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Cortical and trabecular deterioration in mouse models of Roux-en-Y gastric bypass

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Abstract

Roux-en-Y gastric bypass (RYGB) is a profoundly effective treatment for severe obesity, but results in significant bone loss in patients. Developing a murine model that recapitulates this skeletal phenotype will provide a robust tool with which to study the physiologic mechanisms of this bone loss. We studied adult male C57BL/6J mice who underwent either RYGB or sham operation. Twelve weeks after surgery, we characterized biochemical bone markers (parathyroid hormone, PTH; C-telopeptide, CTX; and type 1 procollagen, P1NP) and bone microarchitectural parameters as measured by microcomputed tomography. RYGB-treated mice had significant trabecular and cortical bone deficits compared with sham-operated controls. Although adjustment for final body weight eliminated observed cortical differences, the trabecular bone volume fraction remained significantly lower in RYGB mice even after weight adjustment. PTH levels were similar between groups, but RYGB mice had significantly higher indices of bone turnover than sham controls. These data demonstrate that murine models of RYGB recapitulate patterns of bone loss and turnover that have been observed in human clinical studies. Future studies that exploit this murine model will help delineate the alterations in bone metabolism and mechanisms of bone loss after RYGB.

Keywords

bariatric surgery; gastric bypass; weight loss; obesity; bone loss; bone microarchitecture; mouse models

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1. INTRODUCTION

More than one-third of U.S. adults have obesity and the subpopulation of individuals with severe obesity (body mass index, BMI ≥ 40 kg/m²) continues to increase rapidly (1, 2). Bariatric surgery is an increasingly popular and effective treatment for severe obesity, and Roux-en-Y gastric bypass (RYGB) is one of the most commonly performed bariatric procedures in the U.S. and worldwide (3).

Despite major improvements in body weight and obesity comorbidities after this operation, clinical studies have documented striking declines in bone mineral density (BMD) of up to 10% (4–12). However, clinical studies have been hampered by their observational study designs and by concerns about obesity-related bone imaging artifact (13, 14). Furthermore, these studies have been unable to tease out the physiological mechanisms of RYGB-induced bone loss and have tested associations without the ability to show direct causation.

Rat and mouse models of RYGB have been established that recapitulate the metabolic improvements observed in human clinical studies (15–18). These surgical models have been utilized in genetically modified mice to identify the physiological and molecular mechanisms through which RYGB imparts its powerful effects on weight loss and improved glucose regulation (19–23). Declining bone density has been documented in rat models of RYGB (24–28), whose evaluation suggests that neither skeletal unloading nor calcium and vitamin D malabsorption are the primary drivers of the associated bone loss. These studies have raised intriguing questions about the etiology of RYGB-induced bone loss, but they have been limited in their ability to elucidate the underlying molecular mechanism(s). By their ability to harness the power of genetic manipulation, mouse bariatric surgery models provide the opportunity to explore the molecular pathophysiology of bone loss. In particular, targeted manipulation of individual pathways can determine their effects on surgically-induced bone loss. Robust mouse models of RYGB that demonstrably recapitulate human physiology have only recently been established, and the skeletal consequences of bariatric surgery in the mouse have not yet been evaluated. We sought to assess the relevance of the mouse model of bariatric surgery for bone disease by determining the effects of RYGB on bone microarchitecture and metabolism in adult mice.

2. MATERIALS AND METHODS

2.1 Animals

We evaluated wild-type mice that were part of a larger study examining the effects of 5HT_{2C}-receptor on metabolism after RYGB (29). Male C57BL/6J mice (Jackson Labs, Bar Harbor, ME) were placed on a 60% high fat diet (HFD; Research Diets, D12492) at weaning to induce substantial obesity. All surgically treated animals were individually housed and maintained in a 12-h light, 12-h dark cycle under controlled temperature (19–22°C) and humidity (40–60%). All animal studies were performed under protocols approved by the Massachusetts General Hospital and Harvard Medical School Institutional Animal Care and Use Committees.

