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Suppressor of cytokine signaling 2 (SOCS2) deletion protects bone health of mice with DSS induced inflammatory bowel disease

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Summary Statement – Utilising a mouse model of inflammatory bowel disease this article provides an improved understanding of the relative effects of GH/IGF-1 on bone health in experimental colitis.
Abstract

Individuals with inflammatory bowel disease (IBD) often present with poor bone health. The development of targeted therapies for this bone loss requires a fuller understanding of the underlying cellular mechanisms. Although bone loss in IBD is multifactorial the altered sensitivity and secretion of growth hormone (GH) and insulin-like growth factor-1 (IGF-1) in IBD is understood to be a critical contributing mechanism. The expression of suppressor of cytokine signaling 2 (SOCS2), a well-established negative regulator of GH signaling, is stimulated by pro-inflammatory cytokines. Therefore, it is likely that SOCS2 expression represents a critical mediator through which pro-inflammatory cytokines inhibit GH/IGF-1 signaling and decrease bone quality in IBD.

Utilising the DSS model of colitis we have revealed that endogenously elevated GH function in the Socs2−/− mouse protects the skeleton from osteopenia. Micro-computed tomography assessment of DSS treated wild-type mice revealed a worsened trabecular architecture compared to control mice. Specifically, DSS treated WT mice had significantly decreased bone volume (BV/TV) (41%; p<0.05), trabecular thickness (16%; p<0.05), trabecular number (30%; p<0.05), and a resulting increase in trabecular separation (19%; p<0.05). In comparison, the trabecular bone of Socs2 deficient mice was partially protected from the adverse effects of DSS. The reduction in a number of parameters including BV/TV (21%; p<0.05) was less, and no changes were observed in trabecular thickness or separation. This protected phenotype was unlikely to be a consequence of improved mucosal health in the DSS treated Socs2−/− mice but rather a result of unregulated GH signaling directly on bone.

These studies indicate that the absence of SOCS2 is protective against bone loss typical of IBD. This study also provides an improved understanding of the relative effects of GH/IGF-1 on bone health in experimental colitis, information that is essential before these drugs are explored as bone protective agents in children and adults with IBD.
Introduction

Inflammatory bowel disease (IBD), which includes the chronic intestinal disorders, Crohn’s disease (CD) and ulcerative colitis (UC), is considered to result from an inappropriate inflammatory response to intestinal microbes in genetically susceptible hosts (Abraham and Cho, 2009). They are life-long conditions with a prevalence of 5 per 1000 people and annual health care costs exceeding $1.7 billion in the United States (Sandler et al., 2002, Lakatos et al., 2006). In addition, to the well-recognized gut inflammation associated with IBD, both children and adults have poor bone health and are at increased risk of fractures which cannot solely be attributed to exogenous glucocorticoid exposure (Thearle et al., 2000; van Staa et al., 2003; Wong et al., 2016)). The relative risk of fracture in adults is 40% higher than normal (Compston et al., 1987) and a vertebral fracture may be present in 10% of affected people (Laakso et al., 2012; Wong et al., 2014). Recent studies in children have also shown that trabecular bone density, which was reduced at diagnosis, showed inadequate improvement despite control of the underlying inflammation (Dubner et al., 2009). These patients also have abnormal bone geometry with thinner and smaller bones (Dubner et al., 2009; Tsampalieros et al., 2013). Furthermore, peak bone mass is compromised in childhood onset IBD, despite adequate control of disease and progression through puberty (Laakso et al., 2014).

The etiology of bone loss in IBD is multifactorial, with risk factors including steroid medication, poor nutrient intake/absorption and the underlying inflammatory state (Bernstein et al., 2013). Central to the inflammatory response in IBD is the over production by T lymphocytes and macrophages of various pro-inflammatory cytokines such as interleukin (IL) -1, -2, -6, -8, and tumor necrosis factor (TNF)-α (Ali et al., 2009). IL-6 has been identified as the predominant cytokine mediating the bone abnormalities, and genetic variations in IL-6 correlate well with the clinical course of IBD and the extent of bone loss (Schulte et al., 2000). Although pro-inflammatory cytokines are known to promote bone loss directly, they also lead to altered sensitivity and secretion of growth hormone (GH) and insulin-like growth factor-1 (IGF-1) in IBD which may be another critical mechanism leading to osteoporosis (Wong et al., 2010).

GH and IGF-1 are recognized stimulators of bone mass (Giustina et al., 2008). Transgenic mice over expressing Igf1 in osteoblasts exhibit increased trabecular bone, whereas Igf1 null mutants exhibit reduced cortical bone (Liu et al., 1993; Zhao et al., 2000). GHR<sup>−/−</sup> mice have reduced bone turnover, cross-sectional cortical bone area, and cortical growth, whereas GH overexpression results in increased bone cortical area (Sjogren et al., 2000; Sims et al., 2000; Eckstein et al., 2004). Skeletal
manifestations are also observed in humans with GH deficiency who present with low bone turnover osteoporosis (Doga et al., 2005) which can be ameliorated by rhGH replacement therapy (Hansen et al., 1996). The therapeutic benefits of rhGH administration on bone health of IBD patients remain unknown. Nevertheless, given that both rhGH and rhIGF-1 are available as therapeutic drugs, there is potential for these anabolic drugs to improve bone health in people with IBD.

Suppressor of cytokine signaling 2 (SOCS2) regulates GH signalling during normal growth and development. This physiological role is not restricted to bone. This function is most clearly observed in the Socs2 KO mouse where a number of organs are increased in size and these include liver, heart, lungs, bladder (Metcalf et al., Nature 2000). In another study SOCS2 has been shown to limit intestinal growth (Michaylira et al., 2005). SOCS2 is also induced by a subset of pro-inflammatory cytokines e.g. IL-6, IL-1b and TNFα which are elevated in IBD and mediate the inflammatory response (Starr et al., Nature 1997; Greenhalgh & Hilton Biol 2001; Rico-Bautista et al., 2006; Sanchez-Munoz et al., 2008; MacRae et al., 2009). However, this protection against inflammation may come at the expense of poor bone health through the induction of SOCS2 and the inhibition of GH signalling (Denson et al., 2003; Shi et al., 2004).

SOCS2 is expressed by many cells, including osteoblasts, and inhibits GH signaling via inhibition of the JAK/STAT intracellular signaling pathway (Greenhalgh et al., 2002; Flores-Morales et al., 2006; Michaylira et al., 2006, Pass et al., 2012, Dobie et al., 2014). Socs2 deficient mice, have unrepressed GH signaling and present with an overgrowth and high bone mass phenotype (Metcalf et al., 2000; MacRae et al., 2009; Dobie et al., 2014). These data emphasize the critical role for SOCS2 in controlling GH’s anabolic effects on bone. Therefore, it is possible that increased osteoblast SOCS2 expression represents a critical mediator through which pro-inflammatory cytokines inhibit GH/IGF-1 signaling and decrease bone mass and quality in patients with IBD.

