fibrosis and smooth muscle content (Supplementary Figure 1).

The circumferential extent of mesenteric fat wrapping was increased among strictures in all categories (range, 53%–100%) (Supplementary Table 2). We observed that the extent of fat wrapping among constrictive strictures correlated with decreasing CR ($P = .003$) as determined from measurements performed on 76 serial cross-sections of a multi-strictured specimen (Figure 4).

The density of myenteric plexitis was significantly higher in hypertrophic than in constrictive strictures overall (mean 9.7 vs. 3.9 lymphocytes per hotspot; $P < .0001$), but measurements performed on the aforementioned multistrictured specimen showed significantly higher densities in the strictures than in the intervening diseased but non-strictured segments (3.9 vs 2.15; $P = .02$) (Supplementary Figure 2).

Discussion

Published studies and reviews of the pathologic and radiologic features of intestinal strictures in CD have stressed mural expansion due to intramural fibrosis and proliferation of myocytes and myofibroblasts as the principal cause of luminal stenosis.[3–5,11,13] Applying cross-sectional morphometry to systematically characterize postsurgical ileal strictures from patients with obstructive CD, we determined that strictures are pathologically heterogeneous and identified among them a subset of strictures in which luminal stenosis was attributable instead to mural constriction (ie, reduced external circumference). In contrast with conventional hypertrophic strictures, which featured normal or expanded external circumferences, concentrically thickened walls, increased fibrous tissue and smooth muscle content, and high transmural inflammation scores, the constrictive strictures in our series were characterized