2.2 Surgery and postoperative care

Between 23 to 26 weeks of age, diet-induced obese mice were randomized to receive either RYGB surgery (n=6) or laparotomy sham surgery (n=5) under inhalation anesthesia. For these operations, a 2.0–2.5 cm midline abdominal incision was made into the peritoneum and a self-retaining retractor placed at the laparotomy. RYGB anatomy was created as previously described (18). The total length of the small intestine was measured, and as in the typical human operation, a total of 20–30% of the small intestine was included in the combination of Roux and biliopancreatic limbs (approximately 10–15% in each limb). To construct the Roux limb, the jejunum transected 2–3 cm below the ligament of Treitz. Then, 4–5 cm beyond the transection, a 2 mm incision was made on the anti-mesenteric wall of the jejunum. A running end-to-side jejuno-jejunal anastomosis was created using 8-0 Vicryl suture (Ethicon, Somerville, NJ). The lesser and greater curvature of the stomach were then dissected and minor branch vessels from the main gastric artery were cauterized around the gastro-esophageal junction to facilitate gastric transection. The gastric pouch and distal gastric remnant were created by transecting the stomach, closing the remnant with a running 8-0 Vicryl suture, and constructing an end-to-end gastro-jejunal anastomosis. Finally, the laparotomy was closed using 6-0 Vicryl suture in two layers. Sham-operated mice were treated in a manner similar to the RYGB mice, but the operation was limited to a laparotomy and surgical repair. Mice were provided with liquid diet (40% Vital AF 1.2 Cal, Abbott Laboratories, Abbott Park, IL) during the 2 weeks immediately following surgery, and were gradually weaned back onto the HFD during this time. All mice were continued on the HFD after surgery to avoid an independent or confounding effect of dietary change. Body weight was monitored weekly, and food intake was measured during post-operative weeks 6–8. One mouse in the RYGB group was excluded from the analysis due to apparent poor health (poor body condition, decreased grooming, decreased mobility, and a body weight that declined to less than 25 g), leaving 5 mice in each of the RYGB and sham groups.

2.3 Specimen harvesting and preparation

At 12 weeks after surgery (age 35–38 weeks), mice were fasted for 4 hours and euthanized by CO₂ inhalation. Blood was immediately collected via cardiac puncture. Femurs, tibias, and vertebrae were harvested and cleaned of soft tissue, wrapped in saline-soaked gauze, and stored at –20°C.

2.4 Biochemical assays

Plasma parathyroid hormone (PTH) was assessed by ELISA (Immutopics, San Clemente, CA), an assay with intra-assay precision of 2–6%. Plasma levels of type 1 collagen C-telopeptide (CTX) and amino-terminal propeptide of type I procollagen (PINP) were measured using mouse ELISA kits (IDS, Fountain Hills, AZ), assays with intra-assay precisions of 5–9%. All assays were batched so as to be performed with a single kit and run according to the manufacturers' protocols.

2.5 Microarchitecture

Micro-computed tomographic (μCT) imaging was performed on the tibia, femur, and the 5th lumbar (L5) vertebra of mice from each group using a high-resolution desktop imaging

system (μ CT40, Scanco Medical AG, Brüttisellen, Switzerland). The methods used were in accordance with guidelines for the use of μ CT in rodents (30). Scans were acquired using isotropic voxel sizes of $10 \mu\text{m}^3$ for the tibia and femur and $12 \mu\text{m}^3$ for the the L5vertebra, with 70 kVp peak x-ray tube potential, 200 ms integration time, and were subjected to Gaussian filtration. Trabecular bone microarchitecture was evaluated in semi-automatically contoured regions in the proximal metaphysis of the tibia, distal metaphysis of the femur, and L5 vertebral body. The region of interest for the proximal tibia began $100 \mu\text{m}$ below the proximal growth plate and extended distally 1 mm . The region of interest in the distal femur started $200 \mu\text{m}$ above the peak of the growth plate and extended proximally 1.5 mm . The vertebral body region of interest extended from $120 \mu\text{m}$ below the cranial growth plate to $120 \mu\text{m}$ above the caudal growth plate. Cortical bone microarchitecture was evaluated in the tibia mid-diaphysis in a region that started 2 mm above the inferior tibiofibular joint and extended distally $500 \mu\text{m}$. Cortical bone microarchitecture was also evaluated in the femoral diaphysis in a region that began 55% of the femoral length below the top of the femoral head and extended distally $500 \mu\text{m}$. Segmentation thresholds of 330 and 696 mg HA/cm^3 were used for the evaluations of trabecular and cortical bone, respectively, based on adaptive-iterative thresholding (AIT) that was performed on the sham-operated control group. All analyses were carried out using the scanner manufacturer (Scanco Medical) evaluation software. Cancellous bone outcomes included trabecular bone volume fraction (Tb. BV/TV, %), trabecular thickness (Tb.Th, μm), trabecular number (Tb.N, mm^{-1}), trabecular separation (Tb.Sp, μm), connectivity density (Conn.D, mm^{-3}), and structural model index (SMI). Cortical bone outcomes included cortical tissue mineral density (Ct.TMD, mg HA/mm^3), cortical thickness (Ct.Th, μm), cortical bone area (Ct.Ar, mm^2), total bone area (Tt.Ar, mm^2), cortical bone area fraction (Ct.Ar/Tt.Ar, %), cortical porosity (Ct.Po, %), polar moment of inertia (J, mm^4), and the maximum and minimum moments of inertia (I_{max} and I_{min} , mm^4).