To directly examine this, we studied bones from a mouse model of IBD using dextran sodium sulfate (DSS) induced colitis and investigated the potential of ablating the expression of Socs2 to endogenously elevate GH and IGF-1 function, and correct the bone loss observed in murine colitis. This study is the first-ever in an IBD animal model to investigate whether GH and IGF-1 have bone protective effects. Furthermore, data from this innovative approach will help inform the design of novel therapies that are directed specifically at the mechanism of insult which leads to poor bone health in children and adults with IBD.
Results

Effect of DSS treatment on body-weight of WT and Socs2−/− mice.

To investigate the effects of DSS induced colitis on bone development and the possible role of SOCS2 in mediating bone loss, WT and Socs2−/− mice were treated with 3% DSS for 4 days (Fig. 1). The dose and duration of DSS treatment was based on results from a dosing experiment where mice were given DSS at varying concentrations for 4-5 days (Fig. S1) During the DSS treatment period (0-4 days) no weight loss was observed in WT or Socs2−/− mice (Fig. 1). Independent of genotype, mice exhibited a rapid weight loss from day 4 (Fig. 1). There was no significant difference in maximum weight loss observed between WT (15%) and Socs2−/− (13%) mice (Fig. S1B). Following the period of weight loss, all DSS treated mice continued to gain weight to the end of the study (day 18) (Fig. 1). Pair fed control mice did not show any significant alterations in body weight throughout the experiment (Fig. 1). During the period of DSS treatment, water intake was similar in WT and Socs2−/− mice. Also, food intake during DSS intake and the periods of weight loss and recovery were similar (data not shown).

In terms of weight loss, WT mice showed high individual variation in susceptibility to DSS (Fig. S1C). Maximum body weight loss ranged from 6% to 21% in WT mice treated with 3% DSS. In contrast, body weight loss observed in Socs2−/− mice treated with 3% DSS was more uniform, ranging from 11% to 15% before recovering (Fig. S1C).

Effect of DSS treatment on longitudinal growth of WT and Socs2−/− mice.

Socs2−/− mice are characterised by their overgrowth phenotype (Greenhalgh et al., 2002). In agreement with this, the untreated Socs2−/− mice at the end of this study (day 18) were 39% (p<0.001) heavier than untreated WT mice (Table 1). Also, tibia (6%; p<0.01), and femur (8%; p<0.01) length as well as nose to rump length (13%; p<0.001) were all greater in untreated Socs2−/− mice compared to WT mice (Table 1). DSS treatment had no effect on the body-weight of WT and Socs2−/− mice at the end of the study (Table 1). Similarly, DSS treatment did not result in altered nose to rump length, tibia length, or femur length of WT or Socs2−/− mice (Table 1).

Colon pathology of DSS treated mice

To assess the effects of DSS on mucosal integrity, detailed histological analysis was performed on the colon from control and DSS treated WT and Socs2−/− mice. Histology scores were minimal in the non-DSS treated (control) mice and furthermore, there were no notable differences in colon diameter, morphological differences or differences in histological scoring between the non-DSS treated (control) WT and Socs2−/− mice (Fig. 2A, B&C). This infers that the ablation of Socs2 alone had no
obvious effects on colon morphology. In contrast, histological analysis of the colon from DSS treated WT and Soc2−/− mice, revealed extensive levels of inflammation. DSS treated mice were characterised as having signs of both acute and chronic inflammation throughout their colon (Fig. 2A). Signs of acute inflammation included infiltration of neutrophils into the lamina propria and submucosa (Fig. 2A), and epithelial degeneration (Fig. S2). In a number of sections there were also signs of crypt loss (Fig. S2). In addition to acute inflammation, there were also high levels of mononuclear leucocytes (macrophages, lymphocytes and plasma cells) (Fig. 2A & Fig. S2), and transmural inflammation (Fig. S2), which are recognised markers of chronic inflammation.

Scores for inflammation severity and extent of inflammation were significantly increased in DSS treated mice (Fig. 2B). However, the histological mean scores for inflammation severity (WT: 6.8, Soc2−/−: 5.2) and extent of inflammation (WT: 7.4, Soc2−/−: 6.0) following DSS treatment were not significantly different between genotypes, nor was colon diameter (Fig. 2B&C). In contrast, there was a significant difference in crypt damage/regeneration mean score between DSS treated WT and DSS treated Soc2−/− mice (p<0.05) (Fig. 2B). This difference in crypt damage/regeneration between genotypes following DSS treatment was not sufficient however to alter total pathology scores indicating that the absence of Soc2 was unable to confer overall protection against DSS induced gut inflammation (Fig. 2B). Although not part of the scoring system, the number of goblet cells in the mucosa of the colon was reduced in areas of loss of the epithelium as a result of the inflammatory response, and in areas of crypt damage and regeneration in DSS-treated WT and Soc2 deficient mice.

**Systemic IGF-1 levels in DSS treated mice**

GH resistance has been associated with IBD, and is characterized by a decrease in systemic IGF-1 levels (Ballinger et al., 2000; Katsanos et al., 2001). Analysis of serum from DSS treated animals revealed that IGF-1 levels were similar in control and DSS treated WT (WT control 263.8 ± 6.2ng/ml; DSS treated WT 243.6 ± 10.8ng/ml) and Soc2−/− mice (Soc2−/− control 264 ± 9.8ng/ml; DSS treated Soc2−/− 276.9 ± 17.6ng/ml).

**Socs transcript levels in bones of DSS treated mice**

Socs1, 2, and 3 transcript levels were measured in bone samples from control and DSS treated WT mice. Soc2 (2.7 fold; p<0.05) and Soc3 (4.1 fold; p<0.05) levels were higher in DSS treated mice, but no significant difference was observed in Soc1 levels (Fig. 3).
Bone phenotype of DSS treated mice

DSS induced colitis has previously been shown to have detrimental effects on bone quality, in both juvenile (4 week old) and young adult (10 week old) mice (Hamdani et al., 2008; Harris et al., 2009). In accordance with these studies, DSS treated WT mice showed worsened trabecular architecture compared to control mice as demonstrated by μCT (Fig 4). Specifically, DSS treated WT mice had significantly decreased bone volume/tissue volume (BV/TV) (41%; p<0.05), trabecular thickness (16%; p<0.05), and trabecular number (30%; p<0.05), and a resulting increase in trabecular separation (19%; p<0.05) (Fig. 4). The increase in trabecular pattern factor (40%; p<0.05) and structural model index (14%; p<0.05) indicates a more disconnected ‘rod-like’ trabecular structure, which are associated with reduced trabecular micro-architecture quality (Hildebrand and Rüegsegger, 1997). In comparison, DSS treated Socs2−/− mice showed a much less severe alteration in trabecular architecture compared to control mice (Fig. 4). Specifically, the reduction in BV/TV (21%; p<0.05) and trabecular number (14%; p<0.05), and increase trabecular pattern factor (19%; p<0.05) and structural model index (6%; p<0.05) were between 50%-55% less severe than that noted in DSS treated WT mice (Fig 4B). Furthermore, trabecular thickness and trabecular separation, in contrast to DSS treated WT mice, were not significantly different in DSS treated Socs2−/− mice compared to controls (Fig 4B). These striking data indicate that the level of bone loss in DSS colitis is influenced by Socs2 expression and furthermore suggest that the absence of SOCS2 is partially protective against bone loss typical of IBD.