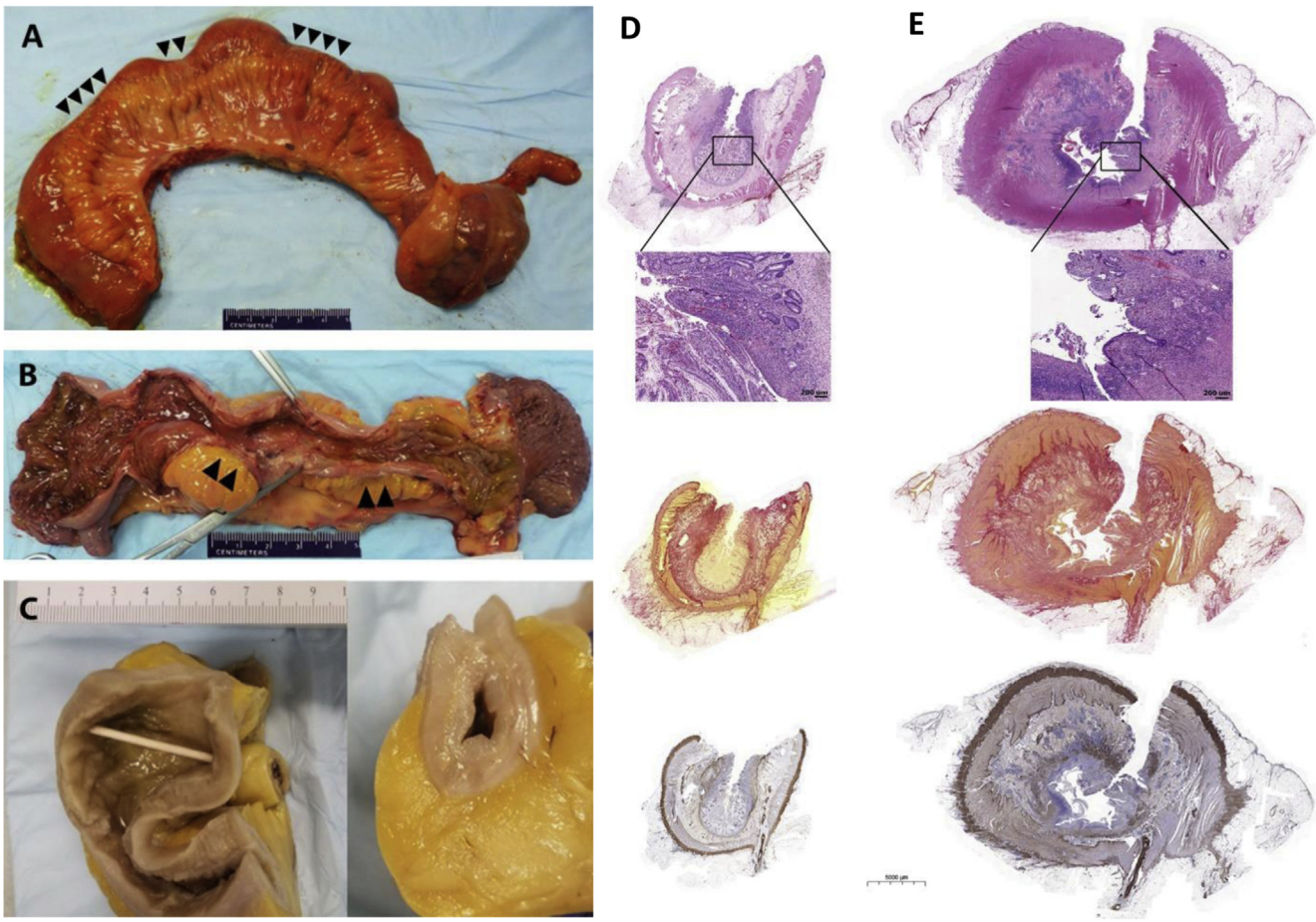

Figure 3. Ileal strictures of patients with obstructive Crohn's disease. *(A)* External view of terminal ileum with 3 constrictive strictures and corresponding serosal fat wrapping (*arrowheads*). *(B)* Mucosal view of terminal ileum with 3 constrictive strictures illustrating near-encasement by serosal fat (*arrows*) and intervening dilated segments. *(C)* Ileal segment with hypertrophic stricture and proximal dilated segment (*left*). The en face view (*right*) illustrates marked mural thickening and extensive serosal fat wrapping. Histologic sections of constrictive *(D)* and hypertrophic strictures *(E)* at equal scale (scale bar shown). Sequentially from top, hematoxylin and eosin (low and high magnifications) stains, SR stains, and SMA immunostains. At low magnification, transmural inflammation is minimal in *D* and marked in *E*, but higher magnification illustrates mucosal inflammation in both. Corresponding sections stained with SR and anti-SMA demonstrate fibromuscular expansion of the wall in the hypertrophic stricture but not in the constrictive stricture.

by circumferences as small as 53% of internal control values, by cross-sectional mural areas and quantities of fibrous tissue and smooth muscle comparable to internal control values, and by low transmural inflammation scores. Among the constrictive strictures, we also noted a subset with mildly reduced circumference but with minimally increased cross-sectional area and stainable fibrous tissue and smooth muscle. Although the increases did not attain statistical significance, they suggest that mural constriction and hypertrophy are not mutually exclusive and can produce stenosis either separately or in combination.

In efforts to account for mural constriction, we investigated whether serosal fat wrapping, a hallmark of CD and universal feature of CD-associated strictures, might be a contributing factor. Previous studies have linked the degree of fat wrapping in CD to the intensity of chronic transmural inflammation and fibrosis.[20,21] Given the paucity of these features in constrictive strictures, it was intriguing that the circumferential extent of serosal fat wrapping in constrictive strictures did not differ significantly from that in hypertrophic strictures, even approaching complete intestinal encasement in some cases. Additionally, we determined that the extent of fat wrapping measured in individual serial cross-sections of constrictive strictures correlated significantly with the corresponding degree of constriction.

These findings support a potential role for serosal fat wrapping in the pathogenesis of constrictive strictures. Hypothetically, it might involve the direct imposition of a fixed physical constraint on intestinal peristalsis, or cocooning, by the mesenteric fat. Alternatively, constriction might result from unidentified interactions between functional components of the intestinal wall and factors elaborated by mesenteric adipocytes. The intestinal mesentery carries out multiple endocrine, metabolic, and immunomodulatory functions that are involved in maintaining intestinal homeostasis and regulating inflammation. In the setting of CD, fat wrapping is associated with loss of barrier functions and immunologic