2.6 Statistical analysis

Statistical analyses were performed with SAS 9.3 (SAS Institute, Cary, NC). All data were checked for normality, and standard descriptive statistics computed. Wilcoxon rank sum tests were used to compare differences between RYGB and sham-operated groups. Multivariate regression using PROC GLM was employed to adjust results using final body weight as a covariate. The threshold of statistical significance was set at $p = 0.05$. Data are reported as mean $\pm$ SD, unless otherwise noted.

3. RESULTS

3.1 Body weight

Pre-operative body weight was similar in RYGB-treated and sham-operated groups ($40.5 \pm 4.7 \text{ g}$ and $39.2 \pm 5.3 \text{ g}$, respectively). Twelve weeks after surgery, RYGB mice weighed an average 38% less than sham controls ($30.1 \pm 2.5 \text{ g}$ vs. $48.2 \pm 5.6 \text{ g}$, $p < 0.0001$; Figure 1). Daily food intake did not differ between groups (RYGB: $3.1 \pm 0.6 \text{ g/day}$; Sham: $2.7 \pm 0.2 \text{ g/day}$).

3.2 Bone microarchitecture

Twelve weeks after surgery, Tb. BV/TV at the L5 vertebra was 58% lower in the RYGB mice compared to the sham-operated controls ($p=0.020$; Table 1). There were concurrent declines in Tb.N and Tb.Th, and an increase in Tb.Sp in the RYGB mice as compared with sham controls ($p=0.020$ for all, Figure 2A). SMI was 93% higher in the RYGB group than sham controls, indicating a more rod-like trabecular architecture in the RYGB group.

At the proximal tibia, Tb. BV/TV was 63% lower in the RYGB mice than in the sham-operated controls ($p<0.001$; Table 1). Tb.N, Tb.Th, and Conn.D were also lower in the RYGB mice, whereas Tb.Sp was higher than in sham-operated controls ($p<0.05$ for all; Figure 2B). At the tibial midshaft, Ct.TMD (-4% , $p=0.022$), Ct.Ar/Tt.Ar (-26% , $p=0.012$) and Ct.Th (-36% , $p<0.012$) were all significantly lower in the RYGB group than in sham controls (Figure 2C). A similar pattern of results were seen at the femur, with disruption of trabecular microarchitecture at the distal femoral metaphysis and weakening of cortical microarchitecture at the femoral midshaft (Table 1).

Adjustment for final body weight diminished the differences between groups in cortical microarchitecture at the tibia and the femur (Table 1). However, weight-adjusted Tb. BV/TV were significantly lower in the RYGB group at the tibia ($p=0.034$) and at the spine ($p=0.006$). Several other trabecular differences between RYGB mice and sham controls persisted after adjusting for differences in body weight.

3.3 Bone turnover markers and PTH

At study end, P1NP and CTX levels were respectively 132% and 109% higher after RYGB than sham-operated controls (Figure 3A/B, $p=0.005$). PTH levels were similar in the RYGB and sham mice (Figure 3C).

4. DISCUSSION

In this study, we demonstrated significant cortical and trabecular bone deficits in obese male mice after RYGB. Indices of bone turnover, including CTX and P1NP, were markedly higher in the RYGB-treated mice than in sham-operated controls, despite a lack of difference in PTH levels between these groups. These data demonstrate that murine models of RYGB reflect similar patterns of bone loss and increases in bone turnover as have been observed in human clinical studies.

