Impact of Therapeutic Strategies on Linear Growth and Bone Health in Children with Crohn's Disease

Mabrouka M A Altowati
MBChB, MSc (Paediatric Clinical Science /Endocrinology) & **MRCPCH**

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Developmental Endocrinology Research Group
Royal Hospital for Children
Faculty of Medicine
University of Glasgow
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Abstract

Growth retardation and impaired bone health are common complications of paediatric inflammatory bowel disease (IBD), especially Crohn's disease (CD). Aetiology may include undernutrition, inflammatory impact on the hormonal growth axis, delayed puberty, the effect of drugs such as glucocorticoids (GC) and low muscle mass. Despite an increase in our knowledge of its pathogenesis, growth retardation and impaired bone health remain common problems for children with CD. Control of disease activity and minimizing the need for GC therapy are necessary measures to facilitate normal growth. However, in many cases these strategies are not sufficient and data on the role of adjuvant therapy with recombinant human growth hormone (rhGH) remain scarce. Moreover, currently there is conflicting evidence on the benefit of anti-tumour necrosis factor-α (anti-TNF-α) on bone health and there are no reports on the effect of rhGH therapy on bone health in children with CD.

To address this knowledge gap, this thesis studied the hypothesis that successful treatment of inflammation with anti-TNF-α would improve linear growth and bone health in children with CD. Additionally, a further objective was to examine the role of adjuvant therapy with rhGH on linear growth and bone health in children with quiescent CD.

A prospective study was first carried out to assess the effect of 12-months anti-TNF-α therapy on linear growth, the growth hormone (GH)-insulin like growth factor (IGF) axis, and bone turnover. The results showed that other than depressed acid label subunit (ALS), markers of GH axis were not particularly abnormal in the majority. Bone turnover markers were also low at baseline. With therapy and improvement in disease, patient height was modestly improved in those with growth potential, and this was associated with increased bone formation but no clear change in markers of the GH-IGF axis. These findings suggest that if growth is of concern then adjuvant therapy combined with other forms of growth-promoting therapy during critical periods of growth warrants further exploration.

Further prospective analyses were conducted in this cohort to examine the effects of anti-TNF-α therapy on bone density and structure, at the time of, and 12 months after initiation of treatment and also to explore the association of IGF-1 axis, cytokines and muscle with bone density in children with CD. These results indicated that although anti-TNF-α therapy was associated with an improvement in disease activity and bone formation, there was insufficient evidence, as assessed by imaging, to show a change in bone health. The observed persistent bone impairment could be related to two possible factors. Firstly, there is a potential lag between growth and bone formation, and secondly, persistent muscle

deficit may partly explain the poor bone health seen in CD. These findings suggest that the anti-TNF-α therapy may not be sufficient for improving musculoskeletal development in children with CD and the role of adjuvant therapy such as nutrition, exercise or manipulation of the GH/IGF axis requires further investigation.

A subsequent analysis was conducted to investigate the effects of 24-months rhGH (67mg/kg/day) therapy on linear growth and insulin sensitivity in 14 children with CD, compared to an equal number of historical controls, matched for age, gender and duration of disease. The results of this analysis demonstrated that the growth-promoting effect of rhGH in children with CD, that were previously observed over a period of 6 months, is sustained over a longer period without a deleterious effect on glucose homeostasis. Improved growth with rhGH therapy was sustained over a two-year period, justifying the need for a randomised clinical trial (RCT) of this therapy. Close monitoring of glucose homeostasis is still recommended with the use of rhGH in children with CD and growth retardation.

Chapter 5 explores a preliminary analysis of the effect of adjuvant therapy with high dose rhGH therapy for 24 months on bone health and body composition by DXA in 8 children with inactive/quiescent CD. The results showed that despite increases in the biomarkers of bone turnover with most children having completed pubertal growth, deficiency in bone mineral density persists. This finding may be explained by partial recovery of the GH-IGF-1 axis and/or decreased muscle mass. These results also underscore the importance of muscle mass for bone health in CD children.

A final set of analyses were performed in a survey conducted to examine the feasibility of a RCT of injectable forms of growth-promoting therapy; and to survey the attitudes of children with CD and their parents to it. The results of this survey showed that by approaching shorter children with CD, as well as alleviating their fears about injections, a future trial would be more likely to achieve higher recruitment rates.

In summary, the body of work presented in this thesis depicted that anti-TNF-α therapy is associated with a modest clinical improvement in height but no observable beneficial effect on musculoskeletal health. The use of high dose rhGH therapy for 24 months was associated with growth-promoting effects but with no discernible influence on bone and body composition. Muscle deficit may partly explain the poor bone health seen in CD.

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Publications

1. **Altowati MA,** Russell RK, Ahmed SF. Endocrine Therapy for Growth Retardation in Paediatric Inflammatory Bowel Disease. Journal of Pediatric Drugs 2014; 16(1):29-42.

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Oral presentations

1. "Experience of Management of Children and Adolescents with Thyrotoxicosis in the West of Scotland 1987–2009". 38th Meeting of the British Society for Paediatric Endocrinology and Diabetes, Manchester (2010).

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3. “Growth & Glucose Homeostasis after 2 Years in Children with Inflammatory Bowel Disease (IBD) Receiving Recombinant Growth Hormone Therapy (rhGH) for Growth Retardation”. Yorkhill Research Day, Yorkhill Hospital, Glasgow (2012).

4. “Growth & Glucose Homeostasis after 2 Years in Children with Inflammatory Bowel Disease (IBD) Receiving Recombinant Growth Hormone Therapy (rhGH) for Growth Retardation”.15th Annual Scientific Meeting of Scottish Paediatric Endocrine Group, Dunkeld (2013).

5. “The Sustained Effects of Recombinant Growth Hormone Therapy on Linear Growth in Children with Crohn's Disease (CD)”. International Conference on Nutrition and Growth Barcelona (2014).

6. “Recombinant Human Growth Hormone in Paediatric Inflammatory Bowel Disease: Short Term Effects on Bone Biomarkers and Long Term Effects on Bone and Lean Mass”. 42nd Meeting of British Society for Paediatric Endocrinology and Diabetes, Winchester (2014).

7. “Attitudes Towards a Clinical Trial of Growth Promoting Therapy in UK Children with Crohn's Disease and Their Parents”. 16th Annual Scientific Meeting of Scottish Paediatric Endocrine Group, Dunkeld (2015).

Posters presentations

1. “Growth & Glucose Homeostasis after 2 Years in Children with Inflammatory Bowel Disease (IBD) Receiving Recombinant Growth Hormone Therapy (rhGH) for Growth Retardation”. 40th Meeting of the British Society for Paediatric Endocrinology and Diabetes, Leeds (2012).

2. “The Longer-Term Effects of Recombinant Growth Hormone Therapy (rhGH) on Growth & Glucose Homeostasis in Poorly Growing Children with Crohn's Disease (CD)”. 52nd Joint Meeting of European Society of Paediatric Endocrinology, Milan (2013).

3. “Low Remission Rates and High Failure Rate for Medical Treatment of Thyrotoxicosis in Childhood and Adolescence-Strategic Implications for Stopping Antithyroid Drugs”. 41st Meeting of British Society for Paediatric Endocrinology and Diabetes, Brighton (2013).

4. “Recombinant Human Growth Hormone in Paediatric Inflammatory Bowel Disease: Short Term Effects on Bone Biomarkers and Long Term Effects on Bone and Lean Mass”. 53rd Joint Meeting of European Society of Paediatric Endocrinology, Dublin (2014).

5. “Recombinant Human Growth Hormone in Paediatric Inflammatory Bowel Disease: Short Term Effects on Bone Biomarkers and Long Term Effects on Bone and Lean Mass”. Yorkhill Research Day, Yorkhill Hospital, Glasgow (2014).

6. “Attitudes Towards a Clinical Trial of Growth Promoting Therapy in UK Children with Crohn's Disease and Their Parents”. 29th Meeting of British Society for Paediatric Gastroenterology, Hepatology and Nutrition, Stratford-upon-Avon (2015).

7. “Bone-Muscle Unit Assessment with pQCT in Children with Inflammatory Bowel Disease Following Treatment with Infliximab”. 7th International Conference on Children’s Bone Health, Salzburg, Austria (2015).

8. “Bone-Muscle Unit Assessment with pQCT in Children with Inflammatory Bowel Disease Following Treatment with Infliximab”. 54th Joint Meeting of European Society of Paediatric Endocrinology, Barcelona (2015).

9. “Persistence of Musculoskeletal Deficits in Paediatric Crohn’s Disease Following Anti-Tumour Necrosis Factor Therapy”. 55th Joint Meeting of European Society of Paediatric Endocrinology, Paris (2016).

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Dedication

This thesis is dedicated to my mother, brothers and sisters for their endless unconditional love, support and encouragement. This work is dedicated to the memory of my father, Mohammed, who was always very supportive, patient, understanding, and encouraging. To the memories of my dearest brothers, Elmantaser, you will always have a place in my heart.

Author’s Declaration

I declare that the above-mentioned thesis embodies the results of my own special work under supervision of Professor Faisal Ahmed, unless otherwise stated. No part of this work has been submitted in support of application for another degree or qualification by this or any other University.

Dr Mabrouka Altowati

I certify that the work reported in this thesis has been performed by Dr. Mabrouka Altowati and that during the period of study she has fulfilled the conditions of ordinance and regulations governing the Degree of Doctor of Philosophy, University of Glasgow.

Prof SF Ahmed

Definitions/Abbreviations

ALS	Acid labile subunits
ADA	Adalimumab
ATI	Antibodies to Infliximab
aBMD	Areal bone mineral density
5-ASA	5-Aminosalicylates
BL	Baseline
BMI	Body mass index
BA	Bone age
BSAP	Bone specific alkaline phosphatase
BA	Bone age
BMAD	Bone mineral apparent density
BMC	Bone mineral content
BMD	Bone mineral density
CA	Chronological age
ΔHt SDS	Change in height standard deviation scores
CRP	C-reactive protein
CD	Crohn's disease
L2	Colonic
CSA	Cross sectional area
CTX	Cross-linked C-terminal telopeptides
NTX	Cross-linked N-terminal telopeptides
PICP	C-terminal pro-peptides of type 1 collagen
DPD	Deoxypyridinoline
DXA	Dual energy X-ray absorptiometry
EEN	Exclusive enteral nutrition
ELISA	Enzyme-linked immunosorbent assay
ESR	Erythrocyte sedimentation rate
Fat-CSA	Fat cross-sectional area
FM	Fat mass
FSH	Follicle-stimulating hormone
FDA	Food and Drug Administration

INF-γ	Gamma interferon
GC	Glucocorticoid
GM-CSF-Ab	Granulocyte macrophage colony stimulating factor autoantibodies
GH	Growth hormone
GHBP	Growth hormone binding protein
GH-IGF-1	Growth hormone/insulin-like growth factor-1
GHRH	Growth hormone releasing hormone
HbA1c	glycosylated haemoglobin
HOMA-IR	Homeostasis model assessment insulin resistance index
HPG axis	Hypothalamic-Pituitary-Gonadal
Ht	Height
Ht SDS	Height standard deviation scores
HV	Height velocity
HV SDS	Height velocity standard deviation scores
IBD	Inflammatory bowel disease
IBDU	IBD unspecified
IFX	Infliximab
IGFBPs	Insulin-like growth factor -binding proteins
IGF-1	Insulin-like growth factor-1
L1	Ileal
L3	Ileocolonic
L4	Isolated upper gastroenterology tract
IGFs	Insulin-like growth factors
IL-2	Interleukin-2
IL-1	Interleukins-1
IL-6	Interleukins-6
IC	Intermediate colitis
ISCD	International Society for Clinical Densitometry
JAK/STAT	Janus kinase/signal transducers and activators of transcription pathway
LH	Luteinizing hormone
LM	Lean mass
LS	Lumbar spine
MIGF	Maximal isometric grip force
6M	6 months

12M	12 months
MPH	midparental height
P1NP	N- terminal pro-peptides of type 1collagen
ND	Non-dominant
n	Number
OPG	Osteoprotegrin
OGTT	Oral glucose tolerance test
ON	Osteonectin
OP	Osteopontin
PTH	Parathyroid hormone
pQCT	Peripheral quantitative computed tomography
PYD	Pyridinoline
PCDAI	Paediatric Crohn's Disease Activity Index
RANKL	Receptor activator of nuclear factor kappa B ligand
RCT	Randomised controlled trial
RhGH	Recombinant human growth hormone
SCOS2	Suppressor of cytokine signalling-2
SH	Sitting height
SILL	. Subischial leg length
SDS	Standard deviation scores
TW2	Tanner-Whitehouse
TRAP5b	Tartrate-resistant acid phosphatase
PCDAI	The Paediatric Crohn's Disease Activity Index
TB	Total body
TGFβ	Transforming growth factor-beta
TNF-α	Tumor Necrosis Factor -Alpha
Th	T-helper cells
UC	Ulcerative colitis
Wt	Weight
2wk	2 weeks
6wk	6 weeks
wPCDAI	Weighted Paediatric Crohn's Disease Activity Index

Chapter One

Introduction

1.1 Crohn's disease

Crohn's disease (CD) is a form of inflammatory bowel disease (IBD); a chronic lifelong remitting and relapsing inflammation of the gastrointestinal tract (GIT) that also includes ulcerative colitis (UC) (1). The findings of endoscopic, histological and radiological investigations, along with the clinical context, can be used to classify children with IBD as CD or UC (2;3). These two conditions differ in the localisation and extent of mucosal inflammation. Whereas UC is defined by inflammation limited to the mucosal layer of the colon, CD is characterized by transmural inflammation which may be localised to any part of the gastrointestinal tract, from oropharynx to anus, and include features such as dissentious inflammation "skip lesions", stricture, and fistula (3;4). Approximately 10% of children present with pathology characteristics of CD but with the affecting tissues limited to the colon only, which can make the differentiation between UC and CD uncertain, even after a complete workup. Such cases are termed IBD unclassified (IBDU) or indeterminate colitis (IC) (1). CD may be defined by age of onset, location, or by pattern of disease (5) and these variables have been combined in the Montréal classification (Table 1-1) (5).

Table 1-1: Definition of Crohn's disease phenotype using the Montréal classification

Age at onset of CD	Location	Behaviour
A1: < 17 years	L1: Distal ileal	**B1:** Non-stricturing, non-penetrating
A2: 17–40 years	L3: Colonic	**B2:** Stricturing
A3: > 40 years	L3: Ileocolonic	**B3:** Penetrating
	L4: Isolated upper gastroenterology tract	**P:** Perianal disease

Adapted and modified from (5).

1.1.1 Aetiology and role of cytokines in pathogenesis of CD

The aetiology of CD remains unclear and appears to involve interplay between genetic susceptibility, environmental factors and the immune system (6;7). A combination of these CD risk factors seems to initiate alterations in epithelial barrier function thus permitting the translocation of luminal antigens (for example, bacterial antigens from the commensal microbiota) into the bowel wall. Subsequently, abnormal and excessive cytokine responses to such environmental triggers cause subclinical or acute mucosal inflammation in a genetically susceptible host (8). Several cytokines have been identified as key factors in the pathogenesis of CD, particularly an imbalance between pro-inflammatory and anti-inflammatory cytokines that hampers the resolution of inflammation and instead leads to disease continuation and tissue destruction (9). Naive CD4+ T cells have been shown to differentiate into T helper -1 (Th1), Th2, Th17 and T regulatory cells, upon recognition of cognate antigen in the context of associated environmental signals, as generated by cytokines or an inflammatory milieu (10). CD is considered a Th1-mediated disease, characterized by increased production of interferon-γ (IFN-γ) and interleukin (IL) -12, activating macrophages and driving the release of key cytokines include tumour necrosis factor-α (TNF-α), IL-1 and IL-6 that contribute to mucosal inflammation in CD (10). TNF-α influences the production of IL-22, which has also been found to be associated with the Th1 pathway (11). In recent years, an additional distinct subset of T cells characterized by secretion of IL-17 (Th-17) under stimulation of IL-23 has been identified and linked to CD pathogenesis. Furthermore, Th17 cells are an important source of IL-21, an IL-22-related cytokine that is up-regulated in inflamed CD mucosa (10;11). T regulatory cells exert a potent anti-inflammatory action through production of IL-10 and transforming growth factor-β (TGFβ) in experimental colitis, and they are depleted in peripheral blood of patients with active CD, relative to quiescent CD patients and control subjects (10). Cytokines not only drive intestinal inflammation and associated symptoms, such as diarrhoea, but may also regulate extra-intestinal disease manifestations (for example, growth failure) and systemic effects in CD, such as impairment of bone health. A more detailed explanation of the role of cytokines in pathogenesis of growth and bone impairment is covered in Section 1.3.3 and 1.4.3.

1.1.2 Epidemiology of CD

Approximately 25% of CD cases present during childhood and adolescence (12). Among children with CD, 4% present before the age of 5 years and 20% before the age of 10 years (12), with the peak onset in adolescence between 10-14 years (12-14). Thus, the disease commonly presents during a short but important period of growth acceleration, which might play a crucial role in final height and peak bone mass. The incidence of CD continues to increase worldwide, with an estimated incidence of 7–10 children per 100,000 developing CD in any given year from studies in UK, Sweden, Norway and the United States (15-19).

1.1.3 Clinical features

Clinical manifestation of CD in children and adolescents can exemplify many similarities to the disease in adults. For instance, typically, CD most commonly presents with abdominal pain, diarrhoea and weight loss (12). However paediatric CD can exhibit diverse symptoms, such that growth impairment and pubertal delay can be the presenting features, without any or only minimal gastrointestinal and other systemic symptoms. Conversely, impaired linear growth is rarely present at diagnosis in UC (20;21). Extra-intestinal manifestations occur in 28-35% of children with CD that can present before, at time of diagnosis, or later during the course of disease. The most common extra-intestinal manifestation in children are arthritis (axial or peripheral), cutaneous disorders (erythema nodosum and pyoderma gangrenosum), eye diseases (such as episcleritis and uveitis), liver disease (primary sclerosing cholangitis, autoimmune hepatitis), impaired bone health (osteopenia /osteoporosis) and disorders of growth and pubertal development (22;23). An in-depth review of impaired growth and bone health in children with CD, the underlying mechanism, and management detailed later in this Chapter (Section 1.3, 1.4, and 1.5).

1.1.4 Assessment of disease activity in CD

The Paediatric Crohn's Disease Activity Index (PCDAI) is the oldest paediatric CD activity score and serves as the primary outcome measure for induction and maintenance of remission (24). PCDAI is based on symptoms (abdominal pain, stool pattern and general wellbeing), physical examination (abdominal and perianal region, growth parameters including weight gain and height velocity), laboratory parameters (serum haematocrit, ESR and albumin), and the presence of extra-intestinal disease, with scores ranging from 0 to

100 (25). Since linear growth is a component of the PCDAI, the interpretation of growth and disease parameters based on PCDAI may confound the finding. Furthermore, the correlation of PCDAI and mucosal inflammation is poor (24). Weighted Paediatric Crohn's Disease Activity Index (wPCDAI) is a multi-measure of disease activity based on a one-week recall of subjective symptoms (abdominal pain, stool pattern and general wellbeing), laboratory parameters (erythrocyte sedimentation rate (ESR) and albumin) and physical examination (presence of extra-intestinal disease e.g. fever, arthritis, rash and ileitis, perirectal disease, and weight), with scores ranging from 0–125 (26). The cut-off point to identify clinical remission is < 12.5, from 12.5-40 for mild, > 40–57.5 for moderate and > 57.5 for severe disease. Improvement (i.e. 'response') is defined by a reduction of 17.5 points or more (26). Since growth is not a parameter of wPCDAI it might be considered more accurate to assess the relationship between disease and growth parameters. In addition, the wPCDAI is shorter, appears more feasible than PCDAI, and it is better at discriminating between the disease activity categories (26). However, none of the PCDAI versions are able to give a valid assessment of mucosal healing (27).

1.1.5 Management

There are two phases of management: active treatment to induce remission and maintenance treatments aimed at keeping the condition in remission and preventing relapse. There are a variety of treatments strategies for each phase supported by national (1;28) and international guidelines (29). Some therapies are effective only for remission induction or maintenance, whereas others are appropriate for both phases. However, due to the differing responses of patients, treatment should be tailored to each individual (1). Medications used for induction and maintenance of CD are summarised in Figure 1-1 based on British Society of Paediatric Gastroenterology, Hepatology and Nutrition (BSPGHAN) guidelines (1). BSPGHAN recommended that surgery should only be considered for those for whom medical treatment has failed or who present with stricture (1). As paediatric IBD occurs during a critical period of growth and development, special considerations in treatment are needed to reverse linear growth failure, delayed puberty, and deficits of bone mineralization.

To better understand the literature on CD-related growth and bone disease and their management, the next part of the introduction will firstly summarise normal bone structure and development, followed by a comprehensive review of the prevalence, aetiology and research specific to management of CD-related growth and bone disease in children and adolescents.

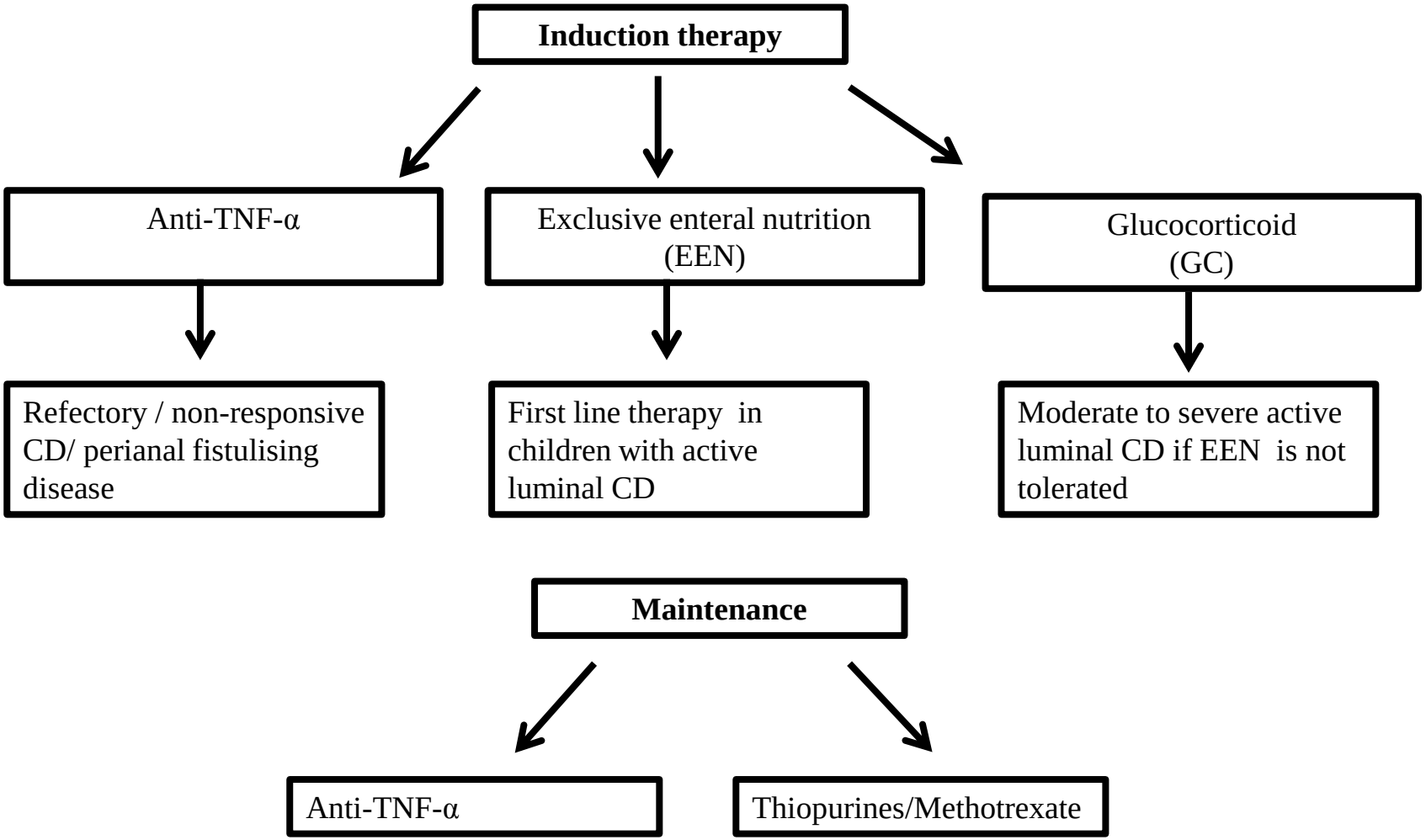

Figure 1-1: Flow chart of the management approach to children with Crohn's disease according to British Society of Paediatric Gastroenterology, Hepatology and Nutrition (BSPGHAN) guidelines (1).

1.2 Bone structure and function

Bone is a complex and dynamic tissue that act as biomechanical support and protection of soft tissue, provides an environment for marrow (both blood forming and fat storage), and plays a key role in mineral homeostasis (30). Bone is composed of type I collagen fibres (25% of bone) packed with hydroxyapatite crystals (65% of bone) that ensures resistance to fracture through an optimal balance of flexibility and stiffness (30).

Morphologically, bone has two components: cortical or compact (external layer) bone making up approximately 80%, and trabecular (internal layer) bone encompassing 20% of total bone mass (Figure 1-2) (31;32). Cortical bone is found in the diaphysis of the shaft of long bones. It also forms the outer shell around trabecular bone at the end of long bones and the vertebrae. Cortical bone serves a structural and protective role. It has a low porosity of between 5% and 10% and a slower turnover rate (annual rate of 3-5%), which gives it a harder structure, with less vulnerability to fracture than trabecular bone (31;32).

Trabecular bone is found within the cortical bone in the metaphysis and epiphysis of long bones and in the vertebrae. Trabecular bone has a significantly higher porosity than cortical bone, of between 50-90%. Due to its porosity, trabecular bone has a large surface that makes the bone tissue more metabolically active, with a remodelling rate of 20–25% annually. Trabecular bone is therefore more sensitive to disturbances in bone formation or bone resorption, and osteoporosis-induced fractures occur mainly in trabecular bone (31;32).