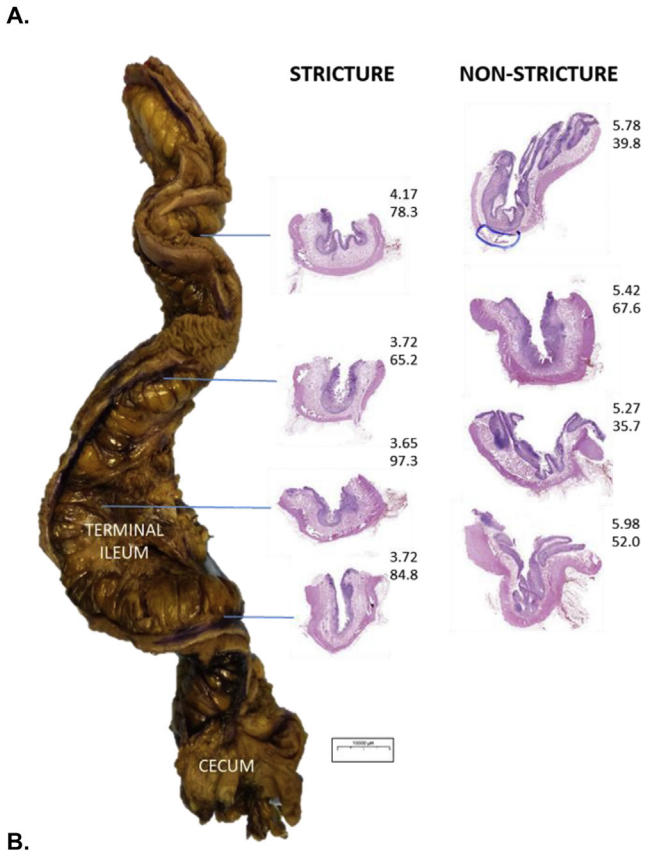

Figure 4. Correlation between increasing serosal fat wrapping and increasing constriction. *(A) Left*, resected terminal ileum with 4 constrictive strictures. *Right*, representative cross sections of the strictures and intervening segments at equal scale (scale bar shown). Each cross section is accompanied by the corresponding circumference in cm (*above*) and percentage of circumference covered by serosal fat (*below*). The control circumference was 4.85 cm. *(B)* Percentage of circumference covered by serosal fat plotted against circumference based on serial sections of the entire specimen.

responsiveness to bacterial translocation.[12,22–28] In these roles, mesenteric adipocytes and immune cells elaborate cytokines, growth factors, neuropeptides, free fatty acids, and other compounds, some of which are involved in modulating transmural inflammation in CD[12,21,23,24,29,30] and in the proliferation and functioning of myocytes and fibroblasts.[12]

We investigated whether constrictive stricturing might result from immune-mediated interactions with myenteric elements in the stricture wall by determining the density of T lymphocyte infiltration (ie, myenteric plexitis). Myenteric plexitis is a recognized feature of CD that predicts accelerated postoperative disease recurrence when present at surgical resection margins.[17,31,32] It is also a feature of various intestinal motility disorders, either as a mediator of cytotoxic neural damage and neuromuscular dysfunction or as a secondary response to neural degeneration.[33] We observed that plexitis was more pronounced in hypertrophic strictures than in constrictive strictures, but that it was more prominent in constrictive strictures than in adjacent diseased but non-strictured segments. The potential relationship between lymphocytic plexitis and stricturing in CD and the question of whether it assumes primary or secondary significance in stricture development are issues that remain to be explored.

In addition to structural differences, constrictive strictures were significantly more likely than hypertrophic strictures to occur multiply within individual resection specimens; two-thirds of the constrictive strictures occurring in groups of 2 to 4 per specimen compared with none of the hypertrophic strictures. Additionally, the interval from disease onset until surgery was shortest among patients with severely constrictive strictures (6.3 years) and progressively longer among those with mildly constrictive and hypertrophic strictures (8.5 and 13.7 years, respectively). Notwithstanding that the timing of surgery for CD can be influenced by a variety of clinical factors, this trend might reflect increasing intervals required for the effects of mural hypertrophy to accumulate and reach a critical clinical threshold.