Analysis of cortical bone in control and DSS treated WT and Socs2 deficient mice revealed no change in any parameters following DSS treatment (Table 2). Nonetheless, the anabolic bone phenotype (characterized by increased cortical tissue area (p<0.05), bone area (p<0.05), cortical thickness (p<0.05), and marrow area (p<0.05) was apparent in untreated Socs2−/− mice (Table 2).

Discussion

Murine models of IBD have been used extensively to better understand disease etiology and therapeutic opportunities. In this study we used the DSS-induced colitis mouse model which has been extensively validated by others to induce acute and chronic forms of IBD (Wirtz et al., 2007; De Roberts et al., 2011; Kanneganti et al., 2011). This model has also been used to study the link between IBD and the risk of colorectal cancer (Bollrath et al., 2009). The strength of this model is that histological changes seen can include a wide range of features that are associated with chronic forms of IBD in man (Perse and Cerrar, 2012). DSS induced colitis is the result of deterioration of the
epithelial barrier through an increase in epithelial cell apoptosis and a decrease in proliferation (Araki et al., 2010). This deterioration allows for the influx of antigens and micro-organisms; prompting an increased expression of inflammatory mediators (e.g. TNF-α, IL-1β, IFN-γ, IL-10, IL-6 and IL-12) which drive the pathogenesis of DSS-induced colitis (Tlaskalova-Hogenova et al., 2005; Perse and Cerar, 2012).

DSS induced acute colitis has previously been shown to result in reduced bone health in juvenile and young adult mice. During active disease and early recovery in juvenile mice, both the trabecular and cortical bone compartments are adversely affected whereas DSS-treated older mice exhibit reduced femoral bone mass and altered micro architecture. No changes were observed in cortical bone indices (Hamdani et al., 2008; Harris et al., 2009).

In the present study we found a severe trabecular bone loss phenotype in WT mice treated with DSS, but no changes in cortical bone parameters. These data are consistent with previous reports in similarly aged adult mice (Hamdani et al., 2008). The trabecular bone phenotype was characterized by lowered BV/TV, trabecular thickness, trabecular number and increased trabecular separation. Taken together, these studies suggest that there is an age-specific effect of DSS induced IBD on bone loss in mice. During early puberty (3 to 5 weeks) there is extensive radial bone expansion and this may explain why the loss of cortical bone is restricted to juvenile mice (Callewaert et al., 2010). DSS treatment did not result in altered nose to rump length, tibia length, or femur length of WT or Soxs2−/− mice. This is contrast to a previous study which reported decreased bone length and growth plate thickness and altered chondrocyte marker gene expression in young mice (four-week-old) treated with DSS (Harris et al., 2009). Whilst bone length was fully restored at the end of the study these data are in accord with human studies that show IBD can stunt growth and diminish final height (Burnham 2004; Sylvester et al., 2007). It is likely that we did not see a reduction in bone length in this study due to the older mice (8-9 weeks of age) studied by which time the rapid phase of linear bone growth was completed.

SOCS2 expression is induced by pro-inflammatory cytokines and is a recognized inhibitor of GH signaling (Denson et al., 2003). Furthermore, Soxs2 deficient mice have uninhibited GH signaling, leading to increased body-weight and a high bone mass phenotype (Metcalf et al., 2002; MacRae et al., 2009; Dobie et al., 2014). GH is a recognized stimulator of bone mass (Giustina et al., 2008). Global GH overexpression results in increased cortical cross-sectional area (Eckstein et al., 2004). Increased cortical area and bone strength is also observed in human growth hormone transgenic mice, where expression of GH is directed specifically to osteoblasts with minimal systemic overproduction (Tseng et al., 1995) Conversely, mice carrying mutations of the GH receptor have
reduced femoral width and tibial cortical thickness, and an associated reduction in periosteal bone growth (Sims et al., 2000). Skeletal manifestations are also observed in humans with GH-deficiency who present with low bone turnover osteoporosis, leading to increased fracture risk (Doga et al., 2014). Using an osteoblast culture model we have previously shown that GH’s promotion of STAT5 phosphorylation and nuclear translocation is enhanced in Socs2 deficient osteoblasts, whereas Socs2 overexpression in osteoblasts blunts GH’s effect (Dobie et al., 2014). These observations stress the key role for SOCS2 in controlling GH’s anabolic skeletal effects and are extended by data from this present study which reveals that Socs2 expression is higher in bone samples from DSS treated WT mice. This increased Socs2 expression, a probable consequence of higher levels of circulating pro-inflammatory cytokines, is likely to repress GH signaling and contribute to the poor bone health noted in colitic (DSS treated) WT mice.

In this study, we examined the hypothesis that elevated GH signaling in mice deficient in SOCS2 protein would offer protection against bone loss in a murine model of IBD. Such protection was observed in trabecular bone where in the absence of SOCS2, DSS treatment resulted less severe changes to the trabecular bone architecture. This underscores the importance of Socs2 ablation in protecting against bone loss in DSS induced IBD. Complete protection of the skeleton was not observed however, and this is likely due to other cellular mechanisms including, increased osteoclastic resorption, direct (non-GH mediated) effects of pro-inflammatory cytokines on osteoblast differentiation/function and diminished GH signaling through elevated Socs3 levels. The effects of ablating both Socs2 and Socs3 in the DSS model would be of interest as it may offer enhanced protection to the skeleton. Previous studies have shown that the removal of GH signaling deletes the Socs2^{-/-} phenotype which includes the normalisation of body weight and bone length (Greenhalgh et al., 2002, 2005). Therefore, it is likely that mice in which both Socs2 and GH signaling are silenced there would be no protection from DSS induced bone loss. The gathering of such evidence would provide functional evidence for GH’s pivotal role in protecting bone health in this animal model of IBD. Furthermore, it is also worth noting that reduced expression of SOCS2 has been associated with increased cancer risk which may be a consequence of increased GH signalling (Hendriksen et al., 2006). Therefore, if GH treatment or novel therapies to target the SOCS2 protein are to be considered as bone protective agents in children and adults with IBD then the potential cancer risk has to be carefully considered.
Histologic scoring in the present study was carried out using a validated scoring scheme, allowing an in depth assessment of the mucosal integrity (Dieleman et al., 1998; Williams et al., 2001). Acute and chronic colitis are characterised by distinct pathological changes to the colon. Acute inflammation is associated with an influx of neutrophils into the lamina propria, and in some cases epithelial degeneration. Chronic inflammation on the other hand is associated with mononuclear leukocyte infiltration, crypt architectural disarray, and crypt regeneration (Melgar et al., 2005; Perse & Cerar 2012). In this study, histological analysis revealed signs of both acute and chronic inflammation in the colons of all DSS treated mice, suggesting that the current experimental design was sufficient to induce chronic inflammation. Previous reports have shown that acute inflammation can progress to chronic in C57BL/6 mice following a single treatment of DSS (Melgar et al., 2005). Importantly, little difference was observed between the pathology scores of colons from DSS treated WT and Socs2−/− mice. This pathological assessment suggests that the absence of SOCS2 does not appear to protect against the deterioration of mucosal integrity in DSS experimental colitis and therefore the improved bone health noted in the DSS treated Socs2 deficient mice is unlikely to be solely attributed to improved disease status. However, it is recognised that further studies to quantify cell apoptosis, proliferation and stem cell number within the colon would allow us to make a more definitive judgement on colon health in the DSS treated WT and SOCS2 deficient mice.