There are two bone surfaces: the periosteum, which is a fibrous connective tissue sheath that surrounds the outer cortical surface of bone, with the exception of joints, and the endosteum, which is a membranous structure on the internal surface of bone covering the trabecular bone and the inner surface of cortical bone (31;32).

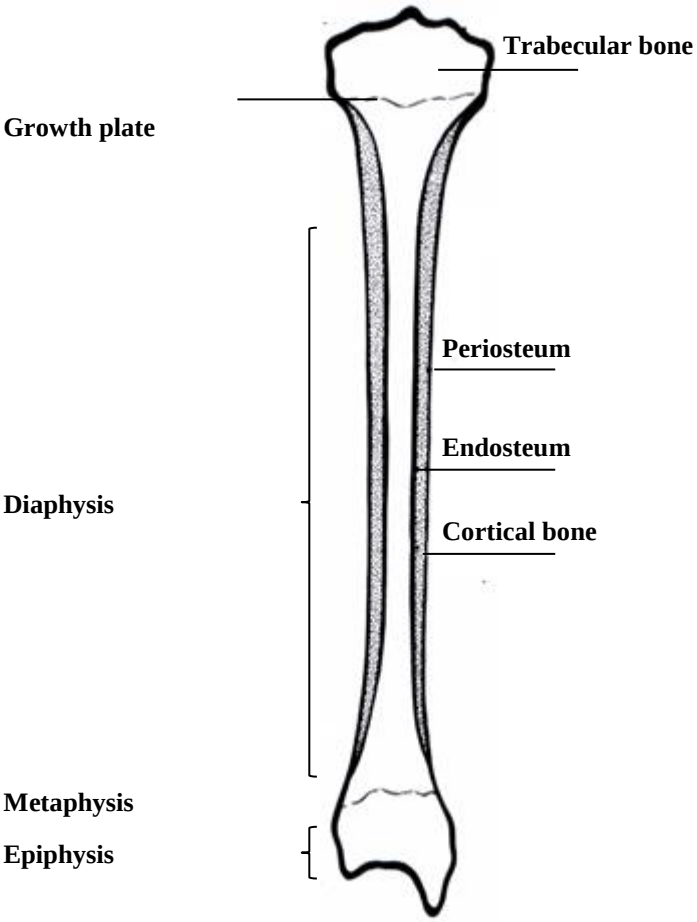

Figure 1-2: Structure of tibia bone illustrating different structures

1.2.1 Bone cells

Bone is a highly metabolic connective tissue and its material is constantly in a state of turnover. There are three main cell types in bone tissue that are responsible for its biological activity: bone-forming osteoblasts, bone-resorbing osteoclasts, and osteocytes for mechanosensing (31).

1.2.1.1 Osteoblasts

Osteoblasts are derived from mesenchymal stem cells (33). Osteoblasts are found on the bone surface and synthesise bone matrix (osteoid) by secreting collagen, non-collagen proteins (osteocalcin (OC), bone sialoprotein (BSP), osteopontin (OP) and osteonectin (ON)), bone specific alkaline phosphatase (BSAP) and ultimately stimulate bone mineralization (34). Whereas BSAP is used as an early marker, OC is considered to be a late marker for osteoblast differentiation. Osteoblasts also function as regulators for osteoclastogenesis by secreting OPG (ostpeprotegerin; inhibitory) and an activator of nuclear factor- κB ligand (RANKL; stimulatory) (35). Mechanical load, oestrogen, insulin-like growth factor-1 (IGF-1) and parathyroid hormone have been shown to play key roles in stimulating osteoblast function (36).

1.2.1.2 Osteocytes

Osteocytes are derived from osteoblasts and are the most abundant cells in bone (37;38). Osteocytes are found embedded in the bone matrix within lacunae and have several roles. Because of their location and their complex dendritic network, osteocytes act as mechanosensors. They respond to mechanical load by sending inhibitory signals to osteoclasts and stimulatory signals to osteoblasts. Osteocytes are involved in the bone remodelling process through a number of different mechanisms. Apoptotic osteocytes trigger signals that attract osteoclast precursors to specific areas of bone, which in turn differentiate to mature, bone-resorbing osteoclasts and initiate the bone remodelling cycle. Osteocytes are also the source of molecules that regulate the generation and activity of osteoclasts, such as OPG and RANKL. Osteocytes can also inhibit bone formation by secretion of sclerostin (37-39).

1.2.1.3 Osteoclasts

Osteoclasts are found on the bone surface and within lacuna. They are unique cells that possess an ability to resorb both the organic and inorganic matrices of bone tissue by secreting acid, proteolytic enzymes (cathepsin K), and collagenase (36). RANKL and OPG are the master regulators of osteoclastogenesis (36). RANKL stimulates osteoclast differentiation and activation and inhibits osteoclast apoptosis, thereby dramatically prolonging osteoclast survival (36). Moreover, there are several other factors that are involved in osteoclastogenesis at different stages of development, including 1,25-

dihydroxyvitamin D3, macrophage-colony stimulating factor (M-CSF), IL-1 and TNF-α and parathyroid hormone (PTH).

1.2.2 Bone growth and development

During childhood, bones increase in length and width, and undergo modelling and remodelling. These processes work to develop and maintain bone mass and health (31). Longitudinal bone growth is the result of a process called endochondral ossification, by which the embryonic cartilaginous model of most bones is gradually substituted by calcified bone (40;41). Longitudinal bone growth occurs at the growth plate (Figure 1-2) (40-42).

The growth plate encompasses one cell type, the chondrocyte, distributed at various points of differentiation within zones designated as resting; proliferative or hypertrophic (Figure 1-3). The resting zone is situated adjacent to the epiphyseal bone and as the name implies, consists of resting chondrocytes that do not have a function in bone growth. The resting chondrocytes are found in a scattered pattern, proliferate at a slow rate, and store nutrients for proliferative chondrocytes (43;44). The proliferative zone contains chondrocytes in a high rate of propagation. This region has a characteristic histological appearance wherein chondrocytes are arranged in a manner akin to stacked coins. The thickness of this zone is proportional to the growth rate, with wider zones displaying an increased level of growth (44;45). In the hypertrophic zone, chondrocytes are hypertrophied and increase their height about 6-10 fold, arranged in column-like structures (40;44;46). The newly formed cartilage is invaded by blood vessels and bone cell precursors, which remodel the hypertrophic zone cartilage into bone (40;41).

The process of longitudinal bone growth is complex and tightly regulated by several factors. Hormones play a major role in the regulation of longitudinal bone growth and most central are the growth hormone/insulin like growth factor-1 (GH/IGF-1) axis, thyroid hormone, and sex steroids. Nutrition is also an imperative regulator of growth (47). Growth is rapid in infancy, followed by slower rate until puberty (47;48). Linear growth increases again at puberty, characterised as the individual's growth spurt or peak height velocity (PHV). PHV accounts for 20-25 % of adult height (47;48) and during the pubertal growth spurt almost half of adult bone mass is acquired (49), with the attainment of peak height velocity preceding peak mineral accumulation by about 7 months (50). The relationship between bone mineral gain during childhood and adolescence, and bone mineral loss and skeletal fragility as an adult, however, are still not fully understood (51).

The rate and timing of PHV is different between the genders. In healthy girls, PHV is attained 2 years earlier than boys, at approximately 12 years, coinciding with early onset of puberty (breast stage-2), whereas in healthy boys it occur late in mid-puberty, corresponding to genital stage 4 (47;48). Generally, sex steroids accelerate growth through an increase production of GH and IGF-1 (48). Since the chronological age does not mirror the pubertal maturity, the recommendation is to consider puberty and the potential to grow in the context of skeletal maturation (Bone age (BA)) (52).

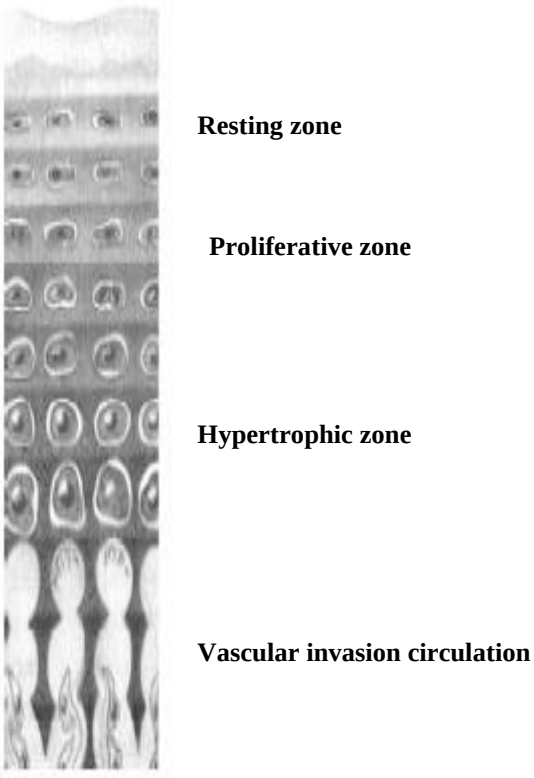

Figure 1-3: Histological structure of a growth plate
Adapted and modified from (53)

1.2.3 Bone modelling and remodelling

Table 1-2 summarises the important characteristics of bone modelling and remodelling (54-56). Bone modelling is defined as either the formation of bone by osteoblasts or resorption of bone by osteoclasts, on a given surface, in an uncoupled manner (54-56). Bone modelling is responsible for changes in the shape and size of the bone, such as those observed during growth, or adaptive responses to a change in mechanical loading patterns (54-56). For example, modelling activity on the diaphyseal cortices during growth, characterised by rapid periosteal formation modelling, is countered by resorptive modelling on the endocortical surface, resulting in a relatively consistent cortical thickness over time while increasing the width of the bone (54-56).

In contrast, remodelling occurs in a coupling manner at the same site on the same bone surface to conserve the bone without any net increase in bone mass (54-56). This process is coordinated by multiple cell types, including osteoclasts, osteoblasts, osteocytes and osteoblast-derived lining cells, which form small packets called basic multicellular units (BMU) under the control of the OPG-RANKL system (54-56). The remodelling cycle includs five basic phases (Figure 1-4). The first stage is the "activation phase" and involves recruitment of osteoclast precursors to an area of bone, differentiation and activation, followed by the "resorption phase", where the mature osteoclast dissolve minerals and liberate collagen fragments to form small cavities in the bone surface. The third, "reversal phase", is characterized by the cessation of osteoclast resorption and the initiation of bone formation, leading directly into the "bone formation phase", where the osteoblasts deposit new un-mineralised bone matrix (osteoid), followed by mineralisation. Osteoblasts incorporated into bone matrix transform into osteocytes to complete bone formation. In the final "resting phase" the bone surface is covered with bone lining cells and the majority of bone surfaces within the bone are in a state of quiescence (54;55). Any interruption in the coupling remodelling process can lead to pathology of the bone such as osteoporosis (54-56). In cortical bone, increased remodelling results in increased cortical porosity and decreased intracortical bone density, whereas in trabecular bone, the net negative balance slowly thins trabeculae over time and result in decrease trabecular bone density (56).

Table 1-2: Characteristic of bone modelling and remodelling

	Modelling	Remodelling
Function	Shape bone and size during childhood Increase bone density	Renew bone after micro-damage Maintain bone density Maintain mineral haemostasis
Bone component	Periosteal, endocortical and trabecular	Periosteal, endocortical, trabecular, and intracortical
Cells involved	Osteoclasts and osteoblasts and precursors	Osteoclasts and osteoblasts and precursors
Mechanism	Uncoupling activation of bone resorption or formation	Coupling activation of bone formation and resorption
Time	Mainly childhood but continues throughout life	Childhood and adulthood

Adapted from (54)

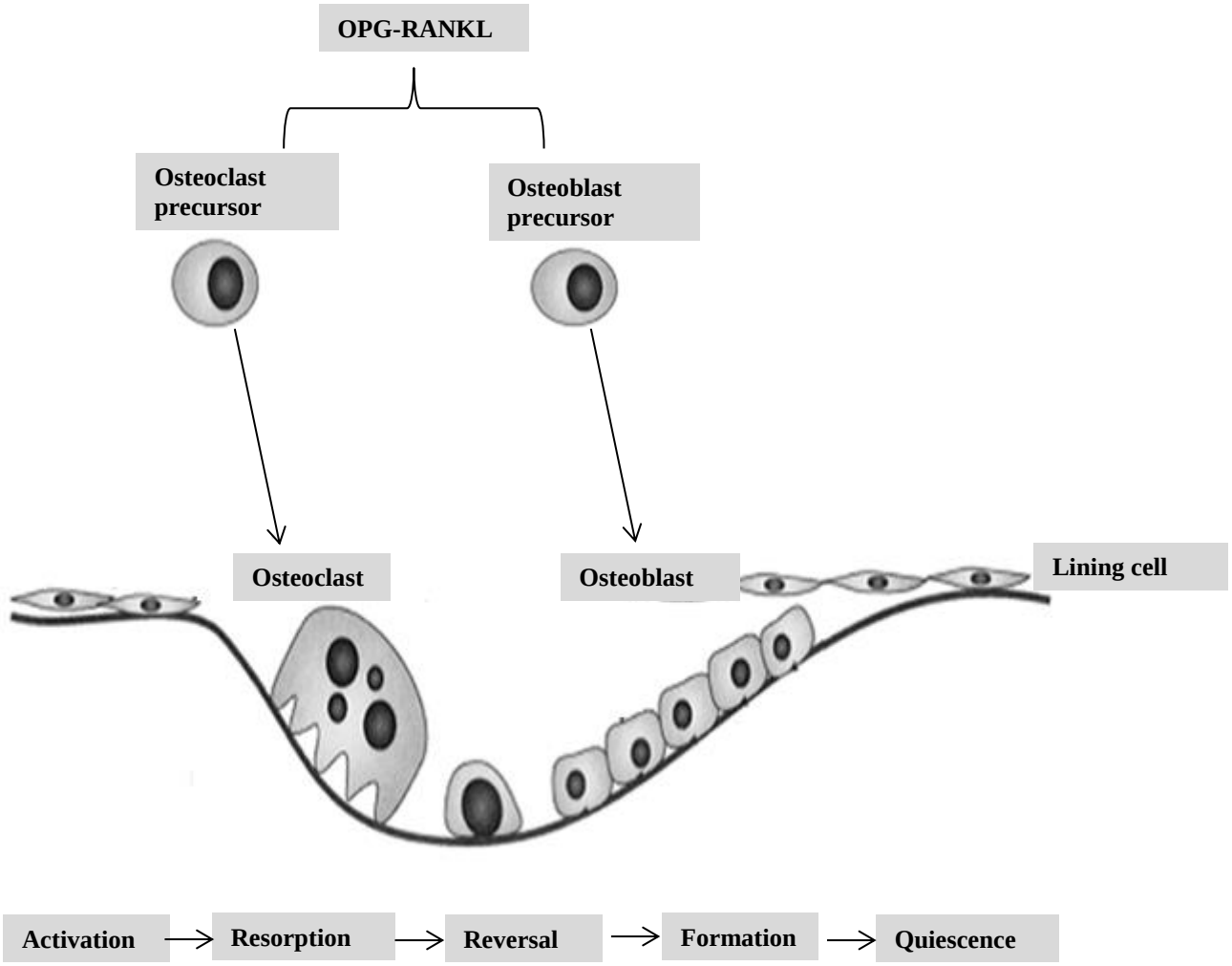

Figure 1-4: Bone remodelling cycle
Activation phase, osteoclasts are attracted to the resorption site; **Resorption phase**, osteoclasts dissolve bone matrix; **Reversal phase**, osteoblast precursors differentiate into mature osteoblasts and migrate into the resorption area; **Formation phase**, osteoblast initiate bone formation; **Resting phase**, the osteoblast initiate bone formation. Adapted and modified from (57).

1.2.4 Biomarkers of bone turnover

Several biomarkers of bone turnover are released into the circulation during the process of bone formation and resorption, reflecting information about the dynamic bone metabolism process (58;59). These biomarkers can be measured in blood or urine (58;59). In adults, it has been documented that accelerated bone turnover is associated with an increased fracture risk (60). However, in children, biomarkers of bone turnover not only reflect bone remodelling but also growth in bone length (endochondral ossification at growth plate), growth in bone width (bone modelling), and pubertal growth spurt. Therefore, an accurate interpretation of bone biomarkers in children should take growth and pubertal maturation in to consideration (61), and this is particularly crucial for children who receive growth-promoting therapy. Additionally, pubertal delay is common in the cohort of IBD children, so the age and gender matched control, without taking puberty in consideration, may not be precise (62). Generally, in children, biomarkers of bone turnover are mostly investigation tools for short-term longitudinal studies to assess the impact of specific intervention and not used for prediction of fracture risk (61). Bone biomarkers complement bone mass evaluation by dual energy x-ray absorptiometry or quantitative computed tomography in children by providing a dynamic picture of whole body bone turnover that can be repeated regularly. This dynamic evaluation allows early detection of disease or therapy consequences, long before changes in bone mass or progression in bone disease can be precisely ascertained (63).

1.2.4.1 Marker of bone formation

Markers of bone formation are either by-products of active osteoblasts expressed during the various phases of their development or osteoblastic enzymes. The most widely used markers of bone formation are measured in serum or plasma and include: bone specific alkaline phosphatase (BSAP), osteocalcin, and the carboxy- and amino-terminal propeptides of type 1 collagen (58;59;64). BSAP has a long half-life, low diurnal variation, clearance is not dependent on the kidney, and food has little effect. In combination with sample stability and a cheap-to-perform assay with lower intraindividual variability than other bone turnover markers, it makes BSAP a valuable and sensitive bone formation marker (58;60). However, BSAP is affected by cross-reactivity with the liver form of alkaline phosphatase, limiting its use in patients with liver disease (58;60). Theoretically, osteocalcin should be the most accurate marker of osteoblast activity. However, diagnostic use of this molecule is disadvantaged by its substantial instability, its circadian variation

and by difficulties in differentiating between the several molecular forms that are found in the circulation (65). Collagen type 1 is by far the most abundant protein synthesized by osteoblasts. Procollagen type 1 Amino terminal Propeptide (P1NP) and Procollagen type 1 Carboxy -terminal Propeptide (P1CP) are propeptides cleaved from type I procollagen during collagen formation and released into circulation (59;60). The advantage of P1NP and P1CP as markers of bone formation is that they reflect the activity of a crucial and well-characterized step of bone formation - the synthesis of collagen type 1. Thus, P1NP represents a real bone formation marker, is stable, its levels are reproducible and independent of the timing of sampling, and no circadian variation has been documented. However, P1NP has a higher cost of measurement compared with other bone turnover markers (58;60).

1.2.4.2 Markers of bone resorption

The majority of markers of bone resorption such as pyridinoline (PYD) and deoxypyridinoline (DPD), and cross-linking telopeptides of collagen type 1 including the C-terminal and N-terminal cross-linking telopeptides are degradation products of bone collagen, whereas tartrateresistant acid phosphatase (TRAP5b) is an enzyme of osteoclast origin (58;59;64). Telopeptides of type 1 collagen are the most extensively studied and used as bone resorption markers. Depending on the cross-link forming site with collagen there are two types, which are released during collagen degradation, Carboxy-terminal (CTX) and amino-terminal (NTX) cross-linking telopeptides. CTX is considered the marker of choice and the international Osteoporosis Foundation has selected serum CTX as the reference marker for bone resorption (60). CTX is released by cathepsin-K cleavage of type I collagen during bone resorption (58). However, CTX is subjected to large circadian variation, thus repeated sampling must be done at the same time of day, under fasting conditions, and immediate freezing is required to minimise degradation (58;60).

1.2.5 Growth hormone–insulin-like growth factor (GH–IGF)-1 axis

GH is secreted from the anterior pituitary gland in a pulsatile manner under control of hypothalamic growth hormone-releasing hormone (GHRH), which stimulates its secretion. GH circulates in the body bound to GH-binding protein (GHBP), where it interacts with hepatic GH receptor to generate IGF-1, the main mediator of GH action. The liver contributes up to 75% of circulating IGF-1 (66). Hepatic-produced IGF-1 circulates bound to one of the six binding proteins, collectively called the insulin-like growth factor binding proteins (IGFBP), which modulate the availability of IGF-1 at the tissue level. The most

abundant IGFBP in serum is IGFBP-3. In serum, most of the IGF-1 is found in a complex formed by IGFs, IGFBP-3, and a non-IGF binding glycoprotein known as the acid labile subunit (ALS) (66). IGFBP-2 is known to be a negative regulator for GH- IGF-1 (67).

1.2.5.1 GH-IGF-1 axis regulation of bone growth and bone mass

Figure 1-5 illustrates the effect of the GH-IGF-1 axis on growth and bone mass. GH–IGF-1 axis is the major regulator of linear growth from postnatal life to throughout puberty (47). GH may acts directly on the growth plate to stimulate the differentiation of chondrocytes or through the intensification of local and systemic IGF-1 secretion (68). Although most of the circulating IGF-1 is produced by the liver under influence of GH, it seems that locally derived IGF-1 may be more important for postnatal growth (69). However, conflicting data exists to challenge this hypothesis (68). In one study, when hepatic IGF-1 secretion was restored in IGF-1 null mice, some growth was also restored (68). Furthermore, GH can promote longitudinal bone growth through IGF-independent mechanisms (70-72). A role for GH acting directly on growth plate cartilage is also suggested by data from suppressor of cytokine signalling 2 (SOCS2) null mice. SOCS2 is expressed by epiphyseal chondrocytes, and is a recognised negative regulator of GH signalling via inhibition of the Janus kinase/signal transducers and activators of transcription (JAK/STAT) pathway (72). On the other hand, it is well documented that the GH/IGF-1 pathway is a critical regulator of osteoblast function, bone homeostasis, and ultimately, bone mass (66;73-75). The GH action on bone mass can be direct (IGF-1 independent) or indirect (IGF-1 dependent). Furthermore, the close connection between GH and IGF-1 hinders deciphering the relative contributions of the systemic and locally derived IGF1 on bone attainment. Based on the data obtained from animal models, systemic IGF-1 appears to predominantly influence cortical bone. Conversely, local IGF1 is important in regulating trabecular architecture, with minimal effects on cortical geometry (76-79). Animal data also provides compelling evidence to support the concept that GH can regulate osteoblast function, and ultimately bone mass, via local mechanisms, that in vivo are perhaps independent of IGF1 production (80).

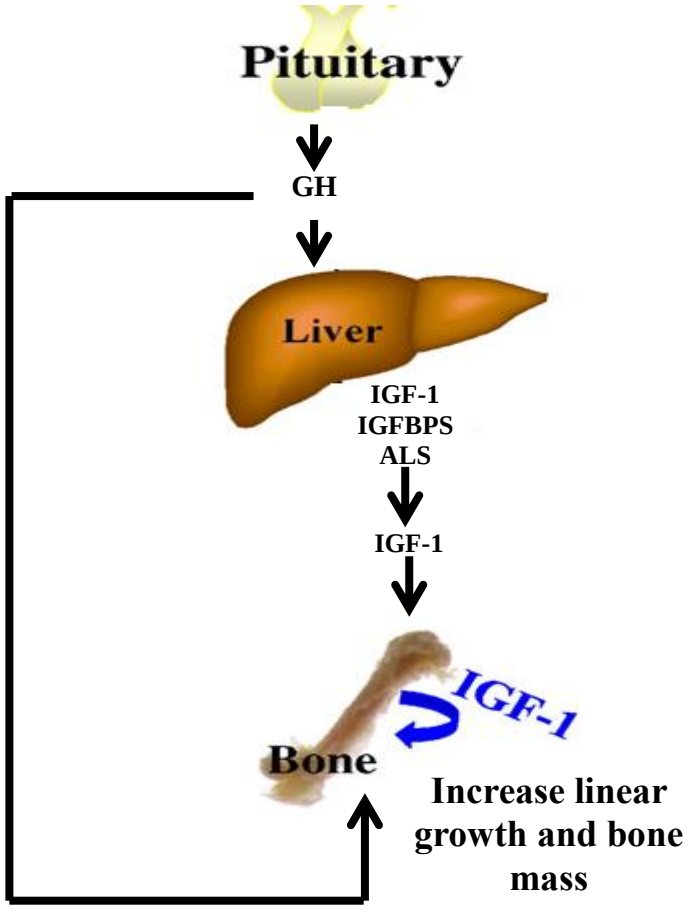

Figure 1-5: The GH-IGF axis and its role in linear growth and bone mass
GH is released from the pituitary. GH receptor is expressed throughout the body, including within the liver and at growth plates and bone cells. GH is the prime regulator of the IGF-1 gene in the liver. The liver is a major contributor to serum IGF-1. The majority of circulating IGF-1 forms a ternary complex with IGFBP-3 and ALS, which regulate its half-life and deliver it to the tissues. GH also stimulates local production of IGF-1 within bone. Adapted and modified from (66). GH, growth hormone; IGF-1, insulin like growth factor-1; IGFBP-3, insulin like growth factor binding protein-3; ALS, acid label subunit.

1.3 Growth impairment in children with CD

As described earlier in this chapter, CD is not limited to the GI tract. The disease can also exhibit extensive and varied extra-intestinal manifestations, such as impaired growth and bone health. Given the age of presentation of IBD, there is a limited window of opportunity for improvement of growth and bone health before skeletal maturation.

1.3.1 Definition of growth failure

As yet there is no universal agreement to define growth failure (81). One commonly used definition is a height (Ht) lower than the third percentile (≤ -2 standard deviation scores (SDS)) (81). However, the formal definitions may underestimate the prevalence of individuals with subnormal growth velocity, that experience a decline in their growth within a certain period of time, but still obtain a height above defined lower limits (82). Another pitfall of these definitions is that static height is primarily influenced by genetic determinants, reflecting both parents' height and thus needs to be interpreted in the context of the child's midparental height (MPH) (83). Whilst some have suggested that growth rate (height velocity, HV) and HV SDS, adjusted for age and gender, are a valid method of identifying growth failure (83), HV represents growth status at a particular point of time and might present a more sensitive marker for the impact of disease on normal growth. However, in a cohort of children where the extent of pubertal delay may be relatively common, comparing HV purely based on age and gender may be deceptive as it varies according to pubertal status. Thus, an understanding of pubertal staging or bone age is needed to interpret HV. Furthermore, the available normative data for HV is based on a small and old reference dataset (84). The use of change in Ht SDS (Δ Ht SDS) has been suggested as a more robust way to report response to growth promoting therapy in children with chronic disease in longitudinal studies, particularly when there is a high prevalence of children of peripubertal age (84). Growth impairment has been defined by some as HV SDS below -1 with Ht SDS below -1 or a reduction from MPH SDS of more than 1.0, and severe growth impairment as reduction from MPH SDS of more than 1.0 combined with a Ht SDS of less than -2 (85). Measurements of bone age may improve the interpretation of growth data, especially when substantial pubertal delay is expected (86).