Our findings suggest that ileal stricture phenotypes in CD reflect a dichotomy between the classical paradigm in which expansion of the intestinal wall results from inappropriate proliferation of intramural mesenchymal cells and an alternate paradigm in which the wall undergoes constriction due to other factors, possibly mesenteric encasement or plexitis. Whatever the processes that account for constrictive stricturing in CD, it is likely that they take effect during early stages of stricture development when normal intestinal wall pliancy remains intact and before mural hypertrophy and rigidity become established. Thus, the ultimate properties of individual strictures might be shaped by the dynamics of different processes that promote constriction and hypertrophy during the course of stricture development.

One of the limitations of our study is skewing of the relative prevalence of constrictive and hypertrophic strictures. Despite its prospective design, constrictive strictures were overrepresented due to their frequent multiplicity, whereas hypertrophic strictures were underrepresented due to our exclusion of strictures with fistulas and dense adhesions. Another limitation is the lack of long-term clinical outcome data and of complete therapeutic histories to permit a more comprehensive

evaluation of the natural histories of strictures of different types and of potential risk factors for their development. A third limitation is the relatively small sample size, which, albeit adequate for a proof-of-concept study, will require validation in the form of larger prospective postsurgical studies. These should be designed to help refine the clinical and pathologic criteria for stricture classification, identify risk factors for stricture development and recurrence, and help develop preoperative diagnostic criteria based on cross-sectional imaging.

Further studies are also needed to evaluate the impact of stricture classification on contemporary treatment modalities for patients with stenosing CD. There are limited published data relating treatment outcomes to stricture characteristics; however, it is plausible that strictures might respond differently to certain treatment modalities or pose different risks depending on their hypetrophic or constrictive phenotype. For example, multiplicity of strictures, which was prevalent among the constrictive strictures in our series, exposes longer lengths of the bowel to risk of surgical resection and is also reported to predict long-term failure among patients undergoing endoscopic balloon dilatation.[34] Conversely, short disease duration, which was also more prevalent among our constrictive strictures, correlates favorably with the durability of endoscopic stricture therapy.[35] Retrospective analysis of outcome data from therapeutic trials of biologic agents that included patients with stenosing CD have identified stricture characteristics that predict favorable therapeutic responses, such as a thin stricture wall and absence of fistulas, both of which characterize constrictive strictures.[36] Furthermore, evidence that biologics are most effective when initiated early in the course of stricture development may well reflect heterogeneity in the pathogenesis and dynamics of stricture formation.[37]

In view of growing interest in the potential application of antifibrotic therapies to stricturing CD based on their successful application to other diseases and the identification of multiple potential molecular targets,[3,36] the existence of paucifibrotic strictures in CD will need to be factored into the design of clinical trials and should further motivate the development of preoperative diagnostic criteria for stricture classification based on cross-sectional imaging modalities.

Given the vast experience of pathologists and surgeons with CD over many decades, it is surprising that the role of constriction in stricture pathogenesis has received so little attention. This gap highlights a currently unmet need for objective histopathologic characterization of strictures in CD based on postsurgical specimens as a gold standard to support advances in diagnostic cross-sectional imaging technologies and to provide endpoints for clinical trials of new therapeutic agents.[38]

Supplementary Material

Note: To access the supplementary material accompanying this article, please click here.