Intriguingly, there have been reports that increased GH activity promotes mucosal repair during IBD associated inflammation. A small clinical trial of patients with active CD reported that therapy with recombinant human GH therapy improved the CD activity index, and decreased the need for other medication (Slonim et al., 2000). Previous studies have also reported that in mice with a mutated gp130 receptor (gp130Y757F mice) there is protection to the colon from the damaging effects of DSS (Bollrath et al., 2009). It is possible that in the gp130Y757F mice in which SOCS3 cannot inhibit JAK/STAT signaling there is a protective pathway(s), involving increased STAT3 activation that may not be active in SOCS2 deficiency.

Furthermore, GH transgenic mice display a similar extent of colon pathology during the onset of inflammation compared to WT mice, but show improved mucosal repair over an extended time period (Williams et al., 2001). Increased mucosal repair in the GH transgenic mice may be a result of increased systemic IGF-1, which has been reported to partially attenuate colonic damage in the DSS rat model (Mathews et al., 1988; Howarth et al., 1998). Whilst we noted subtle improvements in the crypt damage/regeneration score of DSS treated Socs2−/− mice this is unlikely to be related to systemic IGF-1 status of these mice, which were found to be normal. However, it must be noted that
gut IGF-1 levels were not measured in these mice. Therefore the possibility of local upregulation of IGF-1 in response to unregulated GH signalling in Socs2⁻/⁻ mice cannot be ruled out. Despite the slight improvement in the crypt damage/regeneration score of DSS treated Socs2⁻/⁻ mice, their total pathology score was similar to DSS treated WT mice. It is however prudent to note that in our studies systemic IGF-1 levels were measured at the end of the experiment when the DSS treated animals presented with severe inflammation of the colon, but had recovered their body-weight. Previous studies have shown decreased systemic IGF-1 levels with active disease which returned to normal with recovery (Harris et al., 2009). Further studies are required to understand fully the role of systemic IGF-1 in the skeletal response to experimental IBD. In keeping with previous research, systemic IGF-1 levels in WT and Socs2⁻/⁻ control animals were comparable, further confirming the importance of the direct anabolic effects of GH on bone (Metcalf et al., 2000; Lorentzon et al., 2005; MacRae et al., 2009; Dobie et al., 2014, Dobie et al., 2015).

The negative effects of increased Socs2 expression in inflammatory conditions may not be restricted to the skeleton. In rodent models of chronic kidney disease there is increased Socs2 gene expression in liver and muscle which may contribute to impaired phosphorylation and nuclear translocation of GH-activated STAT proteins and the development of GH resistance (Schaefer et al., 2001, Sun et al., 2004; Mak and Cheung 2007, Cheung et al., 2008). Intervention strategies to reduce uremic cachexia are associated with amelioration of the uremia-associated increase in Socs2 expression (Cheung et al., 2008). Recent data has also shown that Socs2 deletion protects against streptozotocin-induced type 1 diabetes in adult male mice possibly through increased β-cell hypersensitivity to GH (Alkharusi et al., 2015). It is therefore likely that SOCS2 signaling represents a generic critical pathway through which pro-inflammatory cytokines alter both GH/IGF-1 signaling and cellular function (Ahmed and Farquharson, 2010; Farquharson and Ahmed, 2013).

In conclusion, these studies suggest that the absence of SOCS2 is protective against bone loss typical of IBD and are consistent with the premise that increased osteoblast SOCS2 expression represents a critical mediator through which pro-inflammatory cytokines inhibit GH signaling and decrease osteoblast function and bone accrual. This study also provides an improved understanding of the relative effects of GH/IGF-1 on bone health in experimental colitis and is consistent with data reporting the beneficial effects of GH on bone in conditions such as juvenile idiopathic arthritis (Bechtold et al. 2009). This accumulation of information is essential before these drugs are explored as bone protective agents in children and adults with IBD.
Materials and Methods

Mice

It is recognised that different genetic strains of mice respond differently to DSS (Melgar et al., 2005; Perse and Cerar 2012). Therefore, we backcrossed our original SOCS2 knockout (KO) mice (76.0% C57BL/6) (MacRae et al., 2009) with pure C57BL/6 mice a further six-times to establish our SOCS2 KO mice and litter mate wild-type (WT) controls on a pure (>99.0%) C57BL/6 background strain. These mice were used in all studies reported. For genotyping, ear biopsied DNA was analysed by PCR for SOCS2 (WT) or the neocassette (Socs2⁻/⁻) using the following primer sequences: SOCS2 - Forward (5'-3') TGTTTGACTGAGCTCGCGC, Reverse (5'-3') CAACTTTAGTGTCTTGGATCT and Neo - Forward (5'-3') ACCCTGCACACTCTCGTTTTG Reverse (5'-3') CCTCGACTAAACACATGTAAAGC. Primers were obtained from Eurofins MWG Operon, London, UK. All animal experiments were approved by Roslin Institute’s Animal Users Committee, and the animals were maintained in accordance with Home Office (UK) guidelines for the care and use of laboratory animals.

Establishment of acute DSS induced colitis model

Male WT and Socs2 KO mice (6 mice per group), 8-9 weeks of age, received 3.0% DSS (molecular weight ~40000kD; Sigma Aldrich, UK) in their drinking water (tap water). They were given DSS treated water ad lib for 4 days, following which they received normal tap water for a 14 day recovery period. Control (non DSS treated) male WT and Socs2 KO mice (6 mice per group) were offered normal tap water for the duration of the study. The health status of the DSS treated mice was scored daily, with particular attention paid to their coat condition, mobility, blood in stools and eye clarity. Body weights of all mice were recorded daily. To establish the weight loss that was due to inflammation and not lowered food intake, the mice were pair-fed. The quantity of food consumed daily (fed ad lib.) by the DSS treated mice was weighed and then provided to control animals (who received no DSS) the following day (Ballinger et al., 2000). All mice were housed individually to allow accurate measurement of food and water intake and health status. Eighteen-days after the initiation of the studies the experiment was stopped, blood collected for serum analysis, and the long bones and the colon dissected.
**Colon histology**

The colon was dissected from WT and *Socs2*−/− mice ± DSS treatment and fixed and stored in 4% paraformaldehyde. Each colon was divided into 5 transverse segments including proximal to distal portions. Tissue processing, wax embedding, sectioning (5µm thick) and H&E staining were done following routine procedures. Colon pathology was graded blind on sections from all 5 segments of each mouse using an established histological grading scheme (Dieleman et al., 1998). Segments of colon were assessed separately for inflammation. Scores from all five segments were averaged to provide an overall pathology score. Colon diameters were measured on the H&E sections. Using image analysis software the diameter of each transverse segment was measured twice and averaged.

**Microcomputed tomography**

To evaluate trabecular architecture and cortical bone geometry of the tibia from control and DSS treated mice we used a µCT system (Skyscan 1172 X-Ray microtomograph, Bruker Corporation, Kontich, Belgium) as described previously (Dobie et al., 2014). In brief, high-resolution scans with an isotropic voxel size of 5 µm (trabecular bone) or 10 µm (cortical bone) were acquired (60 kV, 0.5 mm aluminium filter, 0.6° rotation angle). Two images were averaged at each rotation angle. Scan reconstruction was done using NRecon software (Bruker). A 1 mm section of the metaphysis was taken for the analysis of trabecular bone, using the base of the growth plate as a standard reference point. A 500 µm section of the mid-shaft was taken for the analysis of cortical bone, using the articulation with the fibula as a standard reference point. CTAn software (Bruker) was used to analyse the appropriate parameters (Bouxsein et al., 2010).