1.3.2 Growth failure in children with CD

Growth failure is twice as common in CD compared with UC and may be the first manifestation of disease (87). At diagnosis, growth failure may be present in up to 56 % of children with CD and 10 % of children with UC (20). Ht SDS < -2.0 is present in approximately 10% of children with CD at diagnosis (84;88;89). Furthermore, a quarter of affected children may continue to grow slowly and remain short as adults (90;91). The duration of CD disease negatively correlates with degree of growth retardation, and, in turn, is subsequently associated with reduced adult height (90;91). Thus, early identification and treatment of CD is of the utmost importance in IBD clinics in order to improve growth outcomes (21;91;92). Another factor that studies have consistently shown to correlate with growth failure is disease location, in particular, the proximal small bowel was associated with reduced linear growth, and this may be principally related to a malabsorption of micronutrients and disaccharide intolerance, resulting in shorter gut transit times, pain, and exacerbation of diarrhoea (88;91;93).

Evidence as to whether gender has an influence on growth failure is conflicting. Some reports have demonstrated that males are more likely to be associated with reduced linear growth, and that this may be due to an effect on the pubertal growth spurt which occurs later and lasts longer in boys (94;95). In contrast, other studies have detected no difference in growth between the genders (88;91;96).

Both adults and children with a chronic inflammatory condition such as CD are now living longer, and the adverse effects of chronic inflammation on growth and skeletal development have been ranked by the Crohn's and Colitis Foundation of America as one of the top areas that require further attention (97). A recent study in a cohort of contemporary children and adolescents with IBD demonstrated a negative association of Ht SDS with the body image domain of the pediatric IBD specific quality of life score IMPACT III, with higher scores indicating poorer quality of life (98). However, quality of life is a challenging area in which to obtain meaningful data as there needs to be distinction between the impact of poor growth and the impact of the disease itself.

1.3.3 Pathophysiology of growth impairment in CD

The mechanisms of growth failure are multifactorial and involve the GH–IGF axis at a peripheral and central level (Figure 1-6) (99). Although the exact mechanisms of disturbance of the GH–IGF axis in CD children are still debatable (99-101), there is evidence to show that some children have low levels of IGF-1 despite normal GH secretion (102;103). These findings point to growth hormone resistance as a possible mechanism that may explain growth failure in short children with CD. Recent data has suggested that systemic GH–IGF-1 axis is affected to a variable extent in poorly growing children with CD, and that the abnormalities may range from functional GH deficiency to GH resistance and low IGF-1 level (100;101). A retrospective review of 28 patients with IBD reported that peak GH levels following insulin-induced hypoglycaemia were variable and subnormal in some cases (peak GH<3 mcg/l and IGF-1 SDS<0) but IGF-1 concentrations were almost universally low (101).

The role of specific genetic and immunological factors in growth variation in children with CD continues to be an area of active research (90;93;104). Evidence from an in vivo study reported polymorphisms in the dymeclin gene (DYM) are associated with growth failure in CD children (90). Further evidence to the importance of specific gene polymorphisms comes from a study that investigated genetic variants associated with disease susceptibility that were also associated with growth impairment. They found that patients with a specific organic cation transporter 1/2 (OCTN1/2) haplotype had a significantly lower height at diagnosis (104).

A study of 153 children with CD at time of diagnosis reported that the IL-6 174GG genotype was correlated with a lower Ht SDS score and higher circulating level of C-reactive protein (105). Also, data from Levine et al (106) showed that variations in the TNFα promoter region of the TNFα gene might independently modify linear growth and disease severity in paediatric onset CD. They suggested that the presence of either the TNF 238G/A or 863C/A polymorphism, which is linked to a reduced level of TNFα, was associated with higher mean Ht SDS, and thus had a protective effect on growth retardation, whereas two other polymorphisms were associated with disease severity. A recent in vitro study shows that defects in innate immunity due to granulocyte macrophage colony stimulating factors autoantibodies (GM-CSF Ab) in a CARD15 deficient host is associated with growth impairment in CD and hepatic GH resistance in murine ileitis (93). It is possible that these genetic polymorphisms are associated with variable immune function that modulates disease activity, thus influencing growth.

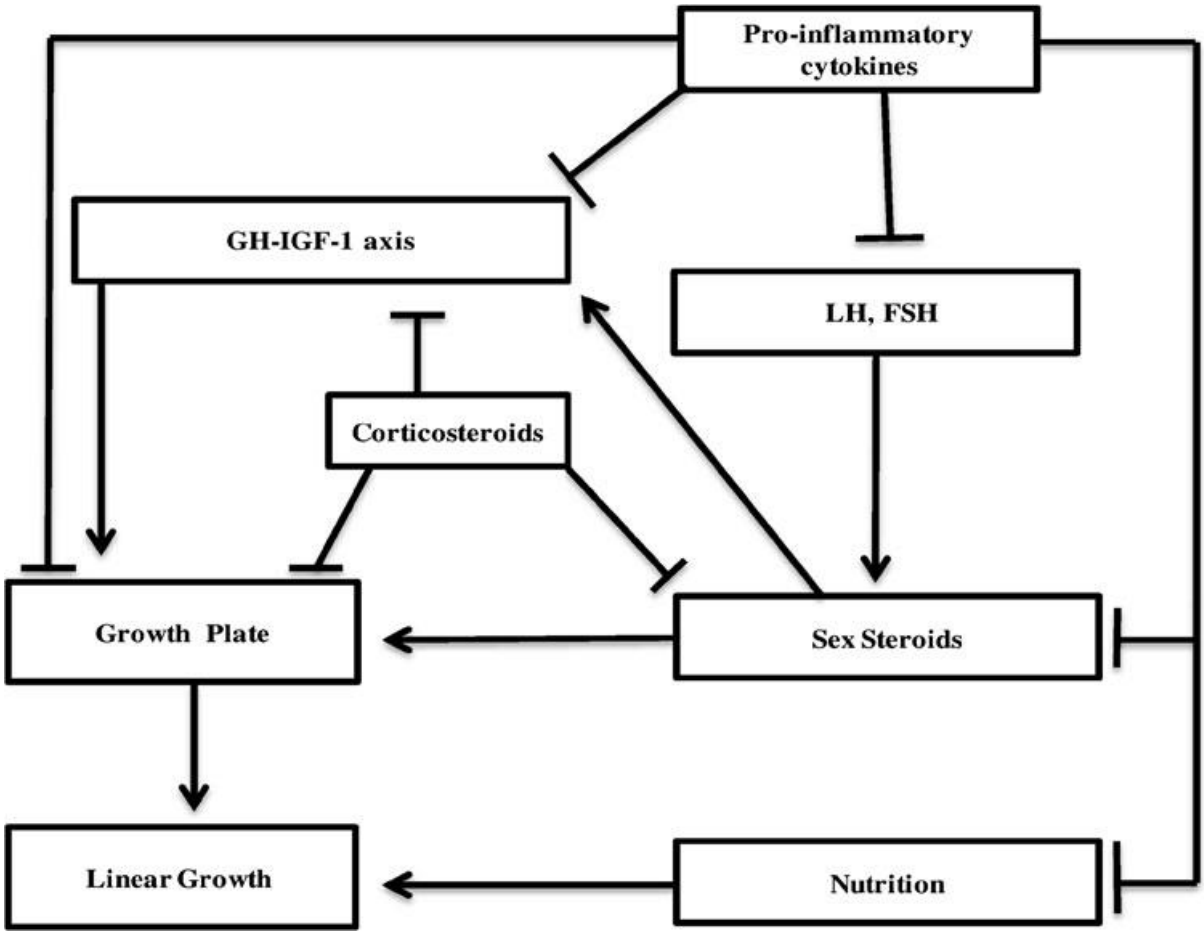

Figure 1-6: Possible mechanism of growth failure in children with Crohn's disease
GH, Growth hormone; IGF-1 insulin-like growth factor-1; LH, luteinizing hormone; FSH, follicle-stimulating hormone. Arrow indicates stimulation and blunted line indicate inhibition.

1.3.3.1 Nutrition

Over 80 % of children with CD are malnourished at the time of diagnosis (107) due to decreased nutrient intake, decreased gut nutrient absorption, increased loss, and increased requirements (108). An abnormal level of serum GH is often found in a state of malnutrition, being high in acute status, but reduced in chronic nutritional deficit. However, in protein-restricted rats, administration of GH was not associated with normalisation of IGF-1 levels, suggesting a degree of GH resistance (109). The nutrition disturbance that occurs in CD may contribute to GH resistance by several mechanisms. Reduced lean body mass is one consequence for children with CD, similar to that seen in cystic fibrosis. Sermet-Gaudelus et al. (110) showed a strong correlation between low serum IGF-1 levels and reduced lean body mass in cystic fibrosis patients. Furthermore, both lower circulatory IGF-1 and IGFBP-3 levels were found in children and adults with restricted energy and protein intake (111). The reduction in both IGF-1 and IGFBP-3 was observed to be associated with high GH levels and the authors suggest a degree of GH resistance at the receptor level in human subjects with energy restriction (111). Thus, nutritional deficit can contribute to both functional GH deficiency and resistance seen in those children. However, improving nutrition has been shown to be associated with only a partial improvement in growth in animal studies, suggesting that poor growth is not solely due to poor nutrition, with over 40 % of impairment in growth explained by inflammation (112).

1.3.3.2 Effect of inflammatory cytokines on the GH-IGF-1 axis

The effect of pro-inflammatory cytokines on growth has been extensively studied (113). A negative correlation between cytokine and IGF-1 levels indicate that cytokines may impair growth through disturbance of the GH–IGF-1 axis in CD and induction of a level of GH resistance (112;114;115). Drug-induced colitis in pair-fed rats is associated with impairment in growth, a normal GH level, and a low IGF-1 level, suggestive of a state of GH resistance. Administration of IGF-1 to the colitis group was associated with an increase in the circulating IGF-1 level and linear growth by approximately 44–60 %, suggesting that the effect of disease on growth due to systemic GH resistance could be partially overcome by increasing IGF-1 (112). The cellular mechanisms by which inflammatory cytokines modulate the GH axis remain unclear but the possible role of suppressor of SOCS has been suggested (116). The existing evidence shows a role for IL-6 in interruption of the GH-IGF-1 axis with transgenic mice studies revealing an inverse

relationship between plasma IL-6 and IGF-1 levels (114). It is of interest to note that administration of IL-6 antibodies to rats with colitis and growth failure was associated with a significant improvement in linear growth and IGF-1 level but that administration of TNF-α had no effect on circulatory IGF-1 levels (105). An association has also been demonstrated in children with IBD between cytokines and physical growth as well as markers of the GH-IGF axis. In 37 children with IBD (17 CD), IGF-1 levels were lower, whilst IGFBP-2 was higher during relapse, compared with controls. IL-1β levels were related to IGF-1 and IGFBP-2 (117). There is also evidence that TNFα may reduce expression of GH receptors by hepatocytes and thus reduce circulatory IGF-1.Furthermore, IL-6 may decrease circulatory IGF-1 half-life by increasing the breakdown of IGFBP-3 and interfering with formation of the IGF-1/IGFBP-3/acid labile subunit (ALS) complex (Figure 1-7) (85). IL-1B may also inhibit local production of IGF-1 (118). An in vivo study showed that greater increases in IGF-1, and decreases in TNF-α and IL-6 levels, were associated with increases in height-SDS in paediatric CD (115). The researchers proposed the possibility of immune-mediated mechanisms in growth failure. However, treatment with IGF-1 could only partially normalise the growth suppressive effects, suggesting that some of the effects may be independent of the IGF-1 system (119).

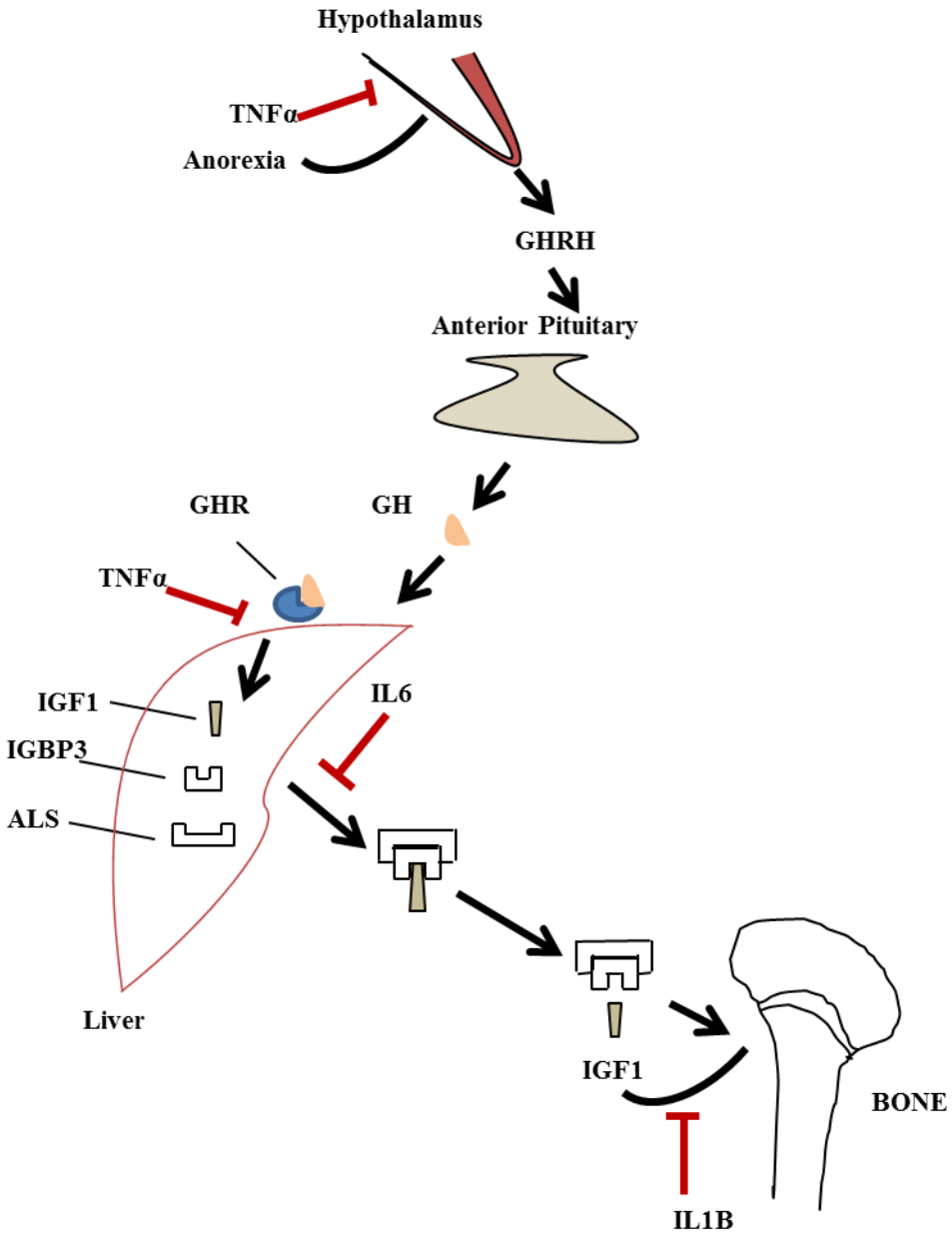

Figure 1-7: Possible mechanism of effect of inflammatory cytokines on systemic GH-IGF1 axis

Tumour necrosis factor (TNFα) may reduce expression of growth hormone (GHR) receptors by hepatocytes and thus reduce circulatory insulin-like growth factor-1 (IGF-1). Interleukin-6 (IL-6) may decrease circulatory IGF-1 half-life by increasing the breakdown of insulin-like growth factor binding protein-3 (IGFBP-3) and interfering with formation of the IGF-1/IGFBP-3/acid labile subunit (ALS) complex. Interleukin-1β (IL-1β) may also inhibit local production of IGF-1. TNF-α may suppress growth by inducing anorexia in patients with chronic inflammation by acting through the appetite regulatory centre at the hypothalamic level. Black arrow indicates stimulation and red line indicates inhibition.

1.3.3.3 Effect of inflammatory cytokines on the growth plate

Animal studies show that both TNF-α and IL-1β inhibit growth plate chondrocyte differentiation and increase apoptosis, and inhibit cartilage-specific proteoglycan synthesis (119;120). TNFα and IL-1β have both synergistic and additive consequences as when combined; they more severely hinder longitudinal growth in the rodent metatarsal model (119;120). Additionally, data from a culture model showed that the duration of exposure to pro-inflammatory cytokines is critical, such that dramatic irreversible decrease in growth was associated with longer duration of exposure (120). This may reflect the clinical findings of greater growth impairment in those children who have a longer period of symptoms prior to diagnosis (21;91;92). In addition, TNF-α may suppress growth by inducing anorexia in patients with chronic inflammation by acting through the appetite regulatory centre at the hypothalamic level (121).

1.3.3.4 Effect of glucocorticoid on GH–IGF axis

The negative effect of glucocorticoid (GC) on growth occurs through multiple mechanisms and is an important drawback of using this medication in children with CD (87). Growth recovery is poorer following the use of GC as compared with enteral nutrition in the early phase of treatment for paediatric CD (122;123). GC can interfere with the GH–IGF axis through attenuation of GH secretion by increasing hypothalamic somatostatin tone and a loss of pulsatile release (124). Steroid exposure can also lead to functional IGF-1 deficiency through downregulation of liver GH receptors, thus suppressing IGF-1 production and inhibiting IGF-1 bioactivity (125). However, it is difficult to separate the effect of GC therapy on growth, weighing its anti-inflammatory properties against its defined adverse impacts. Indeed, no association were found between GC and linear growth in some clinical studies in children with CD (126;127).

1.3.3.5 Effect of glucocorticoid on the growth plate

GC may also inhibit linear growth through pathways that are independent of the systemic GH–IGF axis. They may impair GH receptor expression at the level of the growth plate thus decreasing GH action and local IGF-1 production (128). They may also restrict chondrogenesis by reduced chondrocyte proliferation and increased apoptosis rates, through down regulation of anti-apoptotic proteins (129-131).

1.3.3.6 Pubertal delay

Delayed puberty is a commonly encountered issue in children with CD (14;98;132;133), with potential sequelae, such as poor linear growth (14;98;132;133). Studies of children and adolescents with CD have reported delays in bone age (134), breast development (135), menarche (134;135), testicular enlargement (135), and the pubertal growth spurt (14). The abnormalities of the hypothalamic–pituitary–gonadal (HPG) axis include hypogonadotropic hypogonadism and abnormalities of sex steroid synthesis or action of sex steroid (136-138). Preclinical data has suggested a mechanism in which both under-nutrition and inflammation are postulated to induce central and peripheral hypogonadism (136-138). Generally, it is documented that under nutrition is associated with decreased fat tissue, with a subsequent reduction in levels of leptin, an essential hormone for the normal initiation of puberty (87). However, investigations in rat models with colitis revealed that inflammation appears to have a detrimental effect on pubertal development and either directly inhibits puberty or potentiates the effects of under nutrition (136). A recent study in rats with induced colitis showed that pubertal delay in colitis cannot solely be explained by reduction in leptin level only. They suggest the inhibition of gonadotrophins secretion by inflammatory cytokines as a conceivable explanation of pubertal delay in mice with colitis (137;138). In mice with experimental colitis, treatment with a monoclonal antibody against TNF-α resulted in partial normalization of oestrogen production, as evidenced by earlier vaginal opening, an oestrogen-dependent marker of pubertal progression in murine models (139). This finding was supported by a recent clinical study that showed improvements in disease activity and cytokine levels in children with CD during induction therapy were associated with rapid and significant increases in sex hormone and gonadotropin levels, independent of body mass index or fat mass. The authors suggest a role of inflammatory cytokines in this regulation among individuals with an inflammatory condition (140). Thus, the negative effect of inflammation on the growth axis and puberty may provide a plausible explanation for poor growth and short adult height seen in children with IBD.

In summary, significant growth impairment is often seen in children with CD leading to a short stature at adult height. Depending on how growth failure is defined, approximately 10% of children with CD are considered short at the time of diagnosis. Current data suggests that a proportion of children with CD will not achieve their expected final height. The underlying mechanism is multifactorial and includes poor nutrition, chronic inflammation, and the prolonged use of steroids. It is apparent that systemic abnormalities

in the GH-IGF axis exist in these children, although emerging evidence points to perturbations at the level of the growth plate as a result of cytokine-driven insult and also effects of GC treatment. Given the multifactorial pathogenesis of growth impairment in children with CD, a multifaceted approach may be needed to improve growth.

1.4 Impairment of bone health in children with CD

1.4.1 Definition of osteoporosis

Osteoporosis is a skeletal condition characterised by decreased bone mass and deterioration of the microarchitectures and quality of the skeleton, with a consequental reduction in bone strength and predisposition to fractures (141). An accepted clinical definition of osteoporosis is an adult areal bone mineral density (aBMD) T-score measured by Dual-energy X-ray absorptiometry (DXA) of less than -2.5 (142), however, the interpretation of the standard deviation score (SDS) is less established for assessment of osteoporosis in paediatrics (143). Current paediatric densitometry guidelines (the International Society for Clinical Densitometry (ISCD)) agreed that the presence of one or more vertebral compression fracture (decrease of vertebral height at any point of more than 20%) in the absence of disease or high energy trauma is established as an indicator for the diagnosis of osteoporosis in children and adolescents. In the absence of vertebral fracture, the ISCD stated that the diagnosis of osteoporosis requires the presence of both a clinically significant history of fracture (two or more long bone fractures by the age of ten years or three or more long bone fracture at any age up to 19 years), combined with low BMD or bone mineral content (BMC) (of less than -2) adjusted for age and or body size as appropriate (144). Recently, the expert consensus recommended defining sub-optimal BMD as BMD SDS of less than -1 in children and adolescents with IBD (145).
Bone strength depends not only on bone mineral density (BMD) but also on bone quality (141). Bone quality is determined by microarchitecture, mineralization, and geometry of bone. Bone microarchitecture is determined as width, number and spatial organization of bone trabeculae, and by cortical porosity (146). Bone geometry is determined as persioteal, endosteal circumference, cortical thickness and cortical cross-sectional area (146). Imaging techniques such as peripheral quantitative computed tomography (pQCT), high-resolution pQCT, and micro-MRI that can measure one or more of the determinants of bone strength may enhance clinical management of osteoporosis and enhance new drug development.

Osteoporosis in childhood and adolescence can be primary or secondary due to chronic disease (141). The pathogenesis of secondary osteoporosis is multifactorial. The underlying mechanism of osteoporosis in CD will be detailed in the next section.

1.4.2 Bone health impairment in CD

Poor bone health is a documented complication in children with CD (147-149). Evidence from laboratory, clinical, imaging, and bone biopsy studies suggest that at the time of diagnosis both bone formation and bone resorption are reduced in children with CD, with a significant reduction in markers of bone formation (62;149-151). Recent studies have reported that 43-46% of children with CD had BMD SDS < -1 SD at diagnosis (149;152). In children with newly diagnosed CD, both tibia and radius exhibit decreased trabecular bone mineral density, expanded endocortical surface, and reduced periosteal circumference, resulting in bone that is predicted to be mechanically weaker (153-155). In children with CD, bone mass deficits are apparent at diagnosis, with an incomplete improvement in bone mass after median 12 to 24 months follow-up (149;153-155;155;156). This impairment in bone health may result in an increased risk of fractures. Nevertheless, studies investigating the non-vertebral fracture rate are inconclusive (157;158) and data on vertebral fractures are scarce (145). However, growing evidence suggests an increase rate of vertebral fracture, which may be subclinical in children and adolescents with CD (159;160). The prevalence of vertebral fractures in a cohort of 80 children with IBD was 11% after of an average of three years post-diagnosis, compared to 3% in healthy controls (159). Accordingly, the ISCD Pediatric Official Positions concluded that children with CD should undergo DXA assessment of lumbar spine BMD at diagnosis, and annually thereafter, as indicated (161).

Furthermore, given that paediatric CD most frequently presents during late childhood or early adolescence, at the time when bone mass is acquired at the fastest rate, it may effect bone mass accrual. Premenopausal women with childhood onset CD demonstrated significantly reduced BMD (162). Thus, uncontrolled disease during those years may lead to a reduction in peak bone mass accrual and therefore increased lifetime fracture risk (163;164). Overall, studies addressed the impact of childhood IBD on peak bone mass are still lacking and currently, there is little data on fracture history of adults with paediatric-onset IBD (165).

1.4.3 Pathophysiology of bone impairment in CD

The pathophysiology of skeletal morbidity in CD is complex and may contribute to numerous risk factors, including disease and treatment components (145) (Figure 1-8).