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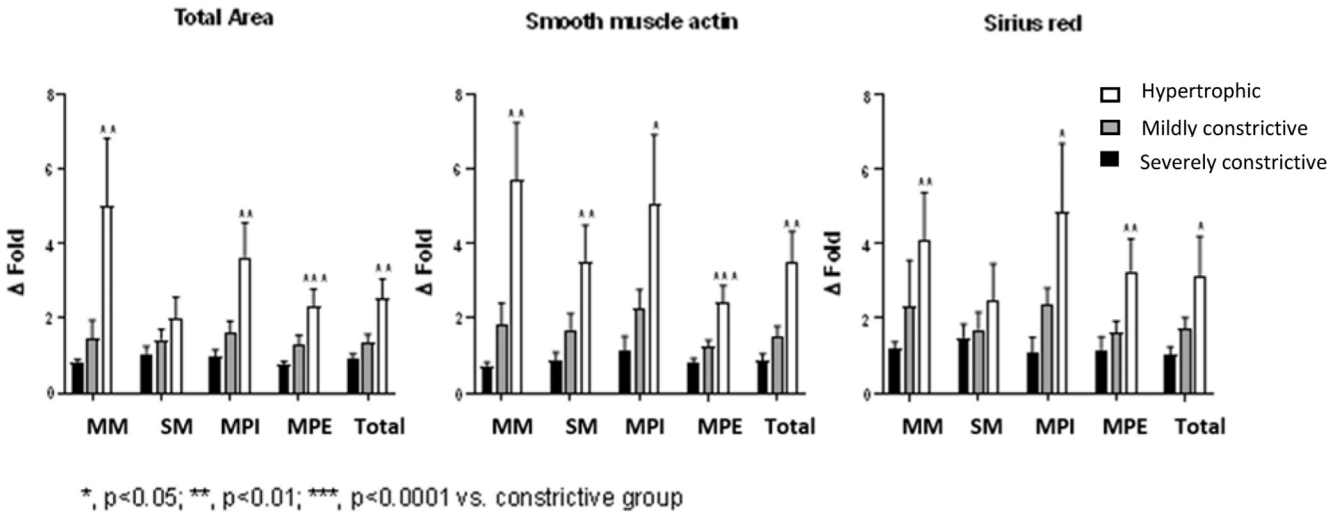
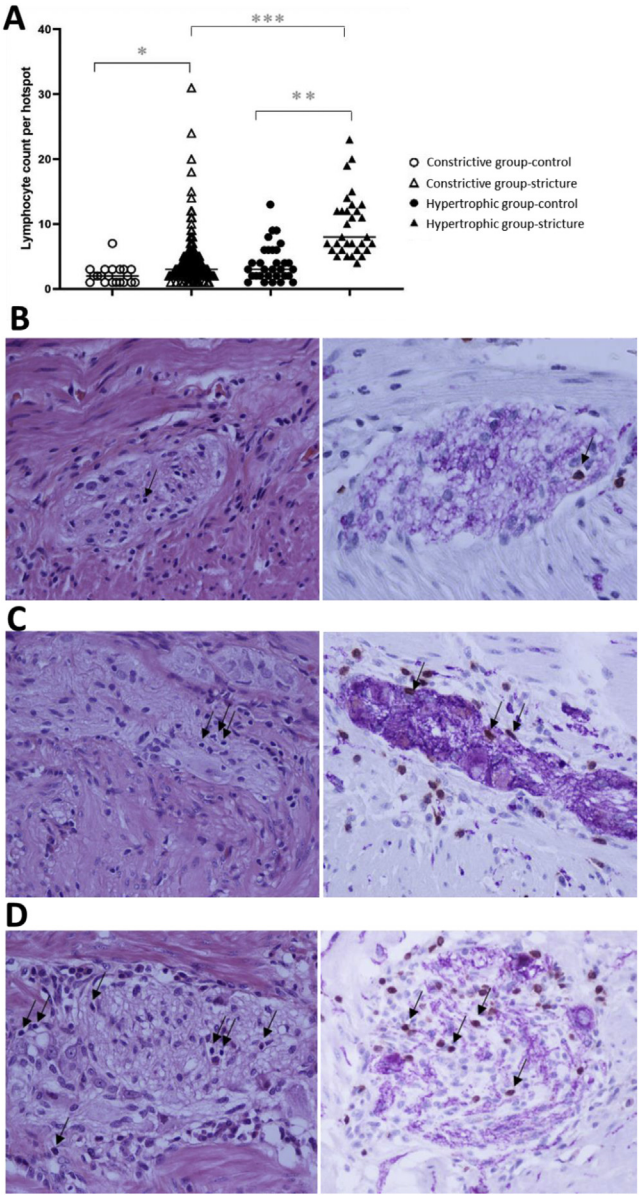


Supplementary Figure 1. Relative cross-sectional areas of strictures and of their individual mural layers after staining with SR and SMA, expressed as ARs. MM, muscularis mucosae; SM, submucosa; MPI, internal muscularis propria; MPE, external muscularis propria.



Supplementary Figure 2. *(A)* Comparison of lymphocyte density within myenteric plexus in strictured and diseased but non-strictured control segments. *$P < .05$, **$P < .005$, ***$P < .0005$. Scattered lymphocytes in the affected myenteric plexus (*arrow*). *Left panel*, hematoxylin and eosin stain; *right panel*, double staining with CD3 immunostaining (*brown*) highlighting lymphocytes, and synaptophysin immunostaining (*purple*) highlighting ganglion cells and nerve fibers in diseased but nonstrictured control *(B)*, constrictive stricture *(C)* and hypertrophic stricture *(D)*, 400×.