**Serum IGF-1 ELISA**

Blood obtained by cardiac puncture was stored in serum tubes (Greiner Bio-One, Gloucestershire, UK) on ice for over 30mins to allow for clotting. Following centrifugation for 10 mins at 1000g, supernatant was removed, aliquotted, and stored at -80°C. IGF-1 levels were assessed by ELISA (Quantikine, R&D Systems, Minneapolis, USA) according to manufacturer’s instructions.

**RNA extraction and RT-qPCR analysis**

Left femur were dissected from WT and *Socs2*−/− mice ± DSS treatment. The femurs had both epiphyses removed and the marrow was spun out by centrifugation and discarded. The bone samples were snap frozen in liquid nitrogen and stored at -80°C. Bone samples were ground using a
mortar and pestle, and homogenised by a hand held homogeniser in QIAzol Lysis Reagent. RNA from extracted using an RNeasy Lipid Tissue Kit (Qiagen Ltd, Manchester UK) following manufacturer’s protocol. RNA content was measured by absorbance at 260 nm, and quality by 260/280 ratios. RNA was stored at -80°C. RT was carried out as described previously (Newton et al 2014). RT-qPCR was performed using the SYBR green (Roche detection method on a Stratagene Mx3000P real-time qPCR machine with MxPro software (Stratagene, Santa Clara, CA, USA). Relative gene expression was calculated using the DDCt method (Livak and Schmittgen, 2001). Each cDNA sample was normalized to housekeeping gene gapdh (Primer Design, Southampton, UK). Reactions were performed with gene of interest primers - Socs1 (For– TCCGATTACCGGCACGCACGC; Rev– CTCCAGCAGCTCGAAAAGGCA), Socs2 (For – TGGCTGCTCAAGATCAAATG; Rev– TGTCCTCCTGGAAATGGAAG) and Socs3 (For– GAGTACCCCCAAGAGAGCTTACTA; Rev– CTCTTAAGTGGAGCATCATCAGT) (MWG Eurofins).

Statistical analysis
All measurements and analysis were done blinded to the researcher. All statistical analysis was completed using GraphPad Prism. Final measurements, IGF-1 ELISA, and histology scoring data were analysed using a two-tailed unpaired t-test with Welch’s correction (equal standard deviations not assumed) or suitable nonparametric test (Mann-Whitney) if the data were not normally distributed. Due to small sample size transcript and μCT data were analysed using a nonparametric test (Mann-Whitney). Data presented as mean ± SEM.
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Competing interest’s statement

The authors declare no conflicts of interest

Author contributions

CF and SFA supervised the project; CF, RD, VEM and SFA conceived and designed the experiments; CF and RD wrote the manuscript; RD, CP and EMM, performed the experiments and analyzed the data; CP, EMM, VEM and SFA helped to prepare the manuscript. All authors read, discussed and edited the manuscript.
References

Abraham, C., Cho, J.H. (2009). Inflammatory bowel disease. N Engl J Med., 361, 2066-2078.

Ahmed, S.F., Farquharson, C. (2010) The effect of GH and IGF1 on linear growth and skeletal development and their modulation by SOCS proteins. J. Endocrinol. 206, 249-59.

Ali, T., Lam, D., Bronze, M.S., Humphrey, M.B. (2009) Osteoporosis in inflammatory bowel disease. Am. J. Med. 122, 599-604.

Alkharusi, A., Mirecki-Garrido, M., Ma, Z., Zadjali, F., Flores-Morales, A., Nyström, T., Castrillo, A., Björklund, A., Norstedt, G., Fernandez-Pérez, L. (2016) Suppressor of cytokine signaling 2 (SOCS2) deletion protects against multiple low dose streptozotocin-induced type 1 diabetes in adult male mice. Horm. Mol. Biol. Clin. Investig. 26, 67-76.

Araki, Y., Mukaisyo, K.I., Sugihara, H., Fujiyama, Y., Hattori, T. (2010) Increased apoptosis and decreased proliferation of colonic epithelium in dextran sulfate sodium-induced colitis in mice. Oncology Rep. 24, 869-874.

Ballinger, A.B., Azooz, O., El-Hajh, T., Poole, S., Farthing, M.J.G. (2000) Growth failure occurs through a decrease in insulin-like growth factor 1 which is independent of undernutrition in a rat model of colitis. Gut. 46, 694-700.

Bechtold, S., Dalla Pozza, R., Schwarz, H.P., Simon, D. (2009) Effects of growth hormone treatment in juvenile idiopathic arthritis: bone and body composition. Horm Res. 72, Suppl 1:60-4.

Bernstein, C.N., Leslie, W.D. (2003) The pathophysiology of bone disease in gastrointestinal disease. Eur. J. Gastroenterol. Hepatol. 15, 857-864.

Bollrath, J., Phesse, T.J., von Burstin, V.A., Putoczki, T., Bennecke, M., Bateman, T., Nebelsiek, T., Lundgren-May, T., Canli, O., Schwitalla, S., Matthews, V., Schmid, R.M., Kirchner, T., Arkan, M.C., Ernst, M., Greten, F.R. (2010) gp130-mediated Stat3 activation in enterocytes regulates cell survival and cell-cycle progression during colitis-associated tumorigenesis. Cancer Cell. 15, 91-102.

Bouxsein, M.L., Boyd, S.K., Christiansen, B.A., Guldberg, R.E., Jepsen, K.J., Müller, R. (2010) Guidelines for assessment of bone microstructure in rodents using micro-computed tomography. J. Bone Miner. Res. 25, 1468-1486.

Rico-Bautista, E., Flores-Morales, A., Fernández-Pérez, L. (2006) Suppressor of cytokine signaling (SOCS) 2, a protein with multiple functions. Cytokine Growth Factor Rev. 17, 431-439.

Burnham, J.M., Shults, J., Semeao, E., Foster, B., Zemel, B.S., Stallings, V.A., Leonard, M.B. (2004) Whole body BMC in pediatric Crohn disease: independent effects of altered growth, maturation, and body composition. J Bone Miner. Res. 19, 1961–1968.

Callewaert, F., Venken, K., Kopchick, J.J., Torcasio, A., van Lente, G.H., Boonen, S., Vanderschueren, D. (2010) Sexual Dimorphism in Cortical Bone Size and Strength But Not Density Is Determined by Independent and Time-Specific Actions of Sex Steroids and IGF-1: Evidence From Pubertal Mouse Models. J. Bone Min. Res. 25, 617-626.

Cheung, W.W., Rosengren, S., Boyle, D.L., Mak, R.H. (2008) Modulation of melanocortin signaling ameliorates uremic cachexia. Kidney Int. 74, 180–186.

Compston, J.E., Judd, D., Crawley, E.O., Evans, W.D., Evans, C., Church, H.A., Reid, E.M., Rhodes, J. (1987) Osteoporosis in patients with inflammatory bowel disease. Gut. 28, 410-415.