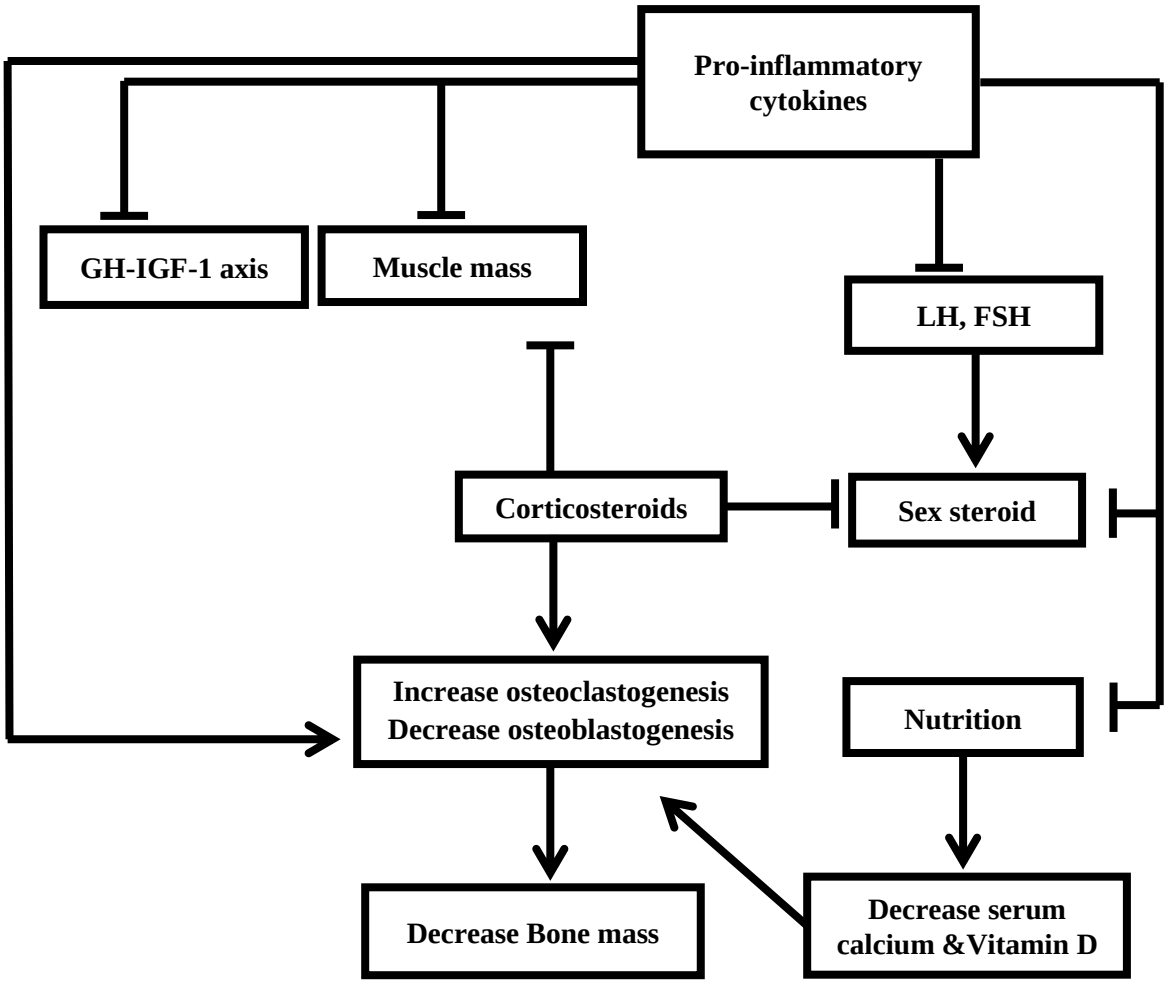

Figure 1-8: Possible mechanism of bone mass impairment in children with Crohn's disease
GH, Growth hormone; IGF-1 insulin-like growth factor-1; LH, luteinizing hormone; FSH, follicle-stimulating hormone. Arrow indicates stimulation and blunted line indicates inhibition.

1.4.3.1 Nutrition

Changes in calcium and vitamin D homeostasis may contribute to bone deficits. Vitamin and mineral deficiencies have been described in patients with CD and are attributed to gut mucosa inflammation and a reduced oral intake (147-149;166-170). Vitamin D and calcium are important for maintaining adequate bone homeostasis and facilitating bone modelling, remodelling and linear growth. However, there is still no strong evidence in the literature of an association between circulating vitamin D levels and bone health outcomes, in particular, improvement in BMD in healthy children (171) as well as children with CD (147-149;172;173). Although randomised controlled trials showed the effectiveness of vitamin D supplementation in treatment of vitamin D deficiency in CD children (174;175), to date, the only experimental study of the effect of vitamin D supplementation on bone mineral density in paediatric CD did not show any benefit (176;177). Based on available data, vitamin D deficiency does not appear to explain the bone health deficient seen in children with IBD. However, BSPGHAN recommended that calcium and vitamin D supplementation should be considered in children with significant nutritional impairment, during the pubertal growth spurt and during steroid treatment (1).

1.4.3.2 Pro-inflammatory cytokines

Evidence from human and experimental studies shows that bone mass deficits present in children with CD, even before treatment is started, suggested the central role of inflammation in the pathogenesis of bone disease (150;178;179). Pro-inflammatory cytokines such as TNF-α, IL-1, and IL-6, are commonly dysregulated in inflammatory disease (10), and can alter osteoblast and osteoclast differentiation and function, resulting in altered bone modelling and remodelling (180). These pro-inflammatory cytokines promote osteoclastogenesis by acting on cells of the osteoclast lineage, or indirectly, by modulating expression in target cells of key molecules such as RANK-L (181;182). TNF-α can also decrease the expression of osteoprotegerin (OPG), a decoy receptor that blocks RANKL (182;183). In addition, TNF-α can directly inhibits osteoblast differentiation and collagen synthesis, and promotes osteoblast and osteocyte apoptosis (Figure 1-9) (181;182;184). Evidence from preclinical data showed that serum from patients with CD impairs osteoblast differentiation and function in vitro, presumably due to these cytokines (178;179;185). Antibody neutralization of serum cytokines has yielded conflicting results for the effects of serum in this model (178;179;185). Limited human studies in children confirmed the negative relationship between IL-6 serum levels and BMD SDS (149;186).

Thus, pro-inflammatory cytokines potentially cause bone loss by inhibition of osteoblast differentiation and stimulation of osteoclastogenesis. Chronic inflammation in CD may also impair bone via other mechanisms such as an increase in renal phosphate excretion and inhibition of renal 25 hydroxyvitamin D3 1 α hydroxylase (187;188), and acute inflammation may increase locally active GC or local GC sensitivity (189). In summary, inflammatory bone loss is a result of the upregulation (and thus hyperactivity) of osteoclasts as well as the downregulation (and thus hypoactivity) of osteoblasts, which leads to a profound net reduction in bone mass.

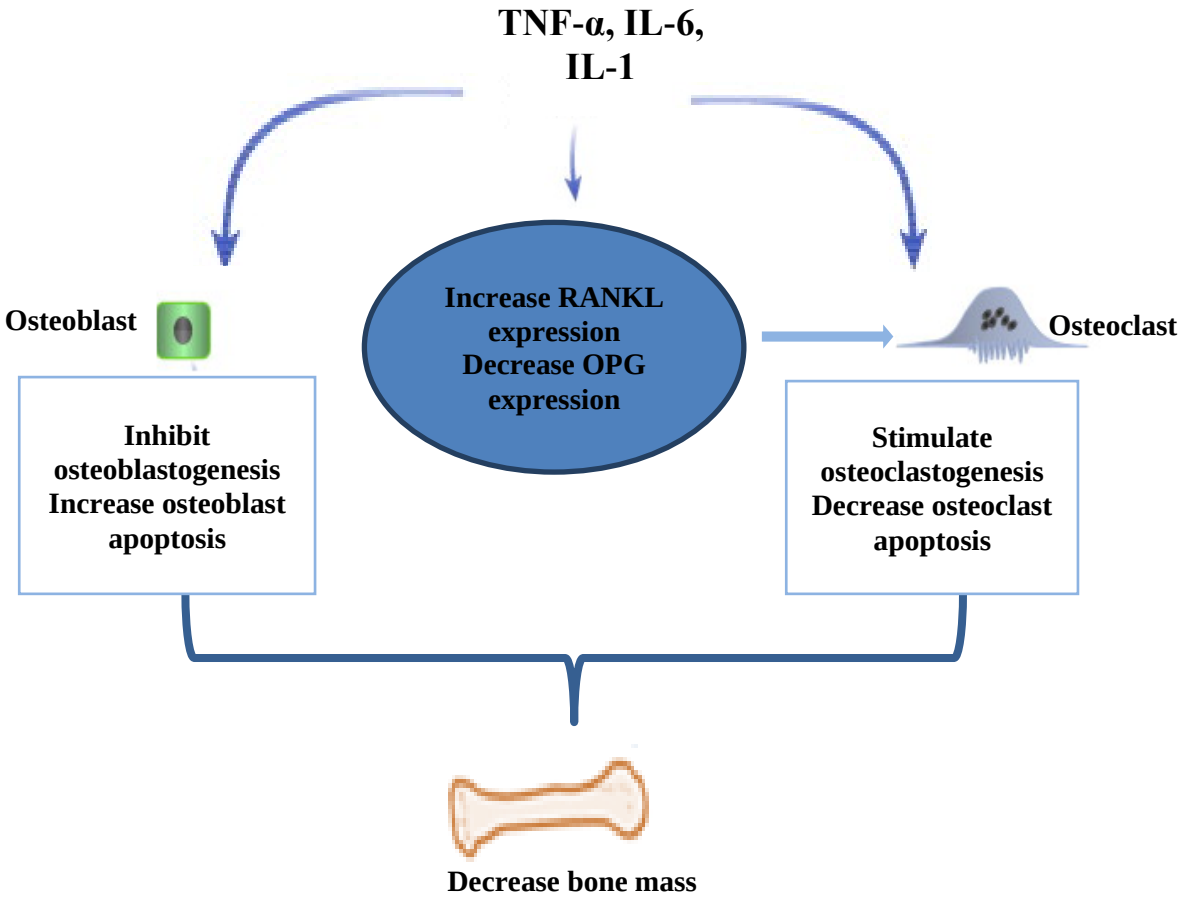

Figure 1-9: Possible mechanism of effect of inflammatory cytokines on bone
Tumour necrosis factor (TNFα, interleukin-6 (IL-6) and interleukin-1β (IL-1β) may alter osteoblast and osteoclast functions through RANKL-OPG system. TNFα can also directly promote osteoclastogenesis and suppresses osteoblastogenesis and promote osteoblast apoptosis.

1.4.3.3 Glucocorticoid

GC is widely used in the treatment of UC and they are also used in children and adolescents with CD, although exclusive enteral nutrition is considered the first line approach for induction of remission in those with mild–moderate CD (1). GC can affect bone health via various mechanisms (190-192). GC can be directly toxic to bone cells by hampering bone formation and stimulating excess bone resorption through inhibition of osteoblastogenesis, promotion of osteoblast/osteocyte apoptosis, prolongation of osteoclast survival (191;192), and promotion of osteoclastogenesis through increased expression of RANKL and decreasing OPG (180). Indirectly, GC use may act to inhibit calcium absorption leading to secondary hyperparathyroidism, impair linear growth, and delay puberty through reduced sex hormone production (190;191). GC also adversely affect bone through their negative effect on muscle function and mass weakness, which may lead to a reduction in bone strain and mechanical stimuli (193;194). GC effects on the growing skeleton may be rather different from their effects in adults due to their influence on bone modelling and remodelling (153;195). A longitudinal cohort study of children with new-onset CD, who subsequently received GC therapy, revealed normal cortical BMD but reduced trabecular BMD and cortical area at baseline. At follow-up, greater linear growth was associated with a greater cortical area improvement, especially among participants with less glucocorticoid exposure and inflammation (153). These findings demonstrate the effect of disease and GC exposure on bone modelling during childhood. Studies of GC effects on BMD in paediatric CD have reported differing observations. Some investigators have found a negative relationship between cumulative GC dose and BMD (62;147;159;196-198). The minimum duration and dose of GC therapy that may cause damage to bones in paediatric patients with CD are currently unresolved from available data. In one study, a cumulative weight-adjusted prednisolone dose of more than 150mg/kg during the preceding 3 years was found to increase the risk of low aBMD at lumbar spine and total body in IBD children (159). Alternately, others investigators failed to confirm a link between GC and BMD in children with CD (149;152-155;186;199). However, in children with CD, the negative effect of GC therapy on bone may be offset to a degree by their ability to combat inflammation, which in itself is detrimental for bone. It is challenging to identify the independent effect of GC. For this reason the role of GC in inducing bone deficits in CD remains controversial and provokes the question of whether cytokines are the main culprit in causing secondary osteoporosis rather than GC alone.

1.4.3.4 Muscle mass and strength

Low muscle mass and strength are a common finding in children with CD compared to healthy controls (115;147;150;153;154;199-203). Low muscle mass may be a consequence of inflammation, malnutrition, glucocorticoid use, or decreased physical activity (204). Some longitudinal evidence demonstrated that CD children might have persistent deficiencies in lean mass, despite treatment and a marked improvement in disease activity (153;155;200;205). Both newly diagnosed young patients and those with a history of CD have demonstrated reduced muscle strength compared to healthy controls, which was reflected in reduced muscle cross-sectional area (154;199). Bone increases in mass, strength and dimension as the muscle mass and strength increase (mechanostat theory) (206). Thus muscle deficit may partly explain the poor bone health seen in CD.

1.4.3.5 The GH-IGF-1 axis and sex steroid

As previously discussed, the GH-IGF-1 axis and sex steroids are frequently disturbed in CD. These hormonal disturbances can affect bone development and maintenance. It has long been known that GH-IGF-1 controls growth, remodelling, and mineralization of the skeleton, in part via their direct actions on bone (68). A close relation between height SD score and BMD is documented based on cross-sectional studies in young patients with CD (147;196;198). In girls during puberty, oestrogen inhibits periosteal apposition of bone while stimulating endocortical bone formation, with consequent narrowing of the medullary cavity. Testosterone enlarges bone size by apposition on the periosteal surface (207). Given the requirement of sex steroids for normal bone mineralization, the absence of sex steroids during early adolescence is likely to worsen BMD accrual (208-211). So far, only one study showing no improvement in bone mass accrual was observed during puberty in children with IBD. Furthermore, postpubertal patients showed significantly decreased aBMD, indicating suboptimal peak bone mass attainment during puberty (165). Although more studies are needed to confirm compromised bone mass accrual in children, puberty may offer a window of opportunity to improve BMD. It is recommended to monitor linear growth and growth velocity, and pubertal development regularly in children or adolescents with CD (145;205). Prompt intervention with GH and sex steroid replacement may have beneficial effects on bone health in children with CD.

In summary, children with IBD, particularly CD, are at risk for low bone mass because of the complex effects of malnutrition, glucocorticoid therapy, cytokines, low muscle mass, physical inactivity, impaired growth, and delayed puberty.

1.5 Therapeutic strategies to improve growth and bone in paediatric Crohn's disease

Based on the described pathophysiology of growth and bone impairment in children with CD it is intuitive that optimization of growth should rely on restoration of appropriate nutrition, minimizing inflammation, and avoiding long-term or frequent corticosteroid therapy. However, the unpredictability of the insult and the limited opportunity for improvement of growth, at least in some adolescents, pose a challenge for decision-making about therapies to improve growth and bone. The first step in management of growth impairment is early identification of growth impairment and close collaboration between the clinical teams overseeing the child’s care. This will help in providing prompt management for children in whom growth and puberty are significant clinical issues. The European Society of Pediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) and the European Crohn's and Colitis Organization (ECCO) guidelines published in 2014 outline the practical approach for treatment of children with CD related growth failure (Figure 1-10) (212). Currently, an expert opinion recommended that the prevention of bone disease is best accomplished by controlling inflammation using GC-sparing agents and careful attention to nutrition by providing adequate calcium and vitamin D supplementation to promote growth, puberty and encourage physical activity (145).

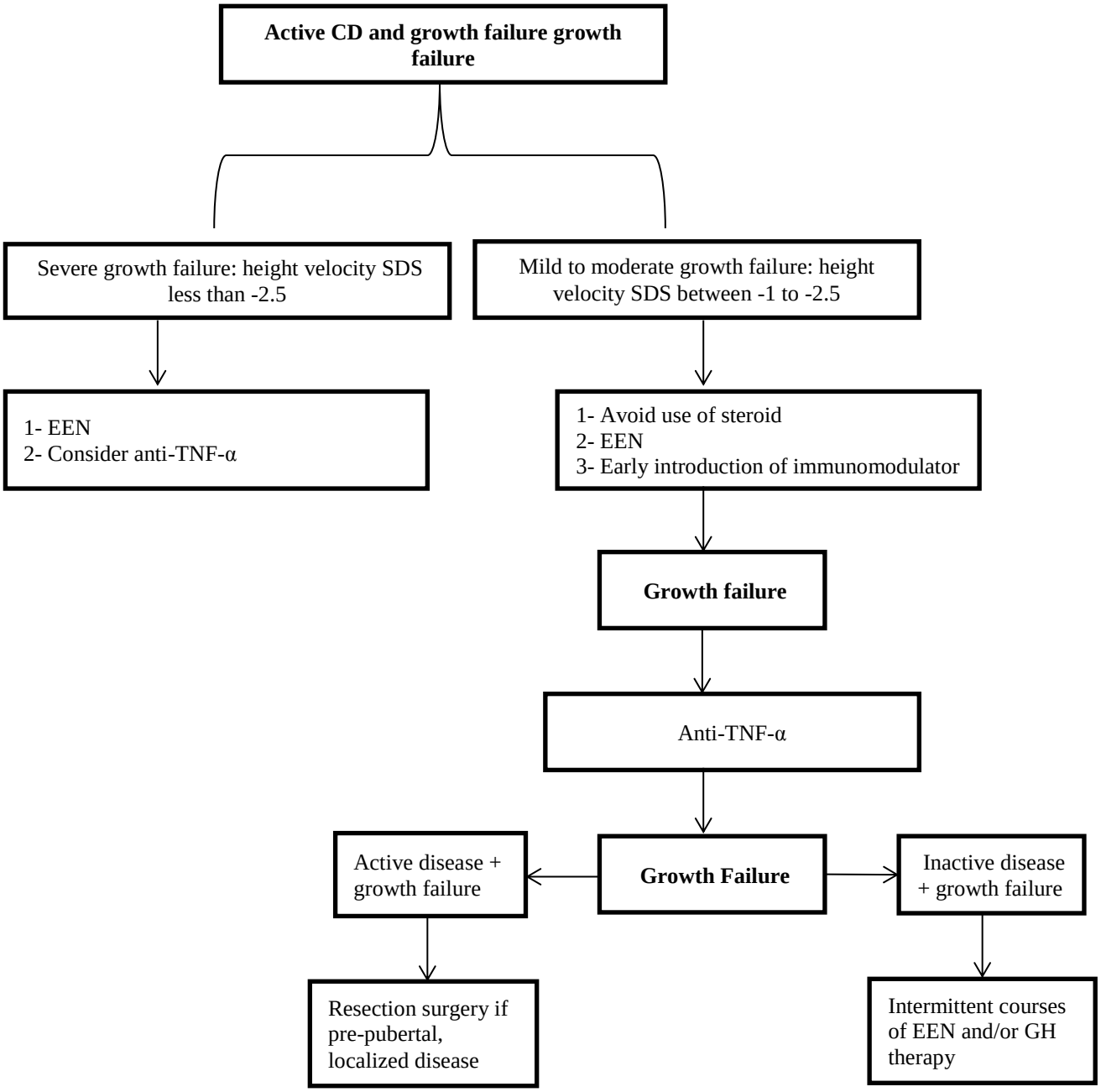

Figure 1-10: Schema for the therapeutic approach to children with active Crohn's disease and growth failure according to European Society of Pediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) and the European Crohn's and Colitis Organization (ECCO).
Adapted and modified from (212). EEN, exclusive enteral nutrition; anti-TNF-α, anti-tumour necrosis factor-α; GH, growth hormone

1.5.1 Improvement of nutritional status

Exclusive enteral nutrition (EEN) is increasingly considered as an effective initial method of controlling inflammation in children with active CD (20), with reported remission rates of about 80 % (213). By controlling inflammation, enteral nutrition may avoid the use of GC and their negative impact on growth and bone (214). Over the last few years, improvement of nutritional status by EEN has also been associated with a short-term improvement in growth velocity and a reduction in inflammation and disease activity. This suggests that some forms of nutritional regimens may lead to growth improvement by mechanisms other than simply improving nutritional status (215;216). A short-term study of 12 children with active CD treated with EEN for 6 weeks showed early improvement in inflammatory markers (erythrocyte sedimentation rate (ESR), IL-6) after 3 days and in IGF-1 after 1 week of starting nutritional treatment, without any significant change in nutritional status, measured by weight SDS, mid upper arm circumference and triceps skin-fold thickness (217). The authors linked this improvement in IGF-1 to the anti-inflammatory effect of EEN rather than nutritional resuscitation (217). A possible mechanism by which EEN may improve growth and bone is induction of mucosal healing and reduced mucosal production of pro-inflammatory cytokines (218;219). Fell et al (218) reported that 8 weeks of enteral nutrition in paediatric patients with active CD was associated with macroscopic and histological mucosal healing. A report where mucosal biopsies were obtained from patients with CD and controls incubated with elemental diet, casein, or whey, showed that elemental diet may have a direct inhibitory effect on mucosal inflammation by increasing the anti-inflammatory: pro-inflammatory cytokine ratio in CD (219). This anti-inflammatory effect may not be particularly due to the amino acid composition, as diets containing casein have similar anti-inflammatory effects.

While EEN has been shown to improve short- and medium term nutritional status, as well as short-term growth, velocity studies have not shown any long-term effect on height SDS /final adult height (220;221). However, Papadopoulou et al (214) and recently, Lambert et al (123) have shown a significant marginal benefit in height SDS at 1 and 2 years respectively, following a single course of EEN at diagnosis, compared with initial corticosteroid treatment. It may be concluded that where growth is concerned, EEN is superior to corticosteroid therapy in the short-term, and there is a need to investigate other therapeutic strategies to maximise height potential in paediatric CD.

To date only two studies demonstrate an effect of EEN on bone health in children with IBD. Wittin et al (222) showed that an 8-week EEN therapy normalized serum markers of bone turnover, however, other aspects of bone health or bone densitometry were not

considered. A recent small study in 10 children with newly diagnosed CD, induced remission with EEN and maintained patients on immunomodulatory treatment, followed up to 52 weeks, showed that the bone biomarkers, trabecular BMD SDS, and muscle CSA SDS measured at radius by pQCT improved within 3 months after starting with EEN, but with no further normalisation thereafter and no improvement seen in grip strength(223). Additional prospective studies that evaluate serum bone markers and bone density outcomes over time are required to assess the potential impact of EEN on this complication of active CD.

1.5.2 Control of inflammation

Introduction of steroid-sparing immunomodulators such as thiopurines and methotrexate should be considered early in children with elements of growth failure, thereby reducing the use of corticosteroids (224). Some promising data regarding the growth-promoting effect of anti-TNF-α are now available, and the limited evidence about its effect on bone will be discussed more thoroughly in this section.

1.5.3 Anti-TNF-α therapy in CD

As previously outlined, TNF-α is a pro-inflammatory cytokine commonly dysregulated in inflammatory diseases, including CD. Studies investigating the role of TNF-α in CD and other inflammatory diseases have led to the development of therapies designed to neutralise to block the effect of TNF-α in pathogenesis of CD. The proposed mechanisms of action of anti-TNF-α in CD include neutralization of soluble and transmembrane TNF-α, , induction of T cell apoptosis, apoptosis of transmembrane TNF-α expressing cells, downregulation of costimulatory molecules like CD40, reduced production of pro-inflammatory cytokines including IL1, IL6, and IL8, and induction of T-cell population with a regulatory phenotype (225). It is propose that modulation of the inflammatory immune response of anti-TNF-α may restore mucosal integrity and inhibit mucosal angiogenesis in gut mucosa.

Two anti-TNF-α agents have been approved for the induction and maintenance of remission in CD: infliximab (IFX) and adalimumab (ADA) (1;212;226). IFX is a chimeric (75% human and 25% murine sequences) IgG1 monoclonal antibody against TNF-α (227). IFX is typically used for the treatment of patients with moderately to severely acute CD and patients with fistulising CD, who have had an inadequate response to conventional therapy (1;212;226). IFX is also recommended as primary induction therapy for patients with penetrating disease (1;212;226). ADA is a recombinant human IgG1 monoclonal

antibody directed against TNF-α (227). ADA is typically used to decrease signs and symptoms of CD, and to achieve and maintain clinical remission in patients with moderate to severe acute luminal CD who have not responded well to conventional treatments (1;212;226). ADA is also used to treat patients who have lost responsivity to IFX, or who are allergic or unable to tolerate it (1;212;226). IFX is given as a scheduled intravenous infusion at a dose of 5 mg/kg at 0, 2 and 6 weeks, followed by maintenance infusions every 8 weeks. Dose escalation (to 10 mg/kg) and/or interval shortening (from 8 weeks to 6 weeks or 4 weeks) may be implemented as needed, and ADA administrated as subcutaneous injection 80 mg stat followed by 40 mg every two weeks (1;212;226). There are a number of side effects that may occur in anti-TNFα therapy. One of the most common problems of IFX includes its chimeric properties and the formation of antibodies (ATI) to IFX, or to the murine portion of the drug (228;229). Development of ATI is can cause adverse reactions such as acute infusion reactions, delayed hypersensitivity reactions and loss of drug response (228;229). Episodic treatment with IFX is associated with a higher likelihood of developing ATI than maintenance treatment, while concomitant use of immunosuppression therapy may reduce the risk of antibody formation (227;229). Clinical studies, registries, and case reports have highlighted that the risk of infection, particularly opportunistic infections (invasive fungal infections, reactivation of latent tuberculosis), in patients with CD treated with anti-TNF-α, which occurs at a higher incidence when concomitant immunosuppression is used. Thus, screening for latent tuberculosis infection before initiation of anti- TNF-α therapy is mandatory (227). The long-term safety of anti-TNF-α regimen is heralded by a potential risk for malignancy. The overall risk to develop malignancies, such as lymphomas, for children with CD is low, but higher than the general paediatric population. Very recent data on malignancy and mortality in paediatric CD have been provided by an ESPGHAN survey, which retrospectively evaluated the cases of malignancy or mortality among paediatric IBD patients over a 6-year period (230). A total of 18 malignancies were identified, mainly in CD patients. None of these cases had received anti-TNF-α. In children on biologicals, as of April 2008, 48 cases of malignancy including lymphoma and skin cancers, melanoma were identified by the FDA (31 following IFX use, 2 following ADA use, and 15 following etanercept use). This rate of malignancy was higher than background rates in the general U.S. paediatric population, but it is currently impossible to associate the risk to the anti-TNF-α and not to other concomitant medications (231).