Supplementary Table 1. Histologic Inflammation Scores

Stricture type	Zappa method	Adler method
Severely constrictive		
CS05	0	3
CS07	1	2
CS09	0	0
CS11	0	2
CS09	0	2
CS18	1	3
CS09	0	0
CS09	0	1
CS10	0	0
CS11	1	3
Mean	0.3	1.6
SD	0.5	1.3
Mildly oonstrictive		
CS18	2	2
CS22	1	3
CS04	1	3
CS17	2	4
CS08	0	0
CS17	2	4
CS22	1	3
CS02	2	4
Mean	1.4	2.9
SD	0.7	1.3
Hypertrophic		
CS19	1	4
CS14	2	4
CS24	1	3
CS16	2	4
CS23	1	3
CS03	2	4
Mean	1.5	3.7
SD	0.5	0.5
P value	< .001	.007

SD, Standard deviation.

Supplementary Table 2. Histomorphometric Characteristics of Ileal Strictures

Stricture category/specimen number	Circumference, stricture/control	Cross sectional area, stricture/control					Serosal fat index, %
		Total wall	Muscularis mucoae	Submucosa	Internal muscularis propria	External muscularis propria	
Severely constrictive							
CS05	0.53	0.54	1.06	0.47	0.49	0.55	100
CS07	0.57	0.59	0.52	0.75	0.59	0.36	52
CS18	0.69	1.90	0.80	2.65	2.25	1.32	64
CS09 s-1	0.73	0.94	0.76	0.90	1.09	0.94	79
CS11 s-1	0.74	1.07	1.06	1.37	1.00	0.76	61
CS10	0.74	0.63	0.58	0.50	0.66	0.90	88
CS09 s-2	0.74	1.00	0.84	1.00	1.17	0.80	76
CS09 s-3	0.76	0.99	0.70	0.98	1.19	0.87	83
CS11 s-2	0.78	0.88	1.22	1.01	0.91	0.53	74
CS09 s-4	0.72	0.69	0.75	0.98	0.50	0.57	69
Mean	0.7	0.94	1.04	0.99	1.06	0.88	77
SD	0.07	0.36	0.69	0.60	0.52	0.44	15
Mildly constrictive							
CS22 s-1	0.85	0.81	0.74	0.88	0.84	0.68	80
CS04	0.86	0.80	0.55	1.02	0.59	0.89	74
CS17 s-1	0.88	1.22	1.12	0.84	1.76	1.28	57
CS08	0.88	2.07	4.72	1.09	3.00	2.13	77
CS17 s-2	0.95	2.36	1.73	2.86	2.76	1.29	57
CS18	0.95	1.20	0.55	0.95	1.58	2.51	64
CS22 s-2	0.97	1.02	1.05	1.87	0.90	0.63	80
CS02	0.98	1.53	1.28	2.05	1.48	1.09	77
Mean	0.9	1.33	1.86	1.35	1.55	1.28	63
SD	0.06	0.53	1.64	0.69	0.80	0.60	25
Hypertrophic							
CS14	1.05	0.81	1.11	0.58	0.84	1.15	86
CS24	1.01	3.26	5.34	1.60	7.99	2.52	83
CS16	1.11	4.58	4.95	4.91	4.75	3.19	96
CS23	1.13	2.46	2.64	1.81	4.45	1.87	78
CS03	1.22	3.04	1.32	2.83	3.52	4.12	100
CS19	1.27	0.94	4.48	0.51	1.13	0.80	67
Mean	1.13	2.52	3.31	2.04	3.78	2.28	85
SD	0.1	1.15	0.09	1.71	1.50	2.41	12
P value (severely constrictive vs Mildly constrictive)	.0001	.09	.17	.22	.13	.12	.14
P value (severely constrictive vs hypertrophic)	.0001	.003	.002	.08	.004	.004	.28
P value (mildly constrictive vs hypertrophic)	.004	.04	.15	.29	.03	.06	.07

SD, Standard deviation.