Importantly, the results from this study closely parallel the findings of human clinical studies with deterioration of both cortical and trabecular compartments. Clinical studies using high-resolution peripheral quantitative computed tomography (HR-pQCT) have demonstrated that bariatric surgery is accompanied by declines in cortical and trabecular bone density and microarchitecture at both the radius and tibia (8, 12). RYGB also leads to declines in trabecular density at the lumbar spine and proximal femur by QCT (8). In this murine model, RYGB induced significant negative effects on both cortical and trabecular bone at the lumbar spine, distal femur, and proximal tibia. The current study avoids concern about the influence of soft tissue artifact on bone imaging, as we had the opportunity to examine excised specimens directly. These results are similar to skeletal phenotypes

previously observed after RYGB in both obese and non-obese rats (24, 25)(26–28). Further understanding of the mechanisms of RYGB effects on bone morphology and metabolism will benefit from the use of this mouse model as a physiologically relevant means of testing mechanistic hypotheses regarding bone loss after RYGB.

This preliminary study does not directly address the etiology of skeletal changes after RYGB. However, we observed a skeletal phenotype in the RYGB group despite having similar PTH levels as the sham-operated controls. Although sampling limitations precluded determination of serum calcium and 25-hydroxyvitamin D levels, the absence of a difference in PTH between groups suggests that secondary hyperparathyroidism is unlikely to drive the dramatic changes we observed in post-RYGB bone metabolism. Although earlier studies in rats suggested that minor changes in calcium and vitamin D homeostasis may occur in the absence of secondary hyperparathyroidism (26–28), dietary supplementation to prevent calcium and 25-hydroxyvitamin D fluctuations did not fully rescue bone density after RYGB (27). The current findings are also consistent with human studies that demonstrate significant bone loss and increases in bone turnover markers after RYGB in the absence of significant changes in either 25-hydroxyvitamin D or PTH (7–11, 31). Some investigators have proposed that metabolic acidosis, which has been observed in rat models of RYGB (26, 28), may induce hypercalciuria that contributes to bone loss. Metabolic acidosis has not been observed in human studies, however, and clinical studies have documented significant declines in urine calcium after RYGB (32, 33).

Finally, it is important to note that differences in body weight after RYGB may lead to mechanical unloading of the skeleton. In clinical studies, it has been shown that diet-induced weight loss of 10–20% can lead to modest bone loss, with declines in hip BMD of 1–3% (34)(35) and increases in serum CTX of 20–35% (36, 37). In the mouse model, there was a significant post-operative weight difference between RYGB and sham controls, an expected consequence of successful metabolic surgery. Adjustment for body weight diminished the cortical differences between RYGB and sham groups, suggesting that mechanical unloading from weight loss may have contributed to the observed cortical decline. Given the powerful physiological effects of RYGB on energy balance and multiple metabolic functions, however, the coincident loss of weight and cortical bone may reflect common or overlapping physiological effects. Several lines of evidence from both human and animal studies suggest that factors other than skeletal unloading likely contribute to RYGB-induced bone loss. Recent clinical studies in RYGB patients demonstrate inconsistent associations between weight loss and bone loss (4, 5, 38). Moreover, increases in bone resorption observed after RYGB have been shown to far exceed those observed after complete bedrest (39), providing further support that mechanisms beyond mechanical unloading are contributing to the bone loss. Similarly, bone loss in calorie-restricted rats is substantially attenuated in comparison to their weight-matched, RYGB-treated counterparts (26). Lastly, alterations in bone volume are observed in the rat RYGB model but not in a rat sleeve gastrectomy model despite similar weight loss, further supporting the hypothesis that a RYGB-specific mechanism may drive the observed skeletal declines (27). In this mouse model, several of the observed trabecular defects after RYGB remained clinically and statistically significant after adjustment for final body weight, underscoring the likely importance of weight-independent bone loss. It remains to be seen whether bone loss after

RYGB is being driven by one of the multitude of physiological and signaling systems known to be influenced by RYGB, including those mediated by gastrointestinal peptide hormones, bile acids, intestinal microbiota, and enhanced thermogenesis. We have recently demonstrated that increases in bone resorption markers after RYGB in human patients are correlated with increases in peptide YY (40), a neuropeptide hypothesized to have direct effects on the skeleton (41). Future studies in mice genetically modified to enhance or diminish peptide YY secretion or signaling could provide valuable insight into this possibility.