1.5.4 Effect of anti-TNF-α therapy on growth in CD

Current published studies point to the growth-promoting effects of anti-TNF-α therapy in CD (Table 1-3; 1-4). However, its effect on optimization of linear growth seems to be variable. A number of studies have now reported that the use of IFX is associated with improved growth in children with CD, whose disease improves with the introduction of this drug, providing treatment is undertaken early enough prior to or during puberty (232-246). The open-label multicentre RCT (REACH study) to evaluate efficacy and safety of IFX therapy for treatment of moderate to severe paediatric CD in 103 children showed a positive impact of IFX on Ht SDS after 30 and 54 weeks of therapy (238). In addition, it seemed that the growth favourable effect was sustained for longer, but these conclusions may be somewhat guarded given that the number of children studied was 15, 10, and 4, at 2 years, 3 years and 4 years, respectively, with a greater beneficial effect in children with at least 1-year delay in bone age and those who were on GC at enrolment. Furthermore, growth outcome was not the main objective of the study, the subjects were not blinded and no pubertal data were available.

An increase in Ht SDS has also been observed after 6 months of therapy in treated subjects compared to an untreated cohort (233). The Assa group (232) recently elucidated that a tendency towards enhanced growth velocity was found in responders compared to non-responders. Furthermore, they highlighted the gender predilection based on the observation that only male responders showed a considerable amplify in height velocity during treatment. The authors supported the need for future study that may clarify sex-specific growth responses during IFX treatment of CD patients. However, growth data from this report need to be approached with caution as a large proportion of the cohort were also taking glucocorticoids, which may have impaired growth.

Interestingly, Walters et al (244) reported that IFX therapy is associated with improvement in mean HV SDS and Ht SDS in responder participants providing that IFX commenced before or during early puberty. Thus, they argued that the time of starting IFX is crucial in gaining control over disease when height is an issue. A comparable observation was reported in a small prospective study by Cezard and colleagues (234), where HV SDS was significantly enhanced in 10 children who had not finished their pubertal growth after one year of commenced IFX therapy, compared to one year before treatment. However, minimal amount of growth is expected for children in late puberty.

A recent retrospective study conducted by Malik et al (239) confirmed that median Ht SDS and HV SDS of a group of 21 children responding to IFX treatment improved significantly over a period of 1 year, even in children who remain in pre-pubertal stage for 6 months

prior and after commencing IFX, compared to non-responders whose growth rate remain unchanged. They verified that the growth-promoting effect of IFX in responders could be independent of reduction in glucocorticoid use and pubertal progress, emphasising the role played by controlling inflammation. This retrospective study also showed median HV in children who were receiving methorexate therapy before starting IFX was significantly increased after 6 months of commencing biologics, compared with a control group who did not receive methotrexate therapy. The authors suggest the beneficial effect of IFX on growth may be enhanced when a child is also receiving methotrexate, possibly due to their synergistic effect on disease activity.

Crombe et al (235) found a significant improvement in mean Ht SDS in a group of 41 CD children responders over a period of follow-up compared to 41 CD children who failed to respond to IFX therapy. In another retrospective study of 33 children with IBD, 21/33 of children experienced catch-up growth, and children in remission showed significantly higher Ht SDS compared with those with mild or moderate-severe disease activity, demonstrated that inflammation has an impact on growth (241). In a study published in 2014, children with matching baseline characteristics were compared in three treatment groups; 68 children receiving immunomodulators, 68 children receiving anti-TNFα, and 68 children with no immunomodulator, within three months of diagnosis for attaining clinical remission and promoting growth in children with CD (245). This study highlighted the benefit of early initiation of anti-TNF therapy for the resumption of growth in paediatric CD patients. In comparison to early immunomodulators or no immunomodulators only, the early anti-TNF treated group increased their Ht SDS at 12 months (a significant difference of +0.25). The study showed that children receiving early anti-TNFα therapy were significantly more likely to be in remission at 1 year compared with those receiving early immunotherapy or no immunotherapy (remission was based on glucocorticosteroids-free clinical remission (PCDAI<10) at 1 year after diagnosis without luminal resection). Interestingly, despite matching the three groups for important confounders (PCDAI, age, HtSDS, puberty, perianal disease, disease location, inflammatory biomarkers and deep ulceration on initial colonoscopy), the proportion of children on glucocorticosteroids in the immunomodulator only and no early immunomodulator groups were higher compared to those on early anti-TNFα at baseline and 12 months. Thus one possible explanation could be that glucocorticosteroids may have had a larger inhibitory effect on growth catch-up in the immunomodulator only and no early immunomodulator groups.

This was not found to be the case in one recent prospective study however, examining the effect of IFX on bone structure. They observed a significant improvement in Ht SDS in 74

CD children after 12 months of treatment, with a greater decrease in PCDAI associated with greater increase in Ht SDS and this improvement was not affected by GC exposure(237). However, the authors did not determine whether this improvement was independent of progress in puberty.

ADA has also recently been reported to be associated with an improvement in disease and growth. A retrospective review of 36 CD children, who started ADA at a median age of 14.7 years, demonstrated that clinical response to ADA treatment was associated with a short-term improvement in linear growth. Furthermore, this improvement was more likely in patients who were in early puberty and on immunosuppression, but was independent of steroid use (240). Alternatively, a recent retrospective study failed to confirm any beneficial effect of ADA on growth in 18 patients received ADA for 12 months (247). However, it should be noted that a large proportion of this cohort was already at late puberty by the time of starting anti-TNF-α.

From available evidence on the effect of anti-TNF-α on growth, it is considered the mainstay for therapy of growth failure in children with CD based on its ability to control inflammation. On the other hand, three retrospective studies failed to find any association between use of IFX and improvement of linear growth in CD children (236;242;246). Nevertheless, the retrospective nature of reports, relatively small sample size and lack of cohort pubertal data make it difficult to interpret the results with any certainty. A similar unfavourable outcome on growth was reported by the Pfefferkon group (88). The researchers prospectively investigated 176 newly diagnosed IBD children younger than sixteen with a Tanner score from 1 to 3, to evaluate growth outcomes with current regimes. They found that although disease activity significantly improved in 64 CD patient receiving IFX, height SDS remain impaired despite the improvement in HV SDS in many children with CD after 2 years following diagnosis (88). Malik et al (84) also found that slow growth and short stature remained a problem in 10% CD children, despite advances in treatment of paediatric CD and that this diverse group may benefit from specific growth-promoting treatment or novel therapeutic approaches.

In conclusion, despite the improvement on growth in some studies, there continues to be some controversy about the overall beneficial effects of anti-TNF-α therapy on growth. Prospective studies on anti-TNF-α on growth of CD children are needed as this will not only improve the knowledge of the effect of anti-TNF-α on growth but also might directed future research for elucidating mechanism and exploring novel therapeutic strategies to improve growth outcomes in CD patients.

Table 1-3: Published studies of anti-TNF-α on linear growth in children with CD (retrospective studies)

Reference	Study design	Size	Duration of study (months)	Age at start of therapy (years)	Tanner stage at start of therapy	Results
Pichler et al (2015)[247]	Retrospective	18	12	14.4 (5.3-19.1)	1-5	ΔHt SDS: baseline: -0.1 (-2.5,0.7); 12 months: 0.0 (-2.5,1.3) (p=NS)
Pichler et al (2014)[241]	Retrospective	33	12	14 (4-17.7)	1-4	ΔHt SDS : baseline: -0.2±0.2; 12 months: 0±0.4 (-2.6, 2.47) (p=0.04)
Church et al (2014)[248]	Retrospective	47	36	NK	1-2	Ht SD: Normalised in those received IFX within 18 months of diagnosis
Assa et al (2012)[232]	Retrospective	101	60	13.5±3.9	NK	HV: Only male responders showed increased HV (p=0.007)
Malik et al (2012)[240]	Retrospective	36	6	14.7 (11.3-16.8)	1-5	ΔHt SDS: baseline: -0.2 (-0.7,0.2); 6 months: 0.0 (-0.5,0.8) (p=0.005)
Malik et al (2011)[239]	Retrospective	28	6	13.1 (9.9-15.7)	1-3	Ht SDS: baseline: -0.1 (0.00,-0.1); 6 months: 0.05 (0.00,0.4) (p=0.001) HV: baseline: 2 (0.3,7.1); 6 months: 6.4 (2.3,9.1) in responders (p=0.004)
Crombe et al (2011)[235]	Retrospective	82	32	18.0 (16-21)	NK	Ht SDS: Only responders showed an improvement baseline: -0.57±1.1; 32 months: -0.25± 0.99 (p=0.04)
Sinitsky et al (2010)[242]	Retrospective	16	12	13.0± 4.2	NK	Ht SDS: baseline: -0.5(-2.5, -0.6); 12 months: -0.8 (-2.8,-1.4) (p=NS)
Diamanti et al (2009)[236]	Retrospective	14	10	13.0 (11.5-15)	NK	Ht: Case, baseline: 146.1 cm±0.2; 10 months: 147.8 cm±0.3 Control, baseline: 147.4 cm±0.2; 10 months: 148.5 cm±0.1(P=NS)
Walters et al (2007)[244]	Retrospective	27	12	14.3	1-5	HV SDS (Tanner 1-3): baseline: -2.8±1.7; 12 months: 1.1±3.6 (p<0.001) HV SDS (Tanner 4-5): baseline: 0.46±3.4; 12 months: 0.68±1.1 (p=NS)
Wewer et al (2006)[246]	Retrospective	24	36	≤17	NK	HV: baseline 0.35cm /month; 36 months: 0.38 cm/month (p=NS)

ΔHt SDS, change in Ht SDS; Ht SDS, standard deviation from the mean for height for normal children for the same age and gender; HV, height velocity (cm/year); HVSDS, standard deviation from the mean for height velocity for normal children for the same age and gender; NK, not known; NS, not significant. Age is represented as mean (±SD) or median (range)

Table 1-4: Published studies of anti-TNF-α on linear growth in children with CD (prospective studies)

Reference	Study design	Size	Duration of study (months)	Age at start of therapy(years)	Tanner stage at start of therapy	Results
Griffin et al (2015)[237]	Prospective	74	12	14 (5-21)	1-5	Ht SDS: baseline: -0.2±0.2; 12 months: 0.0 ±0.2 (p=0.04)
Walters et al (2014)[245]	Prospective comparison	68 each group	12	14 (5-21)	1-3	ΔHt SDS: 0.14±0.4 on antiTNF-α (p=0.002), -0.02±0.4 on early immunomodulator therapy only (p=0.6), -0.06±0.4 on neither (p=0.2)
Hyams et al (2011)[249]	Prospective	20	48	13.3±2.5	NK	Ht SDS: baseline: -1.64; 24 months: 0.82; 36 months: 1.01; 48 months: 1.56
Thayu et al (2008)(243)	Prospective RCT	103	12	13.3±2.5	NK	Ht SDS: baseline: -0.76± 1.24; 12 months: -0.49 ± -0.50 (p<0.002)
Hyams et al (2007)[238]	Prospective RCT	20	12	13.3±2.5	NK	Ht SDS: baseline: -1.5; 12 months: 0.5 (<0.001)
Borreli et al (2004)[233]	Prospective	18	6	13.0 (6-18)	NK	Ht SDS (treated group): baseline: -0.99±0.62; 6 months: -0.74±0.71(p<0.01) Ht SDS (untreated group): baseline: -0.86±0.42; 6 months: -0.83±0.40 (p=NS)
Cezard et al (2003)[234]	Prospective	10	12	15.0±2.0	NK	HV SDS: baseline -0.45 (-1; 1.3); 12 months: 0.5 (0; 1.3) (p=0.004)

ΔHt SDS, change in Ht SDS; Ht SDS, standard deviation from the mean for height for normal children for the same age and gender; HV, height velocity (cm/year); HV SDS, number of standard deviation from the mean for height velocity for normal children for the same age and gender; NK, not known; NS, not significant; RCT, randomised controlled trial. Age is represented as mean (±SD) or median (range).

1.5.5 Effects of anti-TNF-α therapy on the GH-IGF-1 axis

There are only a limited number of studies that have investigated the blockade of TNF-α on the GH-IGF-1 axis (250-252). In a murine colitis model, TNF-α neutralization up-regulated liver GHR abundance and GH signalling and serum IGF-1 levels (250). Preliminary data from a controlled study of adults with active CD showed an opposite effect of treatment with IFX on the GH resistance occurring in active CD (251;252). Vespasiani et al (251) studied 14 adult IBD patients with low levels of IGF-I and IGFBP-3 treated with IFX. IGF-1 and IGFBP-3 levels did increase during induction and IGF-1 reached values comparable with those in healthy controls, however, the study failed to demonstrate normalization of IGF-1 levels during maintenance therapy. The derangement of the GH-IGF-1 axis during maintenance therapy is difficult to elucidate, however, the authors hypothesised that during this period some degree of subclinical mucosal inflammation might persist. The study time points were 0, 2, 6, 12, and 20 weeks after IFX infusion. The authors demonstrated that GH-IGF-1 axis derangement occurs in patients presenting a satisfactory nutritional status, comparable with that in controls, thus suggesting a predominant role of systemic inflammation rather than a reduced protein calorie intake, and the result seen is likely related to suppression of the systemic inflammation. The short-term effect of anti-TNF therapy on IGF-1 is supported by the report by Eivindson et al (252) when 13 adult patients with therapy-refractory CD were treated with IFX and age- and gender-matched with 10 controls. The IGF system and markers of inflammation were studied at baseline, on days 2-5 and after 1, 4, and 8 weeks. Treatment with IFX normalized circulating levels of total IGF-I and IGFBP-3, and partially normalized IGFBP-2, whereas free IGF-I remained suppressed. However, comparable data is not yet available for any paediatric cohort. An improved understanding of the systemic GH-IGF-1 axis and how it is affected in chronic inflammation will lead to an improved rationale for developing therapeutic regimens that can improve growth and bone health in children with CD.

1.5.6 Effect of anti-TNF-α therapy on bone health

Consistent with the hypothesis that TNF-α contributes to bone deficits in CD, several studies have demonstrated the significant beneficial effect of monoclonal anti-TNF-α on bone metabolism in adults with CD, with some limited evidence for the effect of anti-TNF-α on bone mineral density (253). To date, only five studies have been published that investigate the effect of anti-TNF-α on either BMD or bone metabolism and results were

conflicting (Table 1-5) (186;237;241;243). The first study to suggest a beneficial effect for IFX on bone density in children with CD was seen in 2007 by Paganellli et al (186). They observed that lumbar spine BMAD, measured by DXA, in 10 children with CD treated with IFX (-1±0.8) was higher than for 25 children with CD not treated with IFX (-1.8±0.8). They matched for age, sex, puberty and age at diagnosis. However, in this study the authors did not look at markers of bone turnover.

Thayu et al (2008) were the first to observe a beneficial effect with anti-TNF-α on bone metabolism in paediatric CD patients (243). In this study, 112 children aged between 6-17 years, with moderate to severe CD disease based on PCDAI (>30), received IFX induction (5 mg/kg/dose) at 0, 2, 6 and 10 weeks. Responders (n, 103) were randomised to IFX every 8 or 12 weeks maintenance therapy. For the purpose of analysis of bone biomarkers, data was limited to 103 children. The biomarkers of bone metabolism; BSAP, P1NP, urinary CTX-1 and DPD were measured at baseline, and at 10 weeks. The PCDAI was also measured at baseline, 10 and 54 weeks as markers of biological response. In this study the authors observed that both BSAP and P1NP increased during 10 weeks, with median increases of 87% and 103% respectively. The change in BSAP was associated with reduction in PCDAI at 10 weeks when adjusted for bone age, gender, and height. The authors suggest that IFX may have beneficial effect on bone formation through primary inhibition of TNF-α. The CTX-1 and DPD also increased at 10 weeks, but the magnitude of changes (18%, 23%) was less than the change in formation markers. Biomarkers of bone resorption were not associated with PCDAI. This is the largest paediatric study showing IFX treatment is associated with an increase in biomarkers of bone formation and resorption; however, this study did not related changes in bone markers to subsequent changes in bone mass. In addition, because of the short duration of the study, questions remains as to whether this improvement is sustained over a longer period of time, and if there is any associated improvement in BMD.

A retrospective study of the effect of IFX on bone formation (BSAP, Osteocalcin) and resorption markers (CTX), as well as on LS-BMD and bone mineral apparent density (BMAD), over a period of 1 year in 33 children with moderate to severe IBD (24CD/9 IBDU), failed to find any improvement in these parameters (241). The only confounder factor taken into account was clinical response to therapy based on PCDAI, and indeed they found a subgroup of children with BMD SDS<-2, had higher PCDAI. Although this was a 1-year study, not all children received IFX for the full duration. In addition, they did not categorise the patients according to steroid use despite a large proportion of children using corticosteroid at baseline (64%). Although they showed improvement in their

growth, this was not reflected in biomarkers of bone turnover. In a follow-up, data was also reported regarding the effect of ADA on bone health in 18 CD children. The authors reported no significant influence on bone biomarkers after 12 months from the start of ADA. LS-BMD SDS and BMAD SDS remained static in 6 children who had complete remission, and LS-BMD SDS significantly deteriorated in 12 children who had mild or moderate-severe disease at 12 months after starting ADA (254). The authors claim a protective role for IFX and ADA in preventing bone deterioration (241;254).

Recently, the first study reporting bone biomarkers, pQCT measure of tibia bone density and structure, over 1 year after initiation of IFX therapy in 74 children with CD was published (237). At baseline, CD children had lower trabecular BMD, cortical area and muscle area SDS compared with reference data. PCDAI reduced during 10 weeks from induction and was associated with a significant increase in trabecular BMD, cortical area, and muscle area SDS over 12 months. In multivariate analysis, improvement in linear growth, assessed by change in tibial length, was associated with greater increase in cortical area in CD. The use of IFX led to mild improvement in trabecular BMD associated with improvement in bone turnover at follow-up. The improvement in bone parameters was independent of GC use over the study period. The authors conclude that anti-TNF-α during growth and development is associated with improvements in trabecular BMD and cortical structure, and this improvement was greater in younger and growing children, suggesting a window of opportunity for treatment of bone deficient. However, the improvement seen following the IFX in this group of children may largely reflect growth, as the more significant improvement was seen in cortical bone area with a persistent deficient in trabecular BMD which remained relatively low (1 SD below mean) after 1 year of therapy.

Taken together, the limited data available thus far demonstrates contradictory results and the extent of benefit for anti-TNF-α with regard to bone health and architecture is unclear, this warranting further research in this area.

Table 1-5: Published studies of the effect of anti-TNF-α on bone health in children with Crohn's disease

Reference	Age at start of study (years)	Duration of disease (years)	Study design	size	Bone markers	BMD (methods)	Time points measured	Major conclusion
Paganelli et al (2007) [186]	NA	5.2±3.7	Case- controlled	10 on IFX vs 25 IFX naïve	NA	LS-BMAD SDS measured by DXA	IFX for 2 years	BMAD in 10 children with CD who treated with IFX was higher than 25 children with CD IFX naïve.
Thayu et al (2008)[243]	13.3 ±2.5	1.9 ±1.4	Prospective	103 (CD)	BSAP, P1NP urinary CTX-1 DPD	NA	At baseline and 10 weeks	Significant increase in median BSAP, P1NP, CTX, DPD from baseline to week 10.
Pichler et al (2014)[241]	14 (4,17.7)	3.2	Retrospective	33 (24 CD/9 IBDU)	BSAP, Osteocalcin, CTX	LS-BMD and BMAD SDS measured by DXA	At baseline and 1 year	Failed to find any improvement in all these parameters and they remained fairly static during study periods.
Pichler et al (2015)**[254]**	14.4 (5.3, 19.1)	6.6	Retrospective	18 (CD)	BSAP, Osteocalcin, CTX	LS-BMD and BMAD SDS measured by DXA	At baseline and 1 year	No significant influence on bone biomarkers after 12 months of start of ADA. LS BMD SDS and BMAD SDS remained static in 6 children who had complete remission and significantly deteriorated in 12 children who had mild and moderate to severe disease at 12 months after start ADA.
Griffin et al (2015)[237]	14 (5, 21)	2.1 (0.02–9.7)	Prospective	74 (CD)	BSAP, CTX SDS	Tibia bone density and structure measured by pQCT	At baseline, 10 weeks, 6 months and 1 year	Significant increase in trabecular BMD, cortical area and muscle area SDS over 12 months. BSAP and CTX SDS were low at baseline and significantly increased at 10 weeks and this improvement sustained over 12 months.

CD, Crohn's disease; IBDU, inflammatory bowel disease unclassified; BMD, NA, not available; bone mineral density; BMAD, bone mineral apparent density; LS, lumbar spine; DXA, Dual-energy X-ray absorptiometry; pQCT, peripheral quantitative computed tomography; P1NP; Procollagen type 1 Amino terminal Propeptide; BSAP, bone specific alkaline phosphatase; DPD, deoxypyridinoline; CTX, C-terminal cross-linking telopeptides.

1.5.7 Effect of human recombinant growth hormone (rhGH) therapy on growth in CD

GH is a potent anabolic agent known to stimulate linear growth, osteoblast activity and protein synthesis. In the UK, rhGH is licensed in children for treatment of GH deficiency, Turner syndrome, chronic renal insufficiency, Prader Willi syndrome and children who are born small for gestational age (255). Paediatric studies involving use of rhGH children with chronic inflammatory diseases such as juvenile idiopathic rheumatoid arthritis(JIA) and cystic fibrosis show significant improvement of linear growth (256;257).

Table 1-6 summarises studies on the effect of rhGH therapy on linear growth in children with IBD (258-263). The first report that investigated the effect of rhGH on linear growth of IBD children was conducted in 1974 (263). In this study rhGH was used at a dose of 9 mg/week for 6 months in 3 adolescents with IBD and poor growth, and showed no improvement in their height velocity compared to their prior growth rate. However, two of the participants had a bone age of 16 years and 18 years and would have completed most of their growth prior to therapy. In a preliminary study of 10 subjects with CD who received rhGH at dose of 0.05 mg/kg/day for a variable duration (7 for 1 year, 3 for 6 months), the 7 children receiving therapy for 1 year showed significant improvement in their height standard deviation (Ht SDS) from -1.7±0.5 at baseline to -1.2±0.6 by the end of study (p=0.03) (261). Eight of the ten children in the study were prepubertal at time of commencement (n, 1; tanner stage IV, n, 2; tanner stage I). No data was given on puberty at follow up and the dose of GC remained unchanged during period of study (0.27 mg/kg/day).

In another RCT in 7 IBD children with growth impairment, participants were randomised to either rhGH (0.05mg/kg/day) or placebo (Phase 1 for 1 year, in phase 2 all children received rhGH for further 1 year). Investigators show no significant difference in mean Ht SDS between the two groups (258). All patients except one were not on GCs during the study period. Six were on early puberty and one was prepubertal and remained so during the study period.

In 2008, an open labelled controlled trial in 8 children with IBD and short stature, using a rhGH dose of 0.043mg/kg/day for 1 year, showed a significant increase in the height SDS, weight SDS in rhGH treated group ($p<0.01$, $p<0.01$ respectively) compared to the control group of 24 individuals (260). The mean PCDAI in rhGH group was 21.9±21.2 at baseline and 13.1±7.5 after 1 year of treatment.

Recently, a randomized controlled trial was performed in two UK tertiary paediatric hospitals (262). In this study, 22 participants with paediatric IBD were randomly allocated

to either rhGH (0.067mg/kg/day, n=11) or no rhGH (n=11) for six months. They found that HV increases (from 4.5 cm/year (0.6, 8.9) at baseline to 10.8 cm/year (6.1, 15) at 6 months p=0.003) and Ht SDS increases (from - 2.8 (-4.2, -1.5) at baseline to -2.4 (-3.7, -1.2 versus P = 0.003) were significant relative to baseline for the treated group, but not the untreated group. This study also demonstrated a considerable improvement in biomarkers of growth. Median IGF-1 SDS adjusted for bone age improved from -2.9 (-3.7, 2.9) at baseline to -0.1 (-2.7, 2.2) after 6 months in the rhGH group (p = 0.04) whilst it remained unchanged in the control group. The use of steroids in both groups was scanty throughout the period of study, and puberty stayed almost unchanged.

This study also revealed no significant change in disease activity or bone age in both groups at baseline and six months. This was the first study to also assess insulin sensitivity and showed that despite scant use of glucocorticoids, children with IBD were insulin resistant at baseline and the degree of resistance increased in those on rhGH. This increase in insulin resistance was not associated with impaired glucose tolerance as none of the children who had an oral glucose tolerance test 6 months after receiving rhGH injections was found to have asymptomatic diabetes mellitus.

Similar results were reported by Denson and associates (259) who investigated whether rhGH therapy improving disease process (at dose of 0.075mg/kg/day) at 12 weeks. After 12 weeks, 18 subjects aged between 7-18 years who experienced a clinical response received rhGH for 52 weeks, and linear growth was assessed. They found mean height SDS (-1.1(-1, -0.6) at baseline) and HV SDS (-1.3 at baseline) considerably increased to -0.4 (-1, -0.2), p=0.04, and 2.4, p=0.000, respectively, compared to baseline in the GH group. Moreover, a rise in fasting insulin level was documented without impairment in glucose level. The Mauras group (261) found no significant change in oral glucose tolerance test with a trend towards higher fasting insulin concentrations during combined prednisone/ rhGH therapy.

These initial results suggest that rhGH may have a positive effect on growth over the short-term and is not associated with any deterioration in the disease status. However, there is a need to be judicious in the use of this drug especially if used for extended periods given that there are scant data for efficacy and safety over the longer term. Children with JIA, and especially those who receive GC, may develop more profound impairment of glucose tolerance (264). Given that inflammation may itself alter insulin sensitivity, there is a need to perform regular assessment of insulin sensitivity and glucose tolerance in children who receive rhGH.