Denson, L.A., Held, M.A., Menon, R.K. Frank, S.J., Parlow, A.F., Arnold, D.L. (2003) Interleukin-6 inhibits hepatic growth hormone signaling via upregulation of Cis and Socs-3. Amer. J. Physiol. Gastrointest. Liver. Physiol. 284, G646-G654.
De Robertis, M., Massi, E., Poeta, M.L., Carotti, S., Morini, S., Cecchetelli, L., Signori, E., Fazio, V.M. (2011) The AOM/DSS murine model for the study of colon carcinogenesis: From pathways to diagnosis and therapy studies. J. Carcinog.. 10, 9.

Dieleman, L.A., Palmen, M.J.H.J., Akol, H., Bloemena, E., Pena, A.S., Mewissen, S.G.M., van Rees, E.P. (1998) Chronic experimental colitis induced by dextran sulphate sodium (DSS) is characterized by Th1 and Th2 cytokines. Clin. Exp. Immunol. 114, 385-391.

Dobie, R., MacRae, V.E., Huesa, C., van’t Hof, R., Ahmed, S.F., Farquharson, C. (2014) Direct stimulation of bone mass by increased GH signalling in osteoblasts of SOcs2-/- mice. J. Endocrinol. 223, 93-106.

Dobie, R., Ahmed, S.F., Staines, K.A., Pass, C., Jasim, S., MacRae, V.E., Farquharson, C. (2015) Increased linear bone growth by GH in the absence of SOCS2 is independent of IGF-1. J. Cell Physiol. 230, 2796-806

Doga, M., Bonadonna, S., Gola, M. Mazziotti, G., Nuzzo, M., Giustina, A. (2005) GH deficiency in the adult and bone. J. Endocrinol. Invest. 28, 18-23.

Dubner, S.E., Shults, J., Baldassano, R.N., Zemel, B.S., Thayu, M., Burnham, J.M., Herskovitz, R.M., Howard, K.M., Leonard, M.B. (2009) Longitudinal assessment of bone density and structure in an incident cohort of children with Crohn's disease. Gastroenterology. 136, 123-130.

Eckstein, F., Weusten, A., Schmidt, C. (2004) Longitudinal in vivo effects of growth hormone overexpression on bone in transgenic mice. J. Bone Min. Res. 19, 802-810.

Farquharson, C., Ahmed, S.F. (2013) Inflammation and linear bone growth: The inhibitory role of SOCS2 on GH/IGF-1 signaling. Pediart. Nephrol. 28, 547-556

Flores-Morales, A., Greenhalgh, C.J., Norstedt, G., Rico-Bautista, E. (2006) Negative regulation of growth hormone receptor signaling. Mol. Endocrinol. 20, 241-253.

Giustina, A., Mazziotti, G., Canalis, E. (2008) Growth hormone, insulin-like growth factors, and the skeleton. Endocr. Rev. 29, 535-559.

Greenhalgh, C.J., Hilton, D.J. (2001) Negative regulation of cytokine signaling. J Leukoc Biol. 70, 348-56

Greenhalgh, C.J., Bertolino, P., Asa, S.L., Metcalf, D., Corbin, J.E., Adams, T.E., Davey, H.W., Nicola, N.A., Hilton, D.J., Alexander, W.S. (2002) Growth enhancement in suppressor of cytokine signaling 2 (SOCS-2)-deficient mice is dependent on signal transducer and activator of transcription 5b (STAT5b). Mol. Endocrinol. 16, 1394-1406.

Greenhalgh, C.J., Rico-Bautista, E., Lorentzon, M., Thaus, A.L., Morgan, P.O., Willson, T.A., Zervoudakis, P., Metcalf, D., Street, I., Nicola, N.A., Nash, A.D., Fabri, L.J., Norstedt, G., Ohlsson, C., Flores-Morales, A., Alexander, W.S., Hilton, D.J. (2005) SOCS2 negatively regulates growth hormone action in vitro and in vivo. J. Clin. Invest. 115, 397-406.

Hamdani, G., Gabet, Y., Rachmilewitz, D., Karmeli, F., Bab, I., Dresner-Pollak, R. (2008) Dextran sodium sulfate-induced colitis causes rapid bone loss in mice. Bone 43, 945-950.

Hansen, T.B., Brixen, K., Vahl, N., Jørgensen, J.O., Christiansen, J.S., Mosekilde, L., Hagen, C. (1996) Effects of 12 months of growth hormone (GH) treatment on calciotropic hormones, calcium homeostasis, and bone metabolism in adults with acquired GH deficiency: a double blind, randomized, placebo-controlled study. J. Clin. Endocrinol. Metab. 81, 3352-3359.

Harris, L., Senagore, P., Young, V.B., Mccabe, L.R. (2009) Inflammatory bowel disease causes reversible suppression of osteoblast and chondrocyte function in mice. Amer. J. Physiol. Gastrointest. Liver Physiol. 296, G1020-G1029.

Hendriksen, P.J., Dits, N.F., Kokame, K., Veldhoven, A., vanWeerden, W.M., Bangma, C.H., Trapman, J., Jenster, G. (2006) Evolution of the androgen receptor pathway during progression of prostate cancer. Cancer Res. 66, 5012–5020.

Hildebrand, T., Ruesegger, P. (1997) Quantification of bone microarchitecture with the Structure Model Index. Comput Methods Biomed Engin 1(1): 15-23
Howarth, G.S., Xian, C.J., Read, L.C. (1998) Insulin-like growth factor-1 partially attenuates colonic damage in rats with experimental colitis induced by oral dextran sulphate sodium. Scand. J. Gastroenterology 33, 180-190.

Kanneganti, M., Mino-Kenudson, M., Mizoguchi, E. (2011) Animal models of colitis-associated carcinogenesis. J. Biomed. Biotechnol. 342637.

Katsanos, K.H., Tsatsoulis, A., Christodoulou, D., Challia, A., Katsaraki, A., Tsianos, E.V. (2001) Reduced serum insulin-like growth factor-1 (IGF-1) and IGF-binding protein-3 levels in adults with inflammatory bowel disease. Growth Horm. & IGF Res. 11, 364-367.

Laakso, S., Valta, H., Verkasalo, M., Toiviainen-Salo, S., Viljakainen, H., Mäkitie, O. (2012) Impaired bone health in inflammatory bowel disease: a case-control study in 80 pediatric patients. Calcif. Tiss. Int. 91,121-130.

Laakso, S., Valta, H., Verkasalo, M., Toiviainen-Salo, S., Mäkitie, O. (2014) Compromised peak bone mass in patients with inflammatory bowel disease: a prospective study. J. Pediatr. 164,1436-1443.

Lakatos, P.L. (2006). Recent trends in the epidemiology of inflammatory bowel diseases: up or down? World J. Gastroenterology, 12, 6102-6108.

Liu, J.P., Baker, J., Perkins, A.S. (1993) Mice carrying null mutations of the genes encoding insulin-like growth factor I (Igf-1) and type 1 IGF receptor (Igf1r). Cell 75, 59-72.

Livak, K.J., Schmittgen, T.D. (2001) Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. Methods. 25, 402-408.