This preliminary study has several limitations. The absence of a sham-operated control group that was weight-matched to the RYGB-treated mice by calorie restriction prevents certain conclusion that the observed bone loss is weight-loss-independent. Future studies will be needed to explore this potentially important mechanism. Applicability of results from the mouse model to human patients is not a given, as there may be important interspecies differences in gastrointestinal and/or skeletal physiology. Such interspecies differences have been observed in other responses to RYGB including in the relative contribution of thermogenesis to weight loss, and glucose disposal into muscle to ameliorate diabetes. Nonetheless, numerous studies of the response to RYGB in rodents and humans demonstrate highly conserved mechanisms with variations in the relative use of these mechanisms in the different species, thus rendering the mouse a good model for primary mechanistic discovery. Another consideration is the exposure to and maintenance of a very high (60%) fat diet throughout these studies. Maintaining mice on this diet postoperatively was necessary to examine the diet-independent effects of the surgery. While we have observed no effect of this diet alone on bone microarchitecture, we cannot exclude the possibility that the diet used might influence the metabolic outcomes of the surgery, either enhancing or limiting its impact on bone phenotype. Finally, precise assessment of cortical porosity in mice is difficult due to limitations in microCT imaging resolution, and we may therefore miss smaller microscopic cortical pores (<20 μm diameter). Acknowledging this caveat, the ability to detect higher measured cortical porosity in RYGB mice, similar to what has been observed after RYGB in humans utilizing HR-pQCT, suggests that the imaging approach used in these mice is sufficient (8, 12).

5. CONCLUSION

We have demonstrated that a murine model of RYGB recapitulates the skeletal phenotype of bone loss that has been observed in human patients after the same operation. Future studies will be needed to address how bone loss after RYGB may be modulated by age, sex, or gonadal status of the mice, and to further investigate mechanisms by which RYGB may stimulate weight-loss independent bone loss. We expect that this murine model will be a powerful tool to delineate and characterize mechanisms by which gastrointestinal derived signals influence bone metabolism, and will help to identify clinically-relevant targets for the development of treatments that mitigate surgery and diet-related bone decline.

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ABBREVIATIONS

RYGB	Roux-en-Y gastric bypass
PTH	parathyroid hormone
CTX	C-telopeptide, CTX
P1NP	type 1 procollagen
BMI	body mass index
BMD	bone mineral density
μCT	micro-computed tomography
Tb. BV/TV	trabecular bone volume fraction
Tb.Th	trabecular thickness
Tb.N	trabecular number
Tb.Sp	trabecular separation
Conn.D	connectivity density
SMI	structural model index
Ct.TMD	cortical tissue mineral density
Ct.Th	cortical thickness
Ct.Ar	cortical bone area
Tt.Ar	total bone area
Ct.Ar/Tt.Ar	cortical bone area fraction
Ct.Po	cortical porosity
J	polar moment of inertia
I_{max}	maximum moment of inertia
I_{min}	minimum moments of inertia

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HIGHLIGHTS

- Murine models of RYGB recapitulate patterns of bone loss and turnover previously observed in human clinical studies
- RYGB-operated mice had significant trabecular and cortical bone deficits, and increased bone turnover as compared with sham-operated controls
- Future studies can leverage this murine model to delineate alterations in bone metabolism and mechanisms of bone loss after RYGB

Figure 1. Body weight changes in RYGB (solid line) and sham-operated (dashed line) mice Between 23 to 26 weeks of age (average 25 weeks), mice were randomized to RYGB or sham surgery and followed for 12 weeks. RYGB mice lost approximately 30% of their initial weight and maintained the loss thereafter, while sham-operated mice exhibited transient weight loss, with subsequent regain to a body weight approximately 20% greater than their initial weight.

Fig. 2. Representative images of bone microarchitecture in RYGB (right) and sham-operated (left) mice

Defects in trabecular microarchitecture are apparent after RYGB at the L5 vertebrae (A). Abnormal bone geometry and microarchitecture are shown after RYGB at the proximal tibia metaphysis (B) and mid-shaft tibia (C).

Fig. 3. Indices of bone turnover and PTH levels in RYGB and sham-operated mice
Boxplots showing median, interquartile range, maximum and minimum values are displayed. Twelve weeks after surgery, CTX (A) and P1NP (B) levels were significantly greater in the RYGB mice than in sham controls. There was no difference in PTH between RYGB and sham groups (C). ** $p < 0.005$.