Table 1-6: Published studies of rhGH therapy in children with Crohn's disease

Reference	Study Design	Group	rhGHdose (mg/kg/day)	Duration of rhGH therapy (months)	Duration of study (months)	Primary end point	Results
Wong et al (2011)[262]	RCT	11 rhGH 11control (10CD/1UC)	0.067	6	6	Linear growth	HV:baseline: 4.5 cm/yr (0.6,8.9); 6 months: 10.8(6.1,15), p=0.003 Ht SDS: baseline: -2.8 (-4.2,-1.5); 6 months: -2.4(-3.7,-1.2), p=0.003No significant change observed in control group
Denson et al (2010)[259]	Prospective	18 CD	0.075	12	12	Disease activity	Ht SDS: baseline: -1.1(-1.6,-0.6); 12 months: -0.4(-1,0.2), p=0.004
Heyman et al (2008)[260]	Open label prospective	8 CD rhGH 24 no rhGH	0.043	12	12	Linear growth	rhGH group: HV: baseline: 3.00±1.39 cm/yr; 12 months: 8.32±3.20, p=0.01 Δ HtSDS 0.76±0.38, p<0.01 Comparison group:HV: baseline: 4.00±1.39 cm/yr; 12 months: 4.9±3.20, p=0.20 Δ Ht SDS 0.16±0.40, p=0.07
Calenda et al (2005)[258]	Placebo RCT	3 rhGH 4 placebo	0.05	12	12	Linear growth	rhGH: Δ Ht SDS +0.13 Placebo: Δ Ht SDS +0.23 No change in linear growth
Mauras et al (2002)[261]	Open label prospective	9CD, 1 IC	0.05	6	6-12	Linear growth	HV: baseline: 3.5±0.4 cm/yr; 6 months: 7.4±1.1, p=0.001

RhGH: Recombinant growth hormone; RCT: Randomised controlled trial; CD: Crohn's disease; UC: Ulcerative colitis; HV: Height velocity (cm/year); Ht SDS: Number of standard deviation from the mean for height for normal children for the same age and gender.

1.5.8 Effect of rhGH on bone health

Evidence from JIA studies on the effect of rhGH on bone mass have shown that rhGH therapy may have a beneficial effect on bone health (265-267). There are only two studies investigated the effect of rhGH therapy in children with CD (261). In a pilot study by Mauras et al (261) in 10 children with CD, serum markers of bone formation and resorption were not significantly affected by rhGH treatment, except for a significant increase in mean BSAP from 39±8 at baseline to 54±12 at 4 months and a significant decrease in percent fat mass (-3.5%) as measured by DXA scanning after 4 months of rhGH therapy. In an open label study in CD, the authors observed that rhGH therapy for 12 months was associated with a mean increase in LS-BMD SDS for age of 0.3 ±0.3 and mean percent body fat (by DXA scan) was decreased by 2.55±2.58 compared to baseline. Serum alkaline phosphatase level was increased from 127.3±31.4 at baseline to 200.0 ±71.3 at the end of GH treatment (260). In both these studies the increase in bone alkaline phosphatase may reflect linear growth. Furthermore, DXA bone outcomes were not adjusted for growth or puberty and both were short-term studies. Further evidence on the effect of rhGH on bone health in children with inflammation in general, and in CD in particular, are still needed.

1.5.9 Effect of rhGH on disease activity

Other than a growth promoting effect, GH therapy may have an effect on disease status as reported in number of human and animal studies (259;268;269). Slonim et al (269) conducted a randomised controlled trial to investigate the effect of rhGH on disease status in 37 adult patients with moderate to severe CD, allocated to either receive rhGH (n=19) loading dose, 5 mg/day subcutaneously for one week, followed by a maintenance dose of 1.5 mg/day, or placebo (n=18). They found that the Crohn's Disease Activity Index score decreased by mean of 143±144 points in the rhGH group, as compared with a decrease of 19±63 points in the control group (p=0.004) suggesting a beneficial effect of GH in improving disease activity. In a paediatric study by Denson et al (259) 65% of CD children on GH therapy and corticosteroid achieved clinical remission, measured by PCDAI, by 12 weeks, compared to only 20% of patients in control group (p= 0.03). At week 12, 40% of participants in rhGH and corticosteroid arm achieved steroid-free remission compared to 20% of subjects in control group. There was a trend towards improvement in endoscopic disease activity in the rhGH group at week 12; however, this did not reach statistical significance. Furthermore, other markers of disease activity such as faecal calprotectin and

ESR were similar in both groups. Although disease activity index improved in this study, Ht SDS/HVSDS is a component of the paediatric crohn's disease activity index (PCDAI), and the use of PCDAI to assess disease in studies promoting growth may be flawed by a concurrent improvement in growth rate. It is possible that the lower PCDAI in the rhGH group in that study merely reflects improvement in linear growth, independent of reduction of inflammation. The possibility that rhGH may improve inflammation directly in paediatric CD remains an open question.

Data from Wong et al(262) reported no significant difference in disease status, based on an abbreviated paediatric CD activity index (APCDAI) that utilised subjective symptoms and physical examination after six months, between the intervention and control groups. Additionally, other markers of disease activity (such as ESR, CRP, Hb, HCT, albumin and inflammatory cytokines: TNF, IL1 and IL6) were similar between the two groups. However, in this study the children were in remission or had mild disease activity so it is difficult to conclude effect of rhGH therapy on disease activity from this study.

1.6 The rationale for the present work and aims

Impaired growth and bone health are documented detrimental consequences in children with IBD, particularly CD. The underlying mechanism is multifactorial and includes poor nutrition, inflammation, and the prolonged use of steroids. Assuming a multifactorial mechanism, several therapeutic approaches have been proposed that may aid in improving growth and bone health in children with CD. However, currently there is inconsistent evidence regarding the efficacy of available therapeutic agents in this cohort. Therefore, the overall aim of this thesis is to explore the effect of some therapeutic strategies particularly anti-TNF-α and rhGH therapy on growth, the GH-IGF-1 axis, and biomarkers of bone turnover as well as bone mineral density in children with CD. Furthermore the following specific hypothesis and aims are proposed under the scope of this thesis:

Impact of Anti-Tumour Necrosis Factor Therapy on Linear Growth, Insulin like Growth Factor Axis and Bone Turnover Markers in Childhood Crohn's Disease (Chapter-2).

Hypothesis: Childhood-onset CD is associated with impaired growth, disturbance in IGF-1 axis, and bone turnover that is potentially related to TNF-α. We hypothesized that use of anti-TNF-α is associated with improvement in linear growth, IGF-1 axis and biomarkers of bone formation.

Aim: To examine linear growth, growth biomarkers and bone turnover in a cohort of children and adolescents with CD at time of, and 12 months after, initiation of treatment with anti-TNF-α.

Persistence of Musculoskeletal Deficits in Paediatric Crohn's Disease Following Anti-Tumour Necrosis Factor Therapy (Chapter-3).

Hypothesis: TNF-α contributes to deficits in BMD and bone geometry and these parameters would improve during anti-TNF-α therapy.

Aim: (1) To assess change in bone density and structure in a cohort of children and adolescents with CD at time of, and 12 months after, initiation of treatment with anti-TNF-α.

(2) To explore association of IGF-1 axis, cytokines and muscle, with bone density in children with CD.

The Sustained Effects of Recombinant Human Growth Hormone Therapy on Linear Growth in Children with Crohn's Disease (Chapter-4).

Hypothesis: Some children with CD continue to grow slowly despite anti-TNF-α therapy. Poorly growing children with CD may exhibit combined abnormalities of functional GH insufficiency as well as GH resistance. There may, therefore, be a rationale for considering treatment with high dose rhGH in children with CD and ongoing growth retardation.

Aims: To investigate the effects of prolonged rhGH therapy on linear growth and insulin sensitivity in children with CD.

Recombinant Human Growth Hormone Therapy in Children with Crohn's Disease: Impacts on Bone Health (Chapter-5).

Hypotheses: In accordance with an anabolic effect of GH, rhGH therapy may help to reduce detrimental effects of CD on bone health and muscle in children with quiescent CD.

Aim: To determine the effects of prolonged rhGH therapy on BMD by DXA of total body and lumbar spine.

Assessing the Feasibility of Injectable Growth-Promoting Therapy in Crohn's Disease (Chapter-6).

Hypothesis: Dimension in attitude toward RCT in relation to height in both patients with CD and their families.

Aim: To assess the feasibility of RCT of injectable forms of growth promoting therapy; and to survey the attitudes of children with CD and their parents to it.

Chapter Two

Impact of Anti-Tumour Necrosis Factor Therapy on Linear Growth, Insulin Like Growth Factor Axis and Bone Turnover Markers in Childhood Crohn's Disease

2.1 Abstract

Background: Childhood onset CD is associated with impaired growth, disturbance in IGF-1 axis, and bone turnover that is potentially related to TNF-α.

Aims: To examine linear growth, growth biomarkers and bone turn over in a cohort of children and adolescents with CD at baseline and 12 months after initiation of treatment with anti-TNF-α.

Design and participants: Prospective longitudinal study of 19 (12 male) participants with CD, aged 11.2, 17.2 (median 14.9) years, completing a 12 months study.

Main outcomes: Anthropometric data were obtained at baseline and 12 months. IGF-1, IGFBP-3, ALS, BSAP and β-CTX performed at baseline, 2 weeks, 6 weeks, 6 months and 12 months after initiation of anti-TNF-α. All were adjusted for bone age and gender.

Results: wPCDAI decreased significantly from to 40 (0, 95) at baseline to 17.5 (0, 70) during the 2 weeks following induction (p=0.004 vs. baseline). Reduction in wPCDAI was associated with a subsequent improvement in height velocity (p=0.008 vs. baseline) and change in height SDS (p=0.015 vs. baseline) at 12 months in those who still had potential to grow. At baseline, IGF1 SDS was +0.1 (-3.8, 2.1) (p=0.80 vs. zero), IGFBP3 SDS +1.4 (-2.9, 3.0) (p=0.02 vs. zero) and ALS SDS -0.9 (-2.2, 1.5) (p<0.0001 vs. zero). IGF1 SDS was <-2.0 in 5 (26%) at baseline and none at 12 months. At 12 months, IGFBP3 SDS was +0.5 (-1.1, 2.9) and not different from zero (p=0.09). ALS SDS was <-2.0 in 1 (5%) at baseline and none at 12 months. BSAP SDS of -1.7 (-3.6, 1.0) (p<0.0001 vs zero) and β-CTX SDS of -1.1 (-2.6, 0.4) (p=0.01 vs zero) at baseline reflect a low bone turnover state. BSAP SDS increased significantly at 6 weeks (p=0.01), whereas β-CTX remained unchanged, reflecting a net increase in bone formation.

Conclusion: Comprehensive assessment of markers of bone turnover and the IGF-1 axis in children starting anti-TNF-α therapy for CD shows that other than depressed ALS, markers of GH axis were not particularly abnormal in the majority. Bone formation was also low at baseline. With therapy and improvement in disease, the height of the patients improved and this was associated with increased bone formation but no clear change in markers of the GH-IGF axis.

2.2 Introduction

Looking at the literature, it is obvious that children with IBD, and more specifically, CD at risk of poor growth and short stature; both at diagnosis (84;88) and remaining short as adults (90;91).

Various aetiologies have been proposed to be the cause of poor growth in CD that involves abnormalities of the GH-IGF-1 axis and sex steroid, malnutrition, inflammation, and glucocorticoid therapy (270). Clearly all those factors are interlinked, although it is apparent that the inflammatory process mediated by pro-inflammatory cytokines such as TNF-α and interleukins (IL) which are commonly elevated in active CD (10) are central modulating factors. Hence, the first strategy to improve or restore growth is to control inflammation.

The monoclonal anti TNF-α chimeric antibody (IFX) induces and maintains remission in moderate to severe disease and is first line medication in refractory CD (227;249). Loss of the IFX effect can usually be effectively treated with humanised anti TNF-α agent (ADA) (271;272). However, the effect of anti-TNF-α on improvement of linear growth seems to be variable (232-246).

To date, data describing the effects of in vivo and in vitro TNF-α inhibition on growth biomarkers in patients with CD are still limited (250-252). Currently, there is no published evidence about IGF-1 axis in children with CD following TNF-α therapy. Therefore, improvement of disease activity by directly reducing inflammatory cytokines may lead to improvement in linear growth of these children mediated by changes in the GH-IGF axis.

The aim of this prospective study was: To assess linear growth, growth biomarkers, bone turnover in a cohort of children and adolescents with CD at the time and 12 months after initiation of treatment with anti-TNF-α.

2.3 Methods

2.3.1 Study population

This is a prospective cohort study of growth, growth axis and bone metabolism. Between June 2009 and June 2014, children with IBD who had not completed growth (Tanner stage 1-4) attending Royal Hospital for Sick Children (RHC), Yorkhill Glasgow and commencing treatment with anti-TNF-α therapy were approached to see if they satisfied the inclusion criteria. All participants were approached prior to starting anti-TNF-α therapy and provided with information sheets. These participants were patients with long-lasting disease in clinical relapse who failed to reach inactive disease despite conventional therapy. Participants were excluded if received anti-TNF-α therapy in the previous 6 months; pregnancy; and medical illnesses or therapies unrelated to CD that could potentially affect bone, nutrition, or growth such as kidney disease, seizure disorder, diabetes, or chronic liver disease. During the period 27 children with IBD (n, 25 CD: n, 1 UC: n, 1 IBDU started anti-TNF-α therapy (IFX/ADA). 25 of the 27 participants were recruited (n, 24 IFX and n, 1 ADA); two participants declined to take part in the study (n, 1 IFX, n, 1 ADA); two participants decided not to start the study after consenting; one participant was lost to follow up after 6 weeks as she moved to adult care and 3/25 were non responders and stopped IFX after 6 weeks. Therefore, 19 (12 male) participants, aged 11-17 years CD, were included for analysis in this study (Figure 2-1). Disease phenotype was classified using the Montreal criteria (5). None of the participants were on growth or puberty promoting treatments during the period of study. The diagnosis reached in each participant was according to standard criteria after small bowel imaging, endoscopy and colonoscopy with multiple biopsies, and other investigations, as indicated in the revised porto criteria (3).The study protocol was approved by the local research ethics committee (LREC) Reference Number: 09/S0703/58 and the research and development office. Informed consent was obtained from patients and the parents or guardians.

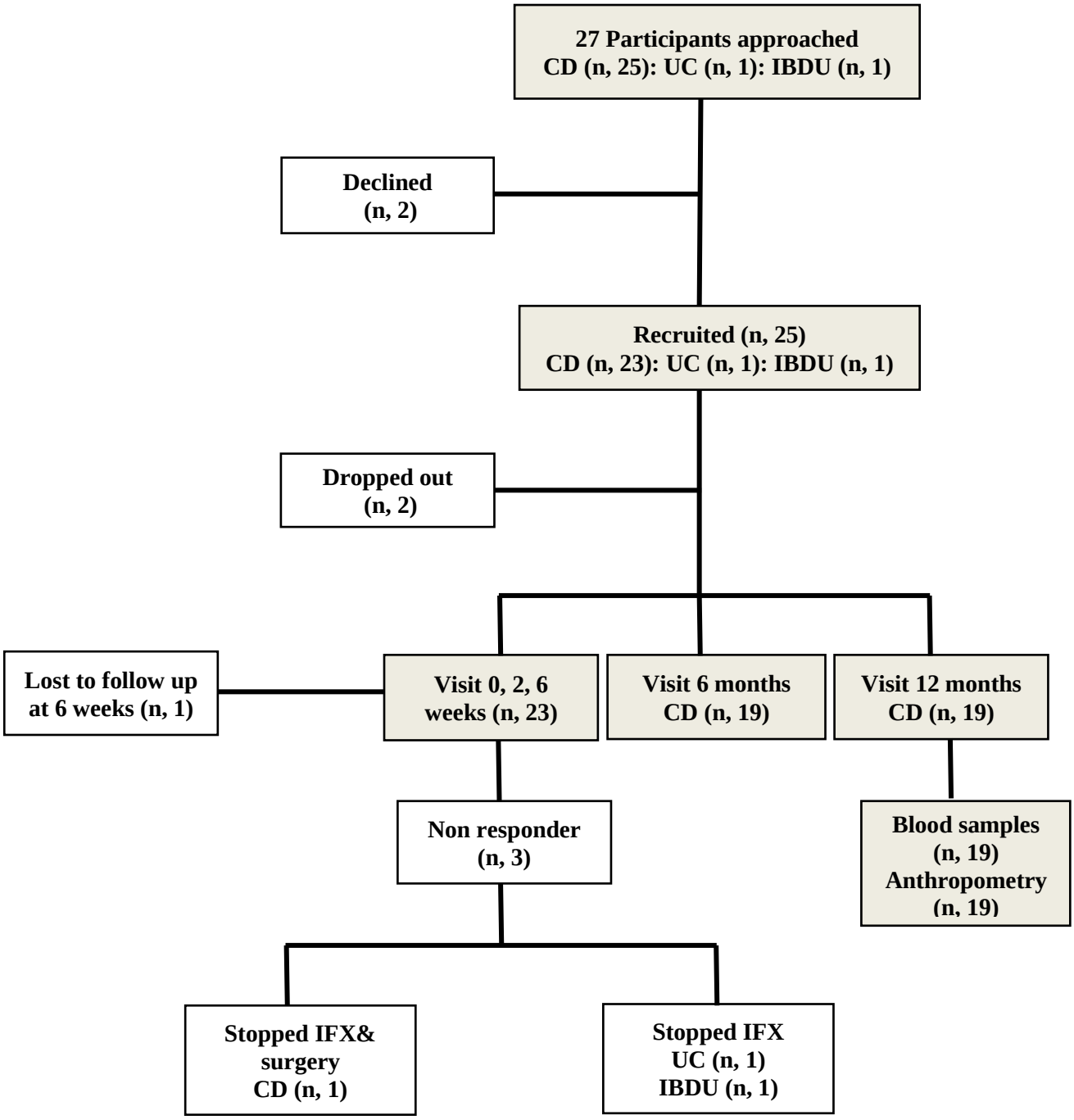

Figure 2-1: Consort diagram for the study.
The total number of eligible participants who were started on anti-TNF-α between 2009 and 2014 was 27; 2 participants declined to take part; 25 participants recruited of whom 2 participants dropped out before starting the study, 1 participant lost to follow up as she moved to adult care and 3 participants stopped anti-TNF-α at 6 weeks as they were non responders. The remaining 19 participants completed 12 months study visits.

2.3.2 Anti-TNF-α

All participants received a baseline schedule of three intravenous infusions of IFX as inpatients (5 mg/kg at baseline, 2 and 6 weeks). Following this, infusions were usually administered every 8 weeks as maintenance therapy for 12 months. Of the 19 participants, 4 had ADA therapy; one had ADA from the start, one switched to ADA after suffering a hypersensitive reaction to IFX; two participants lost response to IFX therapy and switched to ADA between 6 and 8 months. ADA was given intramuscularly (induction dose of 80 mg followed by 40 mg 2 weeks later and the maintenance regimen was every second week).

2.3.3 Disease activity index

Disease activity was assessed by the wPCDAI and was determined at baseline, 2 weeks, 6 weeks, 6 months and 12 months. Disease activity was categorized as remission <12.5, mild (12.5–40), and moderate (>40–57.5), severe (>57.5). These cut-off points of wPCDAI were used to classify the disease status at 12months.

2.3.4 Concomitant medication

At each study visit details of concomitant medication and nutritional support and surgery were obtained. Average oral GC dose over the 5 times points was converted into mg/kg/day. To identify further effects of GC use on changes in IGF-1 axis, bone turnover and bone health, the participants were sub-divided according to GC use during the study period.

2.3.5 Disease biomarkers and cytokines

At baseline, 2 weeks, 6 weeks, 6 months and 12 months the following markers of inflammation were analysed: erythrocyte sedimentation rate (ESR, mm/h), serum C–reactive protein (CRP, mg/l), and albumin (g/l) concentrations, using standard methods in the clinical laboratory. Non fasting blood sample collections coincided with the routine schedule of IFX infusions/ ADA injection visits; usually between 10am-3pm. Samples were immediately centrifuged at 2500 rpm for 5 min, and the serum was subsequently stored at -80C. Numbers of cytokines (TNF-α, IL-6) measured at baseline, 6 weeks, 6 months and 12 months by the Luminex which is a multiplex immunoassay with intra-assay variation of 2.6% and 3.6% respectively. Luminex assay was designed for simultaneous

detection of multiple cytokines in one plate. The assay principle is similar to that of an ELISA.

2.3.6 Anthropometry

At baseline and 12 months, the following data were obtained: Ht and sitting height (SH) using a Harpenden stadiometer, weight using digital weight scale. Subischial leg length (SILL) was calculated from the Ht and SH. Body mass index (BMI) calculated using the formula weight (kg) / height $(m)^2$. Ht and BMI were converted to SDS using 1990 children standards (273;274). SH and SILL were converted to SDS using the 1978 Tanner-Whitehouse17 standards (275). HV (cm/year) and ΔHt SDS were calculated for baseline from the prior 12 months of growth before time points of starting anti-TNF-α therapy and for 12 months after anti-TNF-α therapy from baseline.

2.3.7 Pubertal assessment and skeletal maturation

Tanner stage was ascertained by self-assessment questionnaire (276). Pubertal status data were collected at baseline, 6 and 12 months and participants were categorised as pre-pubertal (Tanner stage 1), early/mid-pubertal (Tanner stage 2-3) and late pubertal (Tanner stage 4-5). Bone age (BA) was determined at baseline and 12 months using the Tanner White House (TW2) the radius-ulna-short bones (RUS) method (277). BA was read on a clinical basis by a paediatric radiologist who was aware of the participant's age but not Tanner stage. However, we found discordant pubertal status using self-assessment with HV because some participants who categorised themselves as early puberty and HV of 0 cm/year, thus we assess the impact of puberty on growth by using BA. To evaluate the effect of anti-TNF- on growth and bone, participants were categorised by 2 groups based on BA: group (A), those with growth potential: BA<13.5 year for girl & <14.5 year for boys (n=9) and group (B) those with little or no growth potential: BA ≥13.5 for girl & ≥14.5 for boys (n=10). ΔBA/ΔCA ratio (the change in bone age (ΔBA) to change in chronological age (ΔCA)) ratio was used as predictors of pubertal progression (52).

2.3.8 Growth biomarkers

Serum IGF-1 (ng/ml) concentration was determined by a serum IGFBP-blocked IGF-1 ELISA kit (Mediagnost, Reutlingen, Germany) with inter- and intra-assay variation coefficients of 11.4% and 9.9% respectively, and low cross-reactivity of the IGF-1 with IGF-2 (< 0.05%). Serum IGFBP-3 (ng/ml) was measured by ELISA (Mediagnost

Reutlingen, Germany) with inter-assay and intra-assay coefficients of 5.3% and 1.6%. Serum ALS (mU/ml) was measured by ELISA using the Mediagnost ELISA Reutlingen, Germany kits with inter-assay variation of 5.1% and intra-assay variation of 14.5% and no cross reactivity with IGF-1 and IGFBP-3. Serum IGFBP-2, (ng/ml) was measured by ELISA (Mediagnost ELISA Reutlingen, Germany). This assay is specific to human IGFBP-2; there is no cross-reactivity with IGFBP-1 or IGFBP-3 and inter- and intra assay coefficients of variability (CV) are 12.6% and 0.76%. To take into account pubertal variation, IGF-1 and IGFBP-3 level were converted to standard deviation scores (SDS) by adjusting to bone age and sex using reference data available from Mediagnost (n, 388) (278) and ALS level was converted to standard deviation scores (SDS) by adjusting to bone age and sex using published reference data (n, 252) (279). The baseline bone age for baseline 2 and 6 week and 12 months bone age for 6 months and 12 months. As IGFBP-2 does not vary with gender or puberty, it was adjusted only for age using published reference data (n, 388) (280).

2.3.9 Bone Biomarkers

Serum BSAP (µg/L) was measured by immunoenzymetric assay (EIA) (Ostase BAP assay, Immunodiagnostic Systems (IDS) UK) (interassay and intra-assay coefficients of variation of 8.9% and 4.5%). Bone age and sex specific standard deviation scores (SDS) calculated using reference data available from Immunodiagnostic (n, 116) (281). Current immunoassays for BSAP possess a low cross-reactivity with the circulating liver isoenzyme. Biomarkers of bone resorption, β-CTX (ng/ml) measured by the Serum CrossLaps® ELISA (Immunodiagnostic Systems (IDS) Ltd), with interassay and intra-assay coefficients of variation of 7.1% and 7.4% respectively. Bone age and sex specific standard deviation scores (SDS) calculated using published reference data (n, 572) (282). Vitamin D (25(OH) vitamin D) was conducted by Mrs Susan Johnston at Glasgow Royal Infirmary Hospital. 25 (OH) vitamin D was measured by liquid chromatography-tandem mass spectrometry (Waters Corporation, Milford, MA, USA) with a sensitivity of 2.5 nmol/L and intra-assay variation coefficients was 5.3%.

2.3.10 Statistics

Analyses were performed using SPSS software version 22 (New York, USA). Non-parametric data were presented with medians and ranges. Changes between parameters assessed at different time points were analysed using repeated measures with Wilcoxon

signed-rank tests and subsequently adjusted for multiple comparison using a Bonferroni correction and $p<0.05$ considered as statistically significant. Mann Whitney U test was used for continuous and Chi-square test for categorical variable to assess inter-group differences divided according to disease, GC use or growth potential. The univariate correlation between variables was assessed using the Spearman's correlation coefficient. To assess the relationship of IGF-1 axis (SDS adjusted for gender and BA) with albumin, wPCDAI, IL-6 and GC exposure, we used a mixed-model approach that takes into account intra individual correlations over the 5 time points of study visits. Linear regression analysis was used to assess the independent predictors for Δ Ht SDS at 12 months in group with growth potential. The statistical analysis was only done for those who had complete data. All graphs were prepared by GraphPad Prism software version 7 (San Diego California, USA) and XSTAT software version 2016.02.27970 (Addinsoft, New York, USA).