Lorentzon, M., Greenhalgh, C.J., Mohan, S., Alexander, W.S., Ohlsson, C. (2005) Reduced bone mineral density in SOCS-2-deficient mice. Pediatr.Res. 57, 223-226.

Macrae, V.E., Horvat, S., Pells, S.C., Dale, H., Collinson, R.S., Pitsillides, A.A., Ahmed, S.F., Farquharson, C. (2009) Increased bone mass, altered trabecular architecture and modified growth plate organization in the growing skeleton of SOCS2 deficient mice. J. Cell Physiol. 218, 276-284.

Mak, R.H., Cheung, W. (2007) Therapeutic strategy for cachexia in chronic kidney disease. Curr. Opin. Nephrol. Hypertens. 16, 542-546.

Mathews, L.S., Hammer, R.E., Brinster, R.L., Palmiter, R.D. (1988) Expression of Insulin-Like Growth Factor-I in Transgenic Mice with Elevated Levels of Growth-Hormone Is Correlated with Growth. Endocrinology 123, 433-437.

Melgar, S., Karlsson, A., Michaelsson, E.M. (2005). Acute colitis induced by dextran sulfate sodium progresses to chronicity in C57BL/6 but not in BALB/c mice: correlation between symptoms and inflammation. Amer. J. Physiol.-Gastrointest. Liver Physiol. 288, G1328-G1338.

Metcalfe, D., Greenhalgh, C.J., Viney, E., Willson, T.A., Starr, R., Nicola, N.A., Hilton, D.J., Alexander, W.S. (2000) Gigantism in mice lacking suppressor of cytokine signalling-2. Nature. 405,1069-1073.

Michaylira, C.Z., Simmons, J.G., Ramocki, N.M., Scull, B.P., McNaughton, K.K., Fuller, C.R., Lund, P.K. (2006) Suppressor of cytokine signaling-2 limits intestinal growth and enterotrophic actions of IGF-I in vivo. Amer. J. Physiol. Gastrointest. Liver Physiol. 291, G472-G481.

Newton, P.T., Staines, K.A., Spevak, L., Boskey, A.L., Teixeira, C.C., MacRae, V.E., Canfield, A.E., Farquharson, C (2012) Development and characterization of a rapidly mineralizing chondrocyte ATDC5 culture model. Int. J. Mol. Med. 30, 1187-1193.

Pass, C., MacRae, V.E., Huesa, C., Ahmed, S.F., Farquharson, C. (2012) SOCS2 is the critical regulator of GH action in murine growth plate chondrogenesis. J. Bone Miner. Res. 27,1055-1066.

Perse, M., Cerar, A. (2012) Dextran sodium sulphate colitis mouse model: traps and tricks. J. Biomed. Biotechnol. 2012, 718617.

Sandler, R.S., Everhart, J.E., Donowitz, M., Adams, E., Cronin, K., Goodman, C., Gemmen, E., Shah, S., Avdic, A., Rubin, R. (2002). The burden of selected digestive diseases in the United States. Gastroenterology,122, 1500-1511.

Sanchez-Munoz, F., Dominguez-Lopez, A., Yamamoto-Furusato, J.K. (2008) Role of cytokines in inflammatory bowel disease. World J. Gastroenterol. 14, 4280-4288.
Schaefer, F., Chen, Y., Tsao, T., Nouri, P., Rabkin, R. (2001) Impaired JAK-STAT signal transduction contributes to growth hormone resistance in chronic uremia. J. Clin. Invest. 108, 467–475.

Schulte, C.M., Dignass, A.U., Goebell, H., Röher, H.D., Schulte, K.M. (2000) Genetic factors determine extent of bone loss in inflammatory bowel disease. Gastroenterology 119, 909-920.

Shi, H., Tzameli, I., Bjørbaek, C., Flier, J.S (2004) Suppressor of cytokine signaling 3 is a physiological regulator of adipocyte insulin signaling. J. Biol. Chem. 279, 34733-34740.

Sims, N.A., Clément-Lacroix, P., Da Ponte, F., Bouali, Y., Binart, N., Moriggl, R., Goffin, V., Coschigano, K., Gaillard-Kelly, M., Kopchick, J., Baron, R., Kelly, P.A. (2000) Bone homeostasis in growth hormone receptor-null mice is restored by IGF-I but independent of Stat5. J. Clin. Invest. 106, 1095-1103.

Sjogren, K., Bohlooly, Y.M., Olsson, B., Coschigano, K., Tornell, J., Mohan, S., Isaksson, O.G.P., Baumann, G., Kopchick, J., Ohlsson, C. (2000) Disproportional skeletal growth and markedly decreased bone mineral content in growth hormone receptor -/- mice. Biochem. Biophys. Res. Commun. 267, 603-608.

Slonim, A.E., Bulone, L., Damore, M.B., Goldberg, T., Wingertzahn, M.A., McKinley, M.J. (2000) A preliminary study of growth hormone therapy for Crohn’s disease. New. Engl. J. Med. 342,1633-1637.

Starr, R., Willson, T.A., Viney, E.M., Murray, L.J., Rayner, J.R., Jenkins, B.J., Gonda TJ, Alexander, W.S., Metcalf, D., Nicola, N.A., Hilton, D.J. (1997) A family of cytokine-inducible inhibitors of signalling. Nature. 387, 917-21.

Sun, D., Zheng, L., Tummala, P., Oh, J., Sun, D., Zheng, L., Tummala, P., Oh, J., Schaefer, F., Rabkin, R. (2004) GH-mediated JAK/STAT signaling is impaired in skeletal muscle in chronic uremia. Growth Horm. IGF Res. 14, 131–132.

Sylvester, F.A., Wyzga, N., Hyams, J.S., Davis, P.M., Lerer, T., Vance, K., Hawker, G., Griffiths, A.M. (2007) Natural history of bone metabolism and bone mineral density in children with inflammatory bowel disease. Inflamm. Bowel Dis. 13, 42–50.

Wirth, S., Neufert, C., Levy, J., Gertner, J.M., Levine, L.S., Harbison, M., Berdon, W., Oberfield, S.E. (2000) Osteoporosis: an unusual presentation of childhood Crohn’s disease. J Clin Endocrinol. Metab. 85, 2122-2126.

Tlaskalova-Hogenova, H., Tuckova, L., Stepankova, R., Hudcovic, T., Palova-Jelinkova, L., Kozakova, H., Rossmann, P., Sanchez, D., Cinova, J., Hrnecir, T., Kverka, M., Frolova, L., Uhlig, H., Powrie, F., Bland, P. (2005) Involvement of innate immunity in the development of inflammatory and autoimmune diseases. Ann. N Y Acad. Sci. 1051, 787-798.

Tsampalieros, A., Lam, C.K., Spencer, J.C., Thayu, M., Shults, J., Zemel, B.S., Herskovitz, R.M., Baldassano, R.N., Leonard, M.B. (2013) Long-term inflammation and glucocorticoid therapy impair skeletal modeling during growth in childhood Crohn disease. J. Clin. Endocrinol. Metab. 98,3438-3445.

Tse, K.F., Bonadio, J.F., Stewart, T.A., Baker, A.R., Goldstein, S.A (1996) Local expression of human growth hormone in bone results in impaired mechanical integrity in the skeletal tissue of transgenic mice. J. Orthop Res 14 598-604.