TABLE 1

Trabecular and Cortical Bone Microarchitecture in L5 Vertebrae, Tibia, and Femur of RYGB-treated and Sham-operated Mice

	RYGB	Sham	Percent difference in group medians
L5 VERTEBRAE			
Tb.BV/TV (%)	9.8 (1.5)	22.6 (2.9)	-57% ^{a,b}
Tb.N (mm ⁻¹)	3.57 (0.51)	4.81 (0.26)	-26% ^a
Tb.Sp (mm)	0.278 (0.044)	0.195 (0.015)	+43% ^a
Tb.Th (mm)	0.040 (0.005)	0.053 (0.004)	-25% ^a
Conn.D (mm ⁻³)	95.6 (26.5)	140.6 (10.3)	-32%
SMI	2.10 (0.18)	1.08 (0.14)	+95% ^{a,b}
TIBIA			
Proximal metaphysis trabecular			
Tb.BV/TV (%)	3.3 (1.0)	8.8 (0.8)	-63% ^{a,b}
Tb.N (mm ⁻¹)	2.61 (0.36)	3.41 (0.20)	-24% ^a
Tb.Sp (mm)	0.388 (0.054)	0.288 (0.017)	+35% ^a
Tb.Th (mm)	0.045 (0.005)	0.062 (0.003)	-28% ^a
Conn.D (mm ⁻³)	5.5 (3.5)	21.7 (9.9)	-75% ^{a,b}
SMI	3.54 (0.10)	3.06 (0.34)	+16%
Midshaft cortical			
Ct.TMD (mgHA/cm ³)	1142 (17)	1187 (13)	-4% ^a
Tt.Ar (mm ²)	1.21 (0.12)	1.25 (0.23)	-4%
Ct.Ar (mm ²)	0.42 (0.03)	0.60 (0.69)	-30% ^a
Ct.Ar/Tt.Ar (%)	37.4 (4.4)	50.3 (6.2)	-26% ^a
Ct.Th (mm)	0.12 (0.01)	0.19 (0.02)	-36% ^a
Ct.Po (%)	0.34 (0.01)	0.22 (0.04)	+52% ^a
J (mm ⁴)	0.14 (0.03)	0.20 (0.05)	-30%
I _{max} (mm ⁴)	0.08 (0.01)	0.12 (0.02)	-31%
I _{min} (mm ⁴)	0.06 (0.01)	0.08 (0.02)	-27%
FEMUR			
Distal metaphysis trabecular			
Tb.BV/TV (%)	3.3 (0.9)	7.6 (1.1)	-57% ^a
Tb.N (mm ⁻¹)	2.42 (0.09)	3.12 (0.27)	-22% ^{a,b}
Tb.Sp (mm)	0.414 (0.012)	0.308 (0.029)	+34% ^{a,b}
Tb.Th (mm)	0.044 (0.005)	0.061 (0.007)	-28% ^a
Conn.D (mm ⁻³)	10.9 (6.9)	26.1 (6.3)	-58% ^a

	RYGB	Sham	Percent difference in group medians
SMI	3.11 (0.20)	2.96 (0.24)	+5%
Midshaft cortical			
Ct.TMD (mgHA/cm ³)	1093 (27)	1153 (21)	−5% ^a
Tt.Ar (mm ²)	2.20 (0.42)	2.35 (0.50)	−6%
Ct.Ar (mm ²)	0.54 (0.11)	0.81 (0.02)	−33% ^a
Ct.Ar/Tt.Ar (%)	24.6 (3.0)	34.5 (6.3)	−29% ^a
Ct.Th (mm)	0.10 (0.02)	0.15 (0.01)	−32% ^a
Ct.Po (%)	0.36 (0.15)	0.20 (0.03)	+77% ^a
J (mm ⁴)	0.31 (0.04)	0.54 (0.13)	−43% ^a
I _{max} (mm ⁴)	0.20 (0.02)	0.38 (0.08)	−46% ^a
I _{min} (mm ⁴)	0.11 (0.03)	0.17 (0.04)	−32%

Values reported are median (interquartile range)

^a indicates unadjusted p-values < 0.05

^b indicates weight-adjusted p-values < 0.05