2.4 Results

2.4.1 Participant characteristics

Table 2-1 summarises the characteristics of the 19 CD participants at the time of starting anti-TNF-α. The median (range) age at diagnosis of CD and age at anti-TNF-α initiation was 11.9 years (5.5, 15.5) and 14.9 years (11.2-17.2) with median interval since diagnosis of 3.1 years (0.2, 10.7); only 3 participants had the disease less than 18 months. 10/19 (53%) of participants had colonic (L2) CD, two had colonic and upper gastro-intestinal disease (L4), 8 /19 (42%) had ileocolic disease (L3) CD and four of them had L3+ L4. 4/19 participants (21%) had perianal disease (p).

Table 2-1: Participant characteristics at time of starting anti-TNF-α.

	n, 19 CD
Male, n (%)	12 (63%)
CA at Diagnosis (year)	11.9 (5.5, 15.5)
CA at starting anti-TNF-α (year)	14.9 (11.2, 17.2)
Duration of disease	3.1 (0.20, 10.7)
BA at starting anti-TNF-α (year)	14.2 (9.3, 16.9)
BA delay (year)	0.5 (-0.8, 2.3)
Tanner stage, n (%)	
Pre-puberty (tanner stage-1)	2 (11)
Mid-puberty (tanner stage 2-3)	8 (42)
Late puberty (tanner stage 4-5)	9 (47)
Ht SDS at starting anti-TNF-α	-0.7 (-2.7, 1.7)
BMI SDS at starting anti-TNF-α	-0.4 (-2.7, 3.2)
Disease location, n (%)	
Ileal (L1)	1 (5)
Colonic (L2)	10 (53)
Ileocolonic (L3)	8 (42)
Isolated upper disease(L4)	6 (32)
Behaviour (non-stricturing, non-penetrating)	19 (100)
Perianal	4 (21)

Data presented as number, n; percentage, %, median (range). CA, chronological age; BA, bone age; Ht SDS, height SD score; BMI SDS, body mass index SD score.

2.4.2 Disease and laboratory characteristics

Table 2-2 summarises disease and treatment characteristic in 19 CD participants at each visit. At baseline, median (range) of wPCDAI scores was 40.0 (0.0, 95.0) and significantly decreased, to 17.5 (0.0, 70.0) at 2 weeks (p=0.004 vs. baseline), 7.5 (0.0, 67.0) at 6 weeks (p=0.008 vs. baseline), 10.0 (0.0, 42.0) at 6 months (p=0.004 vs. baseline) and 15.0 (0.0, 47.0) at 12 months (p=0.038 vs. baseline) (Figure 2-2). Nine (47%) participants had moderate to severe disease activity based on wPCDAI at enrolment with marked improvement to three (16%) at 6 weeks and four (21%) at 12 months (Figure 2-2). ESR, Serum albumin, CRP improved significantly at 6 weeks with no difference between 6-weeks and 12–month values. Overall, median (range) TNF-α (pq/ml) remained unchanged during the study interval. At baseline, median (range) of serum IL-6 (pq/ml) was 52.1 (34.0, 153.0) and changed to 58.6 (21.0, 155.5) at 6 weeks (p=0.60 vs. baseline), reduced to 49.5 (17.5, 151.5) at 6 months (p=0.59 vs. baseline) and 44.4 (22.0, 116.0) at 12 months (p=0.05 vs baseline) (Figure 2-3).

Table 2-2: Changes in disease activity, laboratory results, and treatment over the study interval.

n, 19	Baseline	2 Weeks	6 Weeks	6 Months	12 Months	p-values			
						Baseline vs. 2 weeks	Baseline vs. 6 weeks	Baseline vs. 6 months	Baseline vs. 12 months
wPCDAI,	40.0 (0.0, 95.0)	17.5 (0.0, 70.0)	7.5 (0.0, 67.0)	10.0 (0.0, 42.0)	15.0 (0.0, 47.0)	**0.004**	**0.008**	**0.004**	**0.038**
wPCDAI, n (%)									
Not active (<12.5)	3 (16)	9 (47)	11 (58)	12 (63)	9 (47)				
mild (12.5-40)	7 (37)	8 (42)	5 (26)	5 (26)	6 (32)				0.09
Moderate-severe (>40)	9 (47)	2 (11)	3 (16)	2 (11)	4 (21)	**0.012**	**0.036**	**0.012**	0.09
Albumin (g/l)	34.0 (17.0, 42.0)	35.0 (17.0, 42.0)	36.0 (25.0, 44.0)	38.0 (20.0, 44.0)	36.0 (19.0, 42.0)	0.17	**0.04**	0.20	0.17
ESR (mm/hr)	25.0 (3.0, 70.0)	11.0 (3.0, 70.0)	12.0 (3.0, 86.0)	12.0 (4.0,56.0)	12.0 (2.0, 48.0)	**0.000**	0.075	0.071	0.12
CRP (mg/l)	10.0 (3.0, 95.0)	5.0 (3.0, 98.0)	5.0 (3.0, 51.0)	3.0 (0.0, 39.0)	4.0 (3.0, 90.0)	0.15	**0.042**	**0.042**	0.2
Il-6 (pg/ml)	52.0 (34.0, 153.0)	-	59.0 (21.0, 156.0)	50.0 (18.0, 152.0)	44.0 (22.0, 116.0)	-	0.60	0.59	0.05
TNF-α (pg/ml)	14.0 (10.0, 49.0)	-	19.0 (9.0, 53.0)	19 (9.0, 119.0)	18 (10.0, 92.0)	-	0.13	0.13	0.16
Medication, n (%)									
Glucocorticoid	9 (47)	9 (47)	8 (42)	2 (11)	2 (11)				0.08
Aminosalicylates	10 (53)	9 (47)	10 (53)	8 (42)	5 (26)				0.10
Methotrexate	13 (68)	13 (68)	12 (63)	12 (63)	11 (58)				0.50
Mercaptopurine/Azathioprine	3 (16)	4 (21)	4 (21)	6 (32)	6 (32)				0.25
Exclusive enteral nutrition	6 (37)	4 (21)	3 (16)	2 (11)	2 (11)				0.11

Data presented as number, n; percentage, %, median (range). wPCDAI, weighted paediatric disease activity index; ESR, erythrocyte sedimentation rate; CRP, C-reactive protein; IL-6, interleukin-6; TNF-α, tumour necrosis factor-α. Values in bold indicate statistical significance ($p<0.05$).

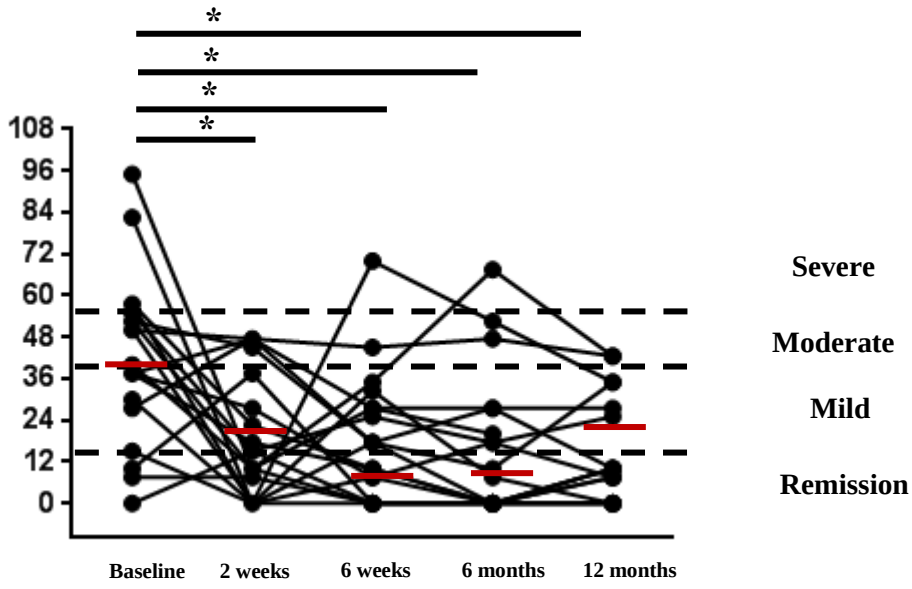

Figure 2-2: Individual change in weighted Paediatric Disease Activity Index (wPCDAI) following 12 months of anti-TNF-α.
Red solid lines represent the median values. Black dotted lines represent the cut off values of wPCDAI. Remission, <12.5; Mild, 12.5-40; Moderate, 40-57.5; Severe, >57.5. *indicate statistical significance ($p<0.05$).

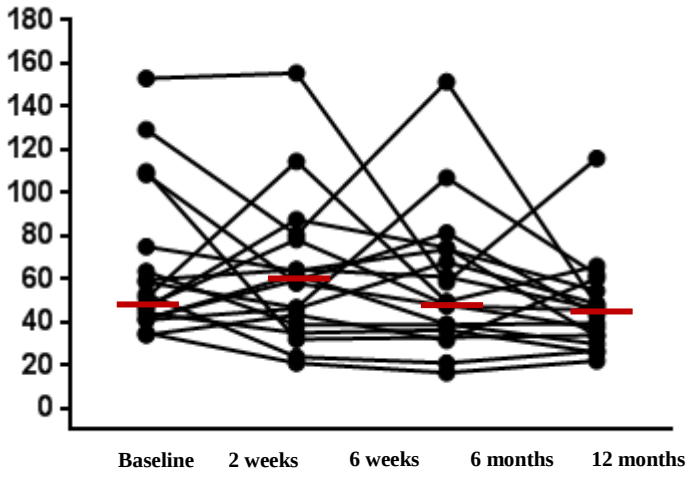

Figure 2-3: Individual change in interleukin-6 (IL-6) following 12 months of anti-TNF-α.
Red solid lines represent the median values.

To identify further effects of disease status (based on wPCDAI) on changes in, IGF-1 axis, bone turnover and growth, the participants were sub-divided into two groups according to their disease status at 12 months following the treatment with anti- TNF-α agents (Table 2-3). There was no significance in gender distribution, duration of disease, GC use and pubertal and anthropometric data at the time of starting anti-TNF-α.

Table 2-3: Participant characteristics according to disease status based on wPCDAI at 12 months.

	Remission (n, 9)	Non-remission (mild/ moderate+ severe disease) (n, 10)	p-value
Male, n (%)	6 (67%)	6 (60%)	0.76
CA at Diagnosis (year)	12.2 (6.5, 15.5)	11.0 (5.5, 15.1)	0.60
CA at starting anti-TNF-α (year)	14.8 (11.6, 16.3)	15.5 (11.2, 17.2)	0.35
Duration of disease	3.0 (0.17, 5.5)	3.6 (0.7, 10.7)	0.39
BA at starting anti-TNF-α (year)	13.5 (11.4, 16.9)	14.4 (9.3, 16.5)	0.65
BA delay (year)	0.1 (-0.9, 1.3)	0.6 (-0.6, 2.3)	0.49
Tanner stage, n (%)			
Pre-puberty (tanner stage-1)	1 (11)	1 (10)	
Mid-puberty (tanner stage2-3)	4 (44)	4 (40)	
Late puberty (tanner stage4-5)	4 (44)	5 (50)	
Ht SDS at starting anti-TNF-α (year)	-0.7 (-2.7, 1.8)	-0.7 (-1.6, 1.5)	0.78
BMI SDS at starting anti-TNF-α (year)	-0.4 (-2.7, 3.2)	-0.1 (-1.4, 1.4)	0.27
Glucocorticoid at baseline	6 (67%)	3 (30%)	0.11
Disease location, n (%)			
Ileal	0 (0)	1 (10)	
Colonic	6 (67)	4 (40)	
Ileocolonic	3 (33)	5 (50)	
Isolated upper disease	2 (22)	4 (40)	
Behaviour (non-stricturing, non-penetrating)	9 (100)	10 (100)	
Perianal	3 (33)	1 (10)	

Data presented as number, n; percentage, %, median (range). CA, chronological age; BA, bone age, Ht SDS, height SDS score; BMI SDS, body mass index SDS.

2.4.3 Treatment characteristics

All participants started the study on anti-TNF-α and all were responders at 6 weeks and had anti-TNF-α for 12 months. Nine (47%) participants were treated with GC at baseline and corresponding data were 9/19 (47%) at 2 weeks, 9/19 (47%) at 6 weeks, 2/19 (10%) at 6 months and 2/19 (10%) at 12 months respectively. None of the participants had surgery during the 12 months of the study interval (Table 2-2).

To identify further effects of GC use on changes in IGF-1 axis, bone turnover, the participants were sub-divided according to GC exposure during the study period into GC naïve (n,10), GC at baseline but stopped at 6 months (n,7), GC at baseline and throughout 12 months (n,2) (Table 2-4). The participants who were on GC had lower BA and all of them were on tanner stage between 1-3. Of the two who were on GC throughout 12 months, one was female (ID18) and another was male (ID19) aged 15.6 and 16 years respectively at the time of starting anti-TNF-α and both were on tanner stage 4. Both of them entered remission at 12 months (wPCDAI; ID 18 was 10, ID 19 was 10).

Table 2-4: Participant characteristics according to GC exposure during study.

	GC naïve (n, 10)	GC at baseline and stopped at 6 months (n, 7)	p-value
Male, n (%)	6 (60%)	5 (71%)	0.63
CA at Diagnosis (year)	11.5 (5.5, 13.1)	10.6 (6.3, 15.1)	0.73
CA at starting anti-TNF-α (year)	15.3 (12.2, 17.2)	12.8 (11.2, 15.8)	0.06
Duration of disease	3.1 (2.7, 10.7)	2.2 (0.73, 5.5)	0.35
BA at starting anti-TNF-α (year)	15.0 (11.4, 16.5)	12.9 (9.3, 15.4)	0.06
BA delay (yr)	0.6 (-0.85, 2.3)	0.5 (-0.5, 1.9)	0.73
Tanner stage, n (%)			
Pre-puberty (tanner stage -1)	1 (10)	1 (14)	0.79
Mid-puberty (tanner stage 2-3)	2 (20)	6 (86)	**0.008**
Late puberty (tanner stage 4-5)	7 (70)	0 (0)	**0.004**
Ht SDS at starting anti-TNF-α	0.2 (-1.5, 1.5)	-0.9 (-2.7, -0.7)	**0.007**
BMI SDS at starting anti-TNF-α	-0.4 (-2.3, 3.2)	-0.03 (-2.3, 0.6)	0.73
wPCDAI	38.0 (0.0, 58.0)	40.0 (7.5, 83.0)	
Disease location, n (%)			
Ileal (L1)	1 (10)	0 (0)	0.39
Colonic (L2)	3 (30)	7 (100)	0.19
Ileocolonic (L3)	6 (60)	0 (50)	0.011
Isolated upper disease (L4)	4 (40)	1 (14)	0.34
Behaviour (nonstricturing, nonpenetrating)	10 (100)	7 (100)	
Perianal	1 (10)	1 (14)	0.87

Data presented as number, n; percentage, %, median (range). CA, chronological age; BA, bone age, Ht SDS, height SD score; BMI SDS, body mass index SDS; GC, glucocorticoid; +/-, GC at baseline and stopped at 6 months. Values in bold indicate statistical significance ($p<0.05$).

2.4.4 Anthropometric characteristics

Table 2-5 summarises anthropometry and pubertal outcomes and figure 2-4 shows individual data at baseline, 2 weeks, 6 weeks, 6 months and 12 months among 19 participants. In 19 participants with active CD, median Ht SDS at time of diagnosis was 0.04 (-3.1, 1.5) and it was not different from median MPH SDS -0.4 (1.5, -1.4) (p=0.55). Median Ht SDS dropped from -0.5 (-2.5, 0.9) at 12 months prior to starting anti-TNF-α to -0.7 (-2.7, 1.7) at the time of starting anti-TNFα. (p=0.87). At baseline the median (range) Ht SDS was not different from zero (p=0.17) and there was no significant improvement in median Ht SDS (p=0.47) 12 months following anti-TNF-α. Among 19 participants, 3 (16%) had Ht SDS <-2 at time of diagnosis, 2 (10%) had Ht SDS <-2 at time of baseline (both of them were male and their BA 16.9 and 11.5 years) and the Ht SDS of those two patients remained <-2 at 12 months. Median HV at baseline was 3.6 cm/year (0.0, 8.6) and dropped to 2.8 cm/year (0.0, 9.5) (p=0.37). There was no significant difference in median ΔHt SDS between baseline and 12 months after initiation of biologic 0.1 (-0.4, 0.5) (p=0.11).

2.4.5 Body mass index, puberty and skeletal maturation

Median BMI SDS at time of diagnosis was -1.1 (-3.6, 2.8); 3 (16%) was underweight (BMI SDS ≤-2) and one (5%) was overweight (BMI SDS ≥ 2). At baseline, median BMI SDS was not different from zero (p=0.136) and there was a trend toward an increase in median BMI SDS at 12 months (p=0.06); none of the participants were underweight and 2 (10%) were overweight at 12 months. Based on self-assessment, 2 (10%) participants were in pre-puberty, 8 (42%) were early puberty and 9 (47%) were late-puberty (all of them were on tanner stage-4). By 12 months the majority of participants 14 (74%) were at late-puberty and 5 (26%) patients were in early puberty.

Table 2-5: Anthropometry, puberty at baseline and 12 months following anti-TNF-α.

n, 19	Baseline	12 Months	p-Value
CA (year)	14.9 (11.2, 17.2)	15.9 (12.2, 18.2)	
BA (year)	14.2 (9.3,16.9)	15.4 (9.7, 18.2)	0.57
CA-BA (year)	0.5 (-0.9, 2.3)	0.4 (-1.1, 3.1)	0.43
Ht SDS	-0.7 (-2.7, 1.7)	-0.5 (-3.1, 1.9)	0.47
Height Velocity (cm/year)	3.6 (0.0, 8.6)	2.8 (0.0, 9.5)	0.37
ΔHt SDS	-0.1 (-0.7, 0.7)	0.1 (-0.4, 0.5)	0.11
SH SDS	-0.9 (-2.2, 1.0)	-0.6 (-2.0, 1.1)	0.45
SILL SDS	0.0 (-3.7,1.7)	-0.6 (-2, 1.1)	0.35
BMI SDS	-0.4 (-2.7, 3.2)	0.2 (-1.8, 2.7)	0.06
Pubertal status			
Pre-pubertal	2(11%)	0(0)	0.15
Early-pubertal	8(42%)	5(26%)	0.31
Late-pubertal	9(47%)	14(74%)	0.097

Data presented as median (range). CA, chronological age ; BA, bone age; Ht SDS, height SD score; HV, height velocity; ΔHt SDS, change in height standard deviation scores; SH SDS; sitting height SD score SILL SDS, sub-ischial leg length SD score; BMI SDS, body mass index SD score. Values in bold indicate statistical significance ($p<0.05$).

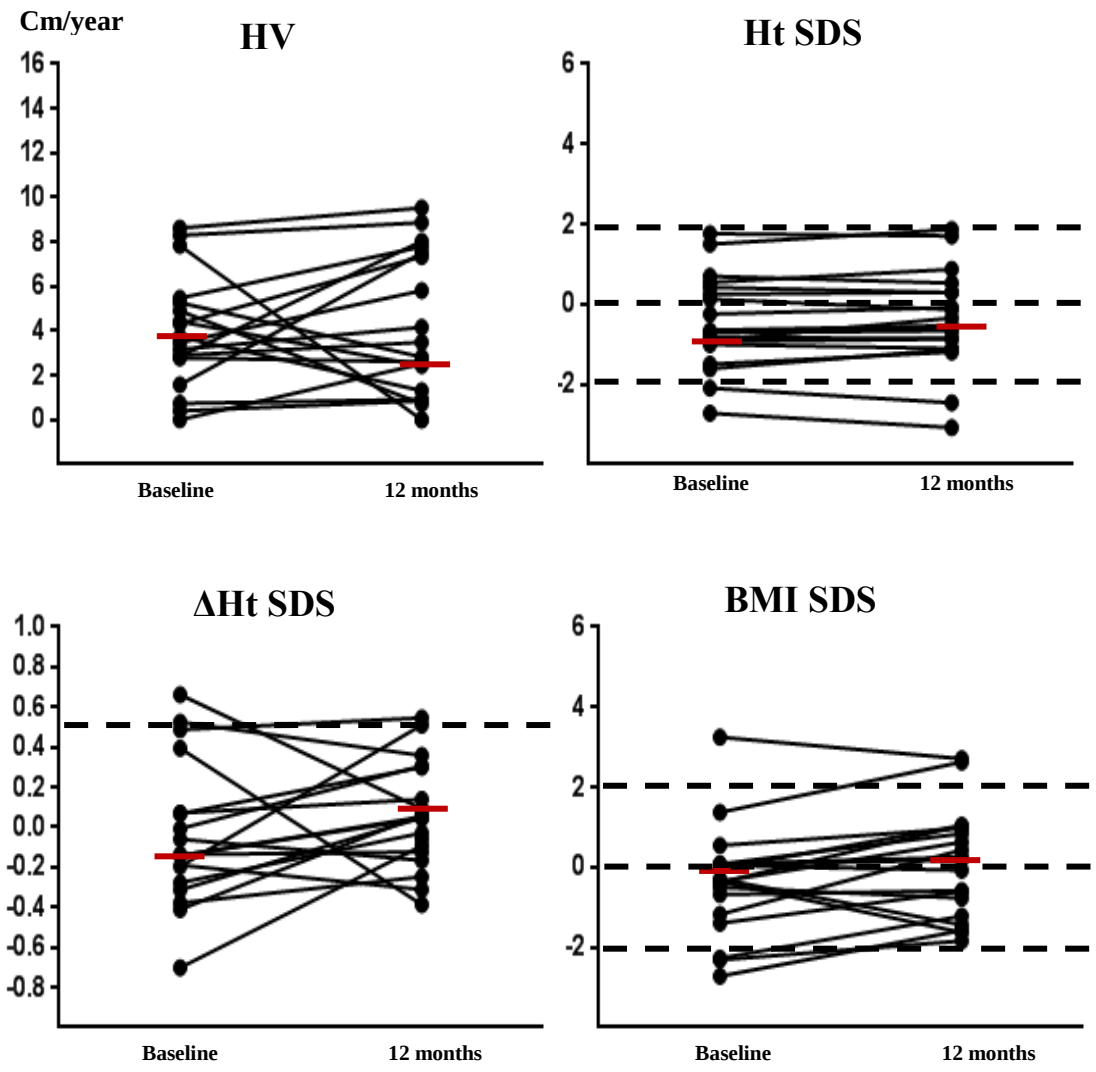

Figure 2-4: Individual change in anthropometric in 19 participants following 12 months of anti-TNF-*α*.

HV, height velocity (cm/year); Ht SDS, height SD score; ΔHt SDS, change in height SD score; BMI SDS, body mass index SD score. Red solid lines represent the median values. Black dotted lines represent the 50th centile (0SDS), 3rd (-2SDS) and 97th (2SDS) centile. *indicate statistical significance ($p<0.05$).

2.4.6 Impact of puberty on growth

The cohort of participants was divided into two groups on whether they have potential to grow at the time of starting anti-TNF-α based on bone age (Table 2-6; Figure 2-5). At baseline, in group (A), the median Ht SDS was below zero (p=0.05). Participants grew at a sub-normal rate over 12 months prior to starting anti-TNF-α and experienced increase in growth rate during 12 months (p=0.008) following anti-TNF-α therapy. The increase in HV was associated with a trend toward increase in median (range) Ht SDS between baseline and 12 months (p=0.07). At 12 months, median Ht SDS stayed below zero (p=0.050). Their MPH SDS was -0.3 (-1.5, 1.1) (p=0.33, Ht SDS at baseline vs MPHSDS) and; p=0.53 (Ht SDS at 12months vs MPH SDS). After 12 months of TNF-α therapy this group experienced a significant increase in median ΔHt SDS (p=0.015) with none of these participants having ΔHt SDS >0.5 at 12 months. At baseline median SH SDS was -0.9 (-2.2, 0.9) and SILL SDS was -1.1 (-3.7, 0.9) and at 12 months was -0.6 (-2.0, 0.9) (p=0.23 vs. baseline) and -0.6 (-2.0, 0.9) (p=0.36 vs. baseline) respectively.

At baseline no significant correlation was observed between Ht SDS in the group (A) with disease activity markers (including albumin, CRP, ESR, wPCDAI), disease duration and cytokines (IL-6, TNF-α). At 12 months a significant negative correlation was observed between Ht SDS and TNF-α (r=-0.81; p=0.02). ΔHt SDS and HV at 12 months were not associated with 12 months change in disease biomarkers/cytokines. The majority of participants (6/9) in group (A) were on GC at baseline and stopped it by 6 months. Based on wPCDAI at 12 months, 6/9 participants in group (A) were in remission, thus categorising the participants based on GC use/wPCDAI was not practical for those participants. Multivariate analysis using linear regression was used to identify predictors of ΔHt SDS at 12 months in group (A)with average wPCDAI over 12 month as markers of disease activity, progress in puberty (ΔBA/ΔCA) and GC (Average dose on mg/kg/d over 12months) as independent variables (R^2=50) showed no significant independent factor.

In-group (B); at baseline the median Ht SDS was not different from zero (p=1.0). Median HV decreased from 3.7cm/year (0.0, 7.8) at baseline to 1.1cm/year (0.0, 2.8) (p=0.12) at 12 months. Median Ht SDS and ΔHt SDS remained static at 12 months. Their MPH SDS was -0.6 (-1.1, 1.4) [p=0.71(Ht SDS at baseline vs MPH SDS); p=0.77 (Ht SDS at 12 months vs MPH SDS)].

Table 2-6: Change in growth following anti-TNF-α. Participants divided into two groups on whether they have potential to grow at time of starting anti-TNF-α based on bone age.