Van Staa, T.P., Cooper, C., Brusse, L.S., Leufkens, H., Javaid, M.K., Arden, N.K. (2003) Inflammatory bowel disease and the risk of fracture. Gastroenterology 125, 1591-7.

Williams, K.L., Fuller, C.R., Dieleman, L.A., DaCosta, C.M., Haldeman, K.M., Sartor, R.B., Lund, P.K. (2001) Enhanced survival and mucosal repair after dextran sodium sulfate-induced colitis in transgenic mice that overexpress sodium sulfate transporters. Gastroenterology 120, 925-937.

Wirtz, S., Neufert, C., Weigmann, B., Neurath, M.F. (2007) Chemically induced mouse models of intestinal inflammation. Nat. Protoc. 2:,541-546.

Wong, S.C., Smyth, A., McNeill, E., Galloway, P.J., Hassan, K., McGrogan, P., Ahmed, S.F. (2010) The growth hormone insulin-like growth factor 1 axis in children and adolescents with inflammatory bowel disease and growth retardation. Clin Endocrinol (Oxf) 73, 220-228.

Wong, S.C., Catto-Smith, A.G., Zacharin, M. (2014). Pathological fractures in paediatric patients with inflammatory bowel disease. Eur. J. Pediatr. 173,141-151.
Wong, S.C., Dobie, R., Altowati, M.A., Werther, G.A., Farquharson, C., Ahmed, S.F. (2016) Growth and the Growth Hormone-Insulin Like Growth Factor 1 Axis in Children With Chronic Inflammation: Current Evidence, Gaps in Knowledge, and Future Directions. Endocr Rev 37(1):62-110.

Zhao, G., Monier-Faugere, M.C., Langub, M.C., Geng, Z., Nakayama, T., Pike, J.W., Chernausek, S.D., Rosen, C.J., Donahue, L.R., Malluche, H.H., Fagin, J.A., Clemens, T.L. (2000) Targeted overexpression of insulin-like growth factor I to osteoblasts of transgenic mice: increased trabecular bone volume without increased osteoblast proliferation. Endocrinology 141, 2674-2682.
Figure 1. **Body weight changes of WT and Socs2−/− mice treated with DSS followed by recovery period.** Percentage change in body weight of WT and Socs2−/− mice treated with 3% DSS for 4 days. Data presented as mean ± SEM (n=6).
Figure 2. **Colon pathology of DSS treated WT and Socs2⁻/⁻ mice.**  
A. Representative H&E stained sections of colon from control and DSS treated WT and Socs2⁻/⁻ mice.  
B. Diameter of DSS treated, and control WT and Socs2⁻/⁻ colons. Numbers 1-5 represent difference segments along the colon.  
C. Histological scoring of DSS treated, and control WT and Socs2⁻/⁻ colons. Data presented as mean ± SEM (n=6). *significantly different from DSS treated WT mice p<0.05; by unpaired t-test or nonparametric test (Mann-Whitney).
Figure 3. *Socs* mRNA expression in bone from DSS treated WT mice. *Socs1*, *2*, and *3* mRNA expression levels in tibia from DSS treated WT mice compared to control mice at day 18 (endpoint) of DSS study. Data presented as mean ± SEM. Control group n=6, DSS treated group n=4. *a* significantly different from control samples, p<0.05; by nonparametric test (Mann-Whitney).
Figure 4. – **Trabecular bone architecture of DSS treated WT and Socs2−/− mice.** **A.** Representative 3D μCT reconstructions showing a less compact trabecular architecture resulting in a more porous structure (*) in DSS treated WT mice compared to WT control mice. A similar alteration in bone architecture was not observed in DSS treated Socs2 deficient mice. **B.** Percentage change of trabecular parameters in DSS treated mice relative to genotype-matched controls. Data presented as mean ± SEM (n=4). *significantly different from genotype-matched control, p<0.05; by nonparametric test (Mann-Whitney).
Table 1. Weight and length measurements of WT and $Socs2^{-/-}$ mice at day 18 of DSS study.

|                      | WT                  |          |          | Socs2^{-/-} |          |          |
|----------------------|---------------------|----------|----------|-------------|----------|----------|
|                      | Control             | DSS Treated | Control | DSS Treated | Control | DSS Treated |
| Weight (g)           | 23.5 ± 0.34         | 22.6 ± 0.38 | 32.6 ± 0.38 | 31.5 ± 0.46 |          |          |
| N to R Length (mm)   | 88.7 ± 1.11         | 87.9 ± 1.40 | 100.5 ± 1.15 | 103.2 ± 1.35 |          |          |
| Tibia (mm)           | 17.7 ± 0.14         | 17.6 ± 0.16 | 18.7 ± 0.18 | 18.9 ± 0.10 |          |          |
| Femur (mm)           | 14.4 ± 0.21         | 14.4 ± 0.10 | 15.6 ± 0.08 | 15.2 ± 0.20 |          |          |

N to R length = nose to rump length. Data presented as mean ± SEM. All groups n=6 except $Socs2^{-/-}$ control femur (n=5).  *significantly different from WT control mice, $p<0.01$,  b significantly different from WT control, $p<0.001$; by unpaired t test.

Table 2. Cortical bone parameters of tibia from control and DSS treated WT and $Socs2^{-/-}$ mice at day 18 of DSS study.

|                      | WT                  |          |          | Socs2^{-/-} |          |          |
|----------------------|---------------------|----------|----------|-------------|----------|----------|
|                      | Control             | DSS Treated | Control | DSS Treated | Control | DSS Treated |
| Tt.Ar (mm³)          | 0.84 ± 0.001        | 0.82 ± 0.002 | 1.03 ± 0.003* | 1.01 ± 0.003 |          |          |
| Ct.Ar (mm²)          | 0.54 ± 0.014        | 0.54 ± 0.012 | 0.67 ± 0.028* | 0.65 ± 0.022 |          |          |
| Ma.Ar (mm²)          | 0.30 ± 0.005        | 0.28 ± 0.005 | 0.37 ± 0.009* | 0.36 ± 0.001 |          |          |
| Ct.Th (mm)           | 0.23 ± 0.003        | 0.23 ± 0.004 | 0.25 ± 0.008* | 0.25 ± 0.006 |          |          |

Tt.Ar = total tissue area, Ct.Ar = cortical bone area, Ma.Ar = medullary area, Ct.Th = cortical thickness. Data presented as mean ± SEM (n=4) *significantly different from WT control mice, $p<0.05$; by nonparametric test (Mann-Whitney).
Supplementary Figure 1. DSS concentration and time dependent changes in body-weight. A. Body weight change of WT mice subjected to varying doses and treatment times of DSS. B. Maximum weight loss observed by WT and Socs2−/− during DSS experiment. Data presented as mean±SEM (n=6). Data non-significant by unpaired t-test. C. Individual weight change observed in WT and Socs2−/− mice treated with 3% DSS.

Supplementary Figure 2. Effects of DSS on mucosal integrity of WT and Socs2−/− mice. Representative image from WT and Socs2−/− mice treated with DSS. Images show epithelial degeneration, crypt loss, high levels of mononuclear leukocytes and transmural inflammation.