	Baseline	12 Months	p-value
Age (year)			
Group (A) (n, 9)	12.8 (11.2, 14.8)	13.9 (12.2, 15.8)	
Group(B) (n, 10)	15.8 (14.9, 17.2)	16.8 (16.0, 18.2)	
Bone age (yr)			
Group (A)	11.9 (9.3, 14.2)	13.6 (9.7, 15.4)	0.74
Group (B)	15.7 (13.9, 16.9)	16.6 (14.4, 18.2)	0.27
Duration of Disease			
Group (A)	3.0 (1.5, 5.5)		
Group (B)	3.6 (0.2, 10.7)		
Height SDS			
Group (A)	-0.9 (-2.7, 0.5)	-0.7 (-3.1, 0.9)	0.07
Group (B)	-0.1 (-2.1, 1.8)	-0.1 (-2.5, 1.9)	0.39
HV (cm/year)			
Group (A)	3.5 (1.6, 8.6)	7.5 (3.5, 9.5)	**0.008**
Group(B)	3.7 (0.0, 7.8)	1.1 (0.0, 2.8)	0.21
ΔHeight SDS			
Group (A)	-0.1 (-0.4, 0.5)	0.1 (-0.3, 0.5)	**0.015**
Group (B)	-0.1 (-0.7, 0.7)	-0.1 (-0.4, 0.4)	0.86
BMI SDS			
Group (A)	-0.3 (-2.3, 0.1)	-0.6 (-1.8, 1.0)	0.37
Group (B)	-0.4 (-2.7, 3.2)	0.5 (-1.6, 2.7)	0.09
GC exposure			
Group (A)	6 (67)	0 (0)	**0.001**
Group (B)	2 (20)	2 (20)	1.0
wPCDAI			
Group (A)	40.0 (0.0, 82.5)	10.0 (0.0, 47.5)	0.05
Group (B)	43.8 (10.0, 95.0)	16.3 (0.0, 47.5)	0.07
Pre-pubertal			
Group (A)	2 (22)	0 (0)	0.11
Group (B)	0 (0)	0 (0)	
Early-pubertal			
Group (A)	6 (66)	5 (55)	0.40
Group (B)	2 (20)	0 (0)	0.14
Late-pubertal			
Group (A)	1 (11)	4 (44)	0.11
Group (B)	8 (80)	10 (100)	0.14

Data presented as median (range).19 Participants, 9 (47%) had growth potential (BA<13.5yrs in girls and <14.5yrs in boys) (Group (A)); 10 (53%) participants had little/no growth potential (BA≥13.5yrs in girls and ≥14.5yrs in boys) (Group (B)). CA, chronological age; BA, bone age; Ht SDS, height SD score; HV, height velocity; ΔHt SDS, change in height standard deviation scores; BMI SDS, body mass index SD score; wPCDAI, weighted paediatric disease activity index; GC, glucocorticoid. Values in bold indicate statistical significance ($p<0.05$).

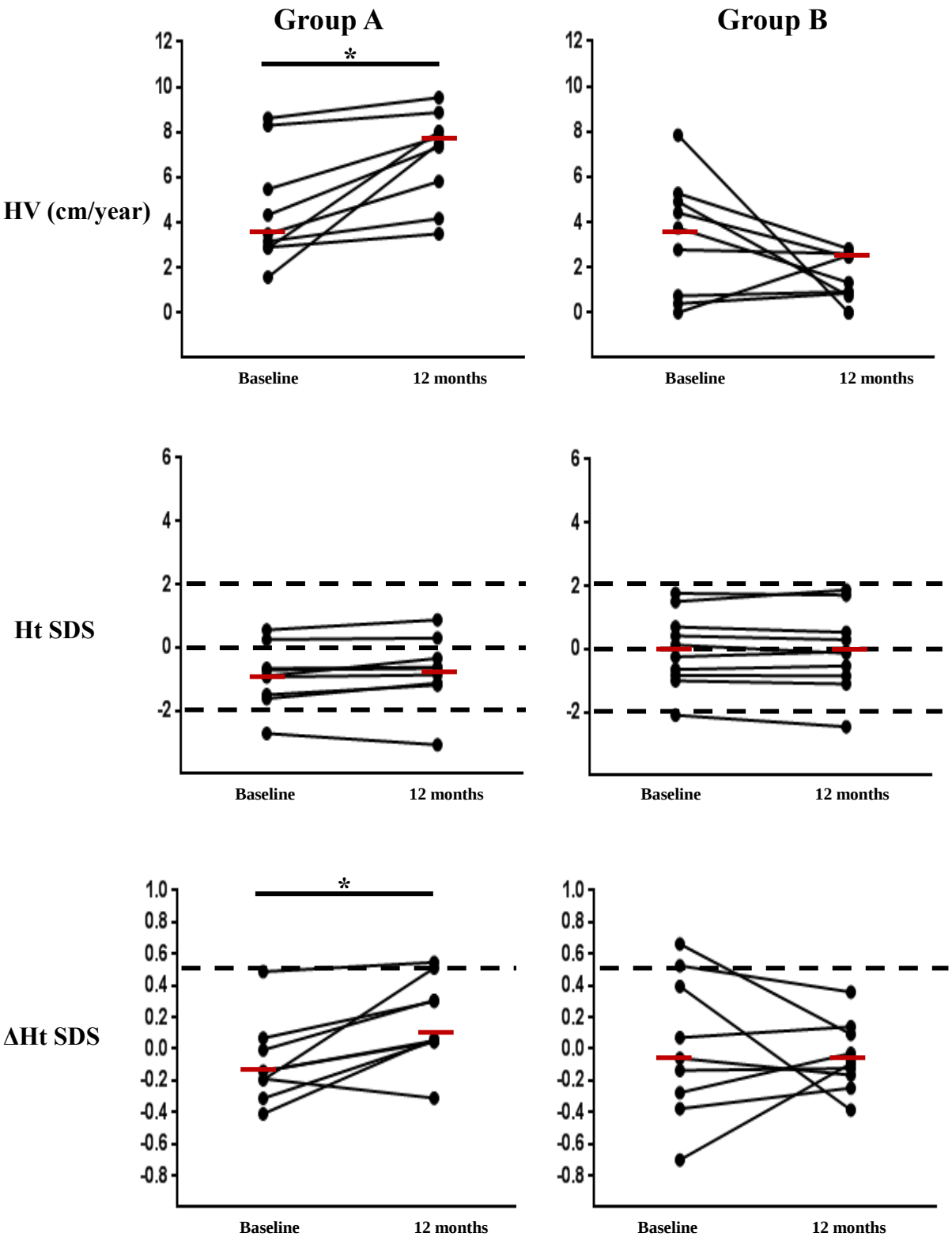

Figure 2-5: Individual change in anthropometric following 12 months of anti-TNF-α. Participants divided into two groups on whether they have potential to grow at time of starting Anti-TNF-α based on bone age.

19 Participants, 9(47%) had growth potential (BA<13.5yrs in girls and <14.5yrs in boys) (Group (A)); 10 (53%) participants had little/no growth potential (BA≥13.5yrs in girls and ≥14.5yrs in boys) (Group (B)). HV, height velocity (cm/years); Ht SDS, height SD score; ΔHt SDS, change in height SD score; BMI SDS, body mass index SD score. Red solid lines represent the median values. Black dotted lines represent the 50th centile (0SDS), 3rd (-2SDS) and 97th (2SDS) centile. *indicate statistical significance ($p<0.05$).

2.4.7 Growth biomarkers

Table 2-7 summarises the median (ranges) changes of growth biomarkers and Figure 2-6 shows individual data of growth biomarkers at baseline, 6 months and 12 months among 19 participants. At baseline, the median IGF-1 SDS was not different compared to zero (p=0.80), median IGFBP-3 SDS was significantly higher compared to zero (p=0.002), the median ALS SDS at baseline was significantly lower compared to zero (p=0.00) and median IGFBP-2 SDS was significantly higher compared to zero (p=0.00). For IGF-1 SDS values did not change through the study interval. The percentage of participants with IGF-1 SDS below minimum reference range (IGF-1 SDS <-2) decreased from 5 (26%) at baseline to 2 (10%) at 2 weeks, 1 (5%) at 6 weeks, 1 (5%) at 6 months and 0 (0%) at 12 months respectively. At 12 months the median IGFBP-3 SDS was not different to zero (p=0.09) with three participants with IGFBP-3 SDS >2 at baseline, and 2 with their values above 2 SDS at 12 months. The median ALS SDS remained unchanged and stayed below zero (p=0.00); one participant had ALS SDS <-2 at baseline. There was a trend toward normalisation of median IGFBP-2 SDS by 12 months. 10 (53%) participants had their IGFBP-2 SDS >2 while for 6 (31%) participants their IGFBP-2 SDS remained >2. IGF-1/IGFBP-3 ratio as a surrogate marker of bioavailable IGF-1 was below one and remained unchanged. When categorised by disease status (Table 2-8; Figure 2-7) or GC exposure, the growth biomarkers yielded the same results (Table 2-9). However, our results showed IGFBP-2 SDS at 12 months was lower in those who had remission compared to those who did not have remission (p=0.042).

Table 2-7: Changes in growth biomarkers following anti-TNF-α.

n, 19	Baseline	2 Weeks	6 Weeks	6 Months	12 Months	p-values			
						Baseline vs. 2weeks	Baseline vs. 6weeks	Baseline vs. 6 months	Baseline vs. 12 months
IGF-1 SDS	0.1 (-3.8, 2.1)	-0.4 (-3.1, 1.7)	-0.04 (-4.8, 1.9)	-0.8 (-2.6, 1.9)	-0.1 (-1.9, 2.4)	0.36	0.29	0.83	0.35
IGFBP-3 SDS	1.4 (-2.9, 3.0)	0.4 (-1.1, 2.9)	0.5 (-1.7, 2.9)	0.6 (-1.2, 2.4)	0.5 (-1.0, 2.9)	0.97	0.42	0.13	0.86
IGF-1/IGFBP-3	0.09 (0.03, 0.2)	0.09 (0.03, 0.2)	0.09 (0.02, 0.2)	0.08 (0.05, 0.2)	0.10 (0.03, 0.2)	0.11	0.06	0.78	0.15
ALS SDS	-0.9 (-2.2, 1.5)	-0.9 (-1.9, 0.3)	-0.7 (-2.3, 0.6)	-1.0 (-2.0, 0.2)	-0.8 (-1.7, 0.2)	0.66	0.69	0.41	0.88
IGFBP-2 SDS	2.2 (-1.2, 4.1)	2.4 (-1.5, 3.9)	1.6 (-0.6, 3.0)	1.8 (-1.3, 3.7)	1.53 (-0.9, 4.1)	0.75	0.08	0.18	0.14

Data presented as median (range) and adjusted for bone age. IGF-1 SDS, serum insulin like growth factor-1SD score; IGFBP-3 SDS, serum insulin like growth factor binding protein-3 SD score; ALS SDS acid-labile subunit; IGFBP-2 SDS, serum Insulin like growth factor binding protein-2.

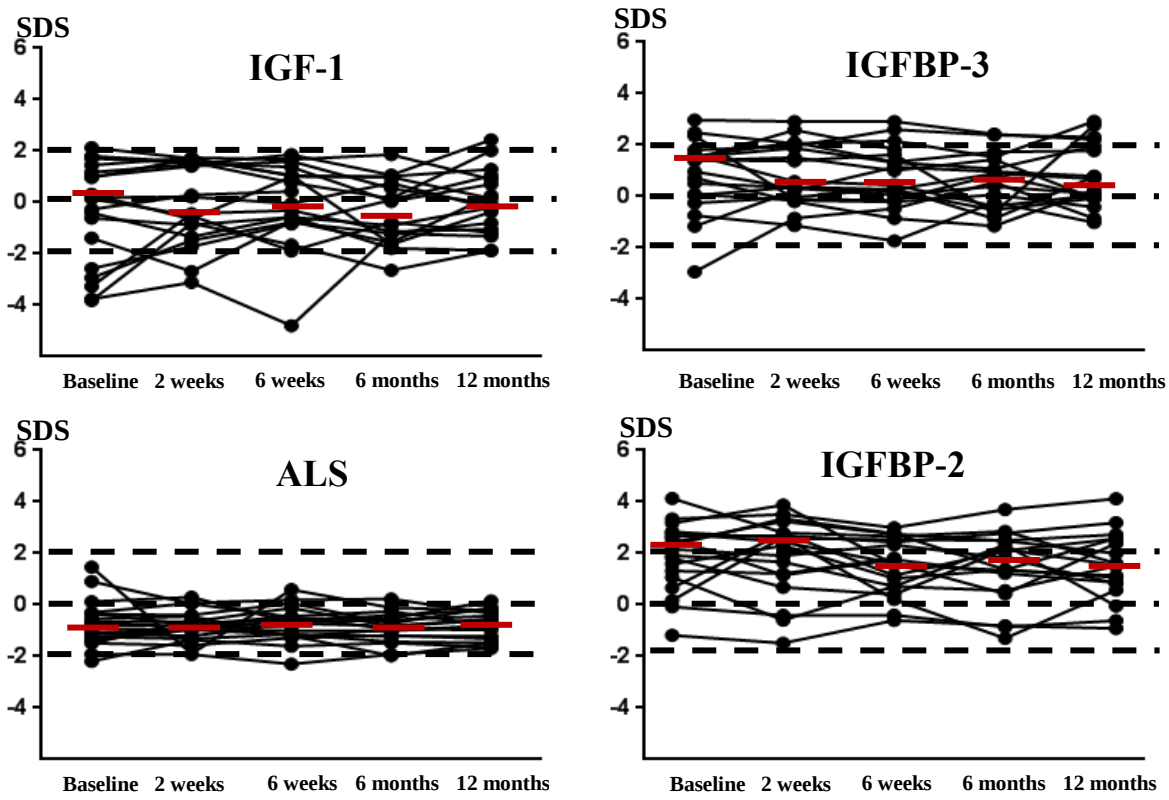

Figure 2-6: Individual change in growth biomarkers following 12 month of anti-TNF-α.
IGF-1 SDS, serum insulin like growth factor-1SD score; IGFBP-3 SDS, serum insulin like growth factor binding protein-3 SD score; ALS SDS, acid-labile subunit SD score; IGFBP-2 SDS, serum Insulin like growth factor binding protein-2 SD score. Red solid lines represent the median values. Black dotted lines represent the 50th centile (0SDS), 3rd (-2SDS) and 97th (2SDS) centile.

Table 2-8: Changes in growth biomarkers following anti-TNF-α. Participants divided according to wPCDAI at 12months.

n, 19	Baseline	2 Weeks	6 Weeks	6 Months	12 Months	p-values				
						Baseline vs. 2 weeks	Baseline vs. 6 weeks	Baseline vs. 6 months	Baseline vs. 12 months	Between groups at 12 months
IGF-1 SDS										
Remission (n, 9)	-0.6 (-3.8, 2.1)	-0.8 (-3.1, 1.7)	-0.6 (-4.8, 1.4)	-0.4 (-2.6, 1.0)	-0.3 (-1.9, 2.0)	0.77	0.77	0.78	0.17	0.33
Non-remission (n, 10)	0.2 (-3.3, 1.5)	0.3 (-1.5, 1.7)	0.2 (-1.9, 1. 9)	-0.8 (-1.8, 1.9)	-0.4 (-1.9, 2.4)	0.17	0.17	0.86	0.86	
IGFPB-3 SDS										
Remission (n, 9)	0.7 (-2.9, 2.5)	0.2 (-1.1, 2.1)	0.2 (-1.7, 2.2)	0.2 (-1.2, 1.7)	0.3 (-1.0, 2.9)	0.78	0.448	0.52	0.78	0.89
Non-remission (n, 10)	1.4 (-0.3, 3.0)	0.9 (-0.2, 2.9)	0.7 (-0.2, 2.9)	0.9 (-0.9, 2.4)	0.7 (-0.4, 2.3)	0.68	0.68	0.41	0.34	
ALS SDS										
Remission (n, 9)	-0.9 (-2.2, 1.5)	-1.0 (-1.9, 0.3)	-0.7 (-2.3, 0.6)	-0.9 (-1.9, -0.1)	-0.5 (-1.7, 0.2)	0.59	0.52	0.59	0.95	0.72
Non-remission (n, 10)	-0.9 (-1.5, -0.3)	-0.7 (-1.6, 0.1)	-0.9 (-1.3, 0.2)	-1.0 (-2.0, 0.2)	-0.8 (-1.5, -0.1)	0.33	0.14	0.77	0.95	
IGFPB-2 SDS										
Remission (n, 9)	2.2 (-1.2, 4.1)	2.6 (-1.5, 3.9)	1.6 (-0.6, 2.7)	2.7 (-1.3, 2.9)	0.8 (-0.9, 2.7)	0.77	0.37	0.33	0.12	**0.042**
Non-remission (n, 10)	2.1 (0.7, 3.3)	2.4 (0.7, 3.5)	1.7 (0.4, 3.0)	1.4 (0.4, 3.7)	2.4 (0.9, 4.1)	0.96	0.11	0.44	0.86	

Data presented as median (range) and adjusted for bone age; IGF-1 SDS, serum insulin like growth factor-1SD score; IGFBP-3 SDS, serum insulin like growth factor binding protein-3 SD score; ALS SDS acid-labile subunit SD score; IGFBP-2 SDS, serum insulin like growth factor binding protein-2 SD score. Values in bold indicate statistical significanc

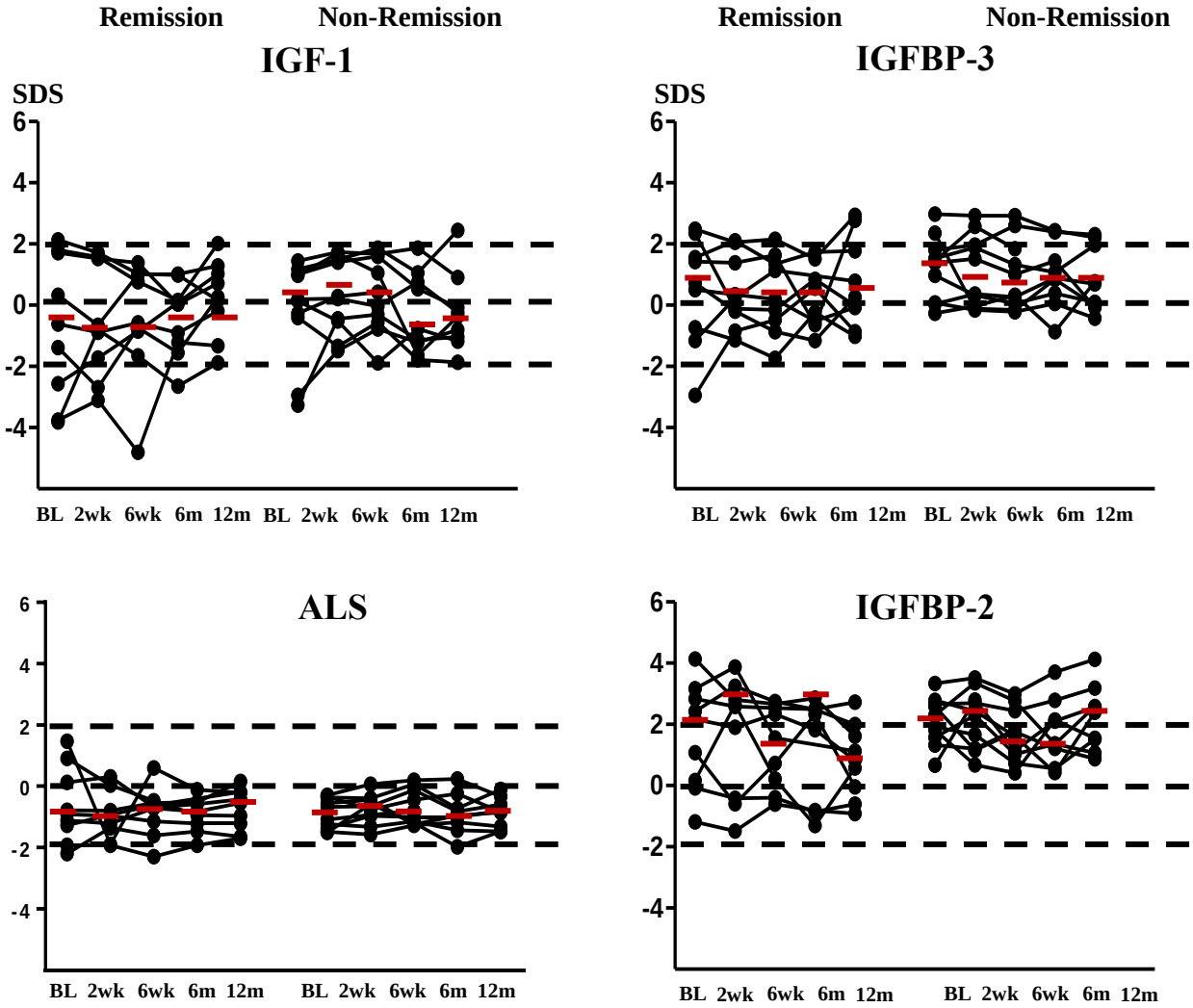

Figure 2-7: Individual change in growth biomarkers following 12 months of anti-TNF-α. Participants divided according to wPCDIA at 12 months (remission (left) and non-remission (right)).

IGF-1 SDS, serum insulin like growth factor-1SD score; IGFBP-3 SDS, serum insulin like growth factor binding protein-3 SD score; ALS SDS, acid-labile subunit SD score; IGFBP-2 SDS, serum Insulin like growth factor binding protein-2 SD score; BL, baseline; 2wks, 2weeks; 6wks, 6week; 6m, 6 months; 12m, 12months. Red solid lines represent the median values. Black dotted lines represent the 50th centile (0SDS), 3rd (-2SDS) and 97th (2SDS) centile.

Table 2-9: Changes in growth biomarkers following Anti-TNF-α. Participants divided according to glucocorticoid use

n, 19	Baseline	2Weeks	6Weeks	6 Months	12 Months	p-values				
						Baseline vs. 2 weeks	Baseline vs. 6 weeks	Baseline vs. 6 months	Baseline vs. 12 months	Between groups at 12 months
IGF-1 SDS										
GC naïve (n, 10)	-0.3	-0.6	-0.4	-0.9	-0.2	0.96	0.17	0.44	0.44	0.24
	(-3.3, 1.5)	(-2.7, 1.7)	(-1.9, 1.7)	(-1.8, 1.9)	(-1.9, 1.3)					
GC +/- (n, 7)	1.0	1.5	1.0	0.3	0.2	0.13	0.87	0.25	0.61	
	(-3.8, 2. 4)	(-3.1, 1.1)	(-4.8, 1.9)	(-1.6, 1.7)	(-1.3, 2.1)					
GC +/+ (ID-18)	1.7	1.5	1.4	0.03	0.7					
GC +/+ (ID-19)	-3.8	-0.81	-1.7	-2.6	-1.9					
IGFPB-3 SDSD										
GC naïve (n, 10)	1.4	1.0	0.9	0.4	0.7	0.80	0.19	0.09	0.74	0.83
	(0.0, 3.0)	(-0.2, 2.9)	(-0.9, 2.9)	(-1.2, 2.4)	(-0.4, 2.8)					
GC +/- (n, 7)	1.4	0.2	1.0	1.1	0.8	0.74	0.87	0.35	0.87	
	(-1.2, 2.5)	(-1.1, 2.1)	(-1.7, 2.6)	(-0.9, 2.4)	(-1.0, 2.9)					
GC +/+ (ID-18)	0.50	0.35	0.2	-0.5	-0.1					
GC +/+ (ID-19)	-2.9	-0.9	-0.5	0.6	-0.9					
ALS SDS										
GC naïve (n, 10)	-0.9	0.9	-0.8	-0.8	-0.8	0.65	0.17	0.37	0.44	0.53
	(-1.5, -0.3)	(-1.6, 0.1)	(-1.3, 0.2)	(-1.2, 0.2)	(-1.3, 0.2)					
GC +/- (n, 7)	-1.2	-0.6	-0.7	-1.2	-1.0	0.74	0.74	0.12	0.87	
	(-2.0, 0.9)	(-2.0, 0.3)	(-2.3, 0.1)	(-2.0, -0.6)	(-1.7, -0.1)					
GC +/+ (ID 18)	1.5	-1.9	0.6	-0.1	-0.2					
GC +/+ (ID19)	-2.2	-1.4	-1.6	-1.5	-1.7					
IGFPB-2 SDSD										
GC naïve (n, 10)	2.4	2.2	2.1	1.4	1.5	0.51	0.33	0.07	0.14	0.83
	(-0.1, 3.3)	(-0.4, 3.5)	(-0.4, 3.0)	(-0.9, 3.7)	(-0.6, 4.1)					
GC +/- (n, 7)	1.6	2.6	1.0	1.7	1.5	0.50	0.24	0.92	0.87	
	(-1.2, 4.1)	(-1.5, 3.9)	(-0.6, 2.7)	(-1.3, 2.9)	(-0.9, 2.6)					
GC +/+ (ID-18)	1.1	-0.6	0.7	2.3	-0.1					
GC +/+ (ID-19)	2.8	2.6	2.5	2.5	2.7					

Data presented as median (range) and adjusted for bone age. IGF-1 SDS, serum insulin like growth factor-1SD score; IGFBP-3 SDS, serum insulin like growth factor binding protein-3 SD score; ALS SDS, acid-labile subunit; IGFBP-2 SDS, serum Insulin like growth factor binding protein-2. GC, glucocorticoid; +/-, glucocorticoid at baseline and stopped by 6 months; GC +/+, glucocorticoid throughout the study period.

2.4.7.1 Correlation between IGF-1 and disease biomarkers

Figure 2-8 shows the correlation between IGF-1 SDS with wPCDAI and albumin; at each time point 19 CD received anti-TNF-α for 12 months. At baseline a significant negative correlation was observed between IGF-1 SDS and ESR (r=–0.48; p=0.037), CRP (r=–0.51; p=0.027), and a positive correlation with albumin (r=0.63; p=0.004). At 2 weeks a significant negative correlation was observed between IGF-1 SDS and CRP (r=–0.56; p=0.013) and wPCDAI (r=–0.52; p=0.023) and a positive correlation with albumin (r=0.50; p=0.031). At 6 weeks a significant negative correlation was observed between IGF-1 SDS and CRP (r=–0.69; p=0.001), and wPCDAI (r=–0.55; p=0.015), and a positive correlation with albumin (r=0.55; p=0.016). At 6 months, a significant negative correlation was observed between IGF-1 SDS and CRP (r=–0.81; p=0.00), and wPCDAI (r=–0.69; p=0.003) and a positive correlation with albumin (r=0.54; p=0.030). At 12 months a significant positive correlation was observed between IGF-1 SDS and albumin (r=0.70; p=0.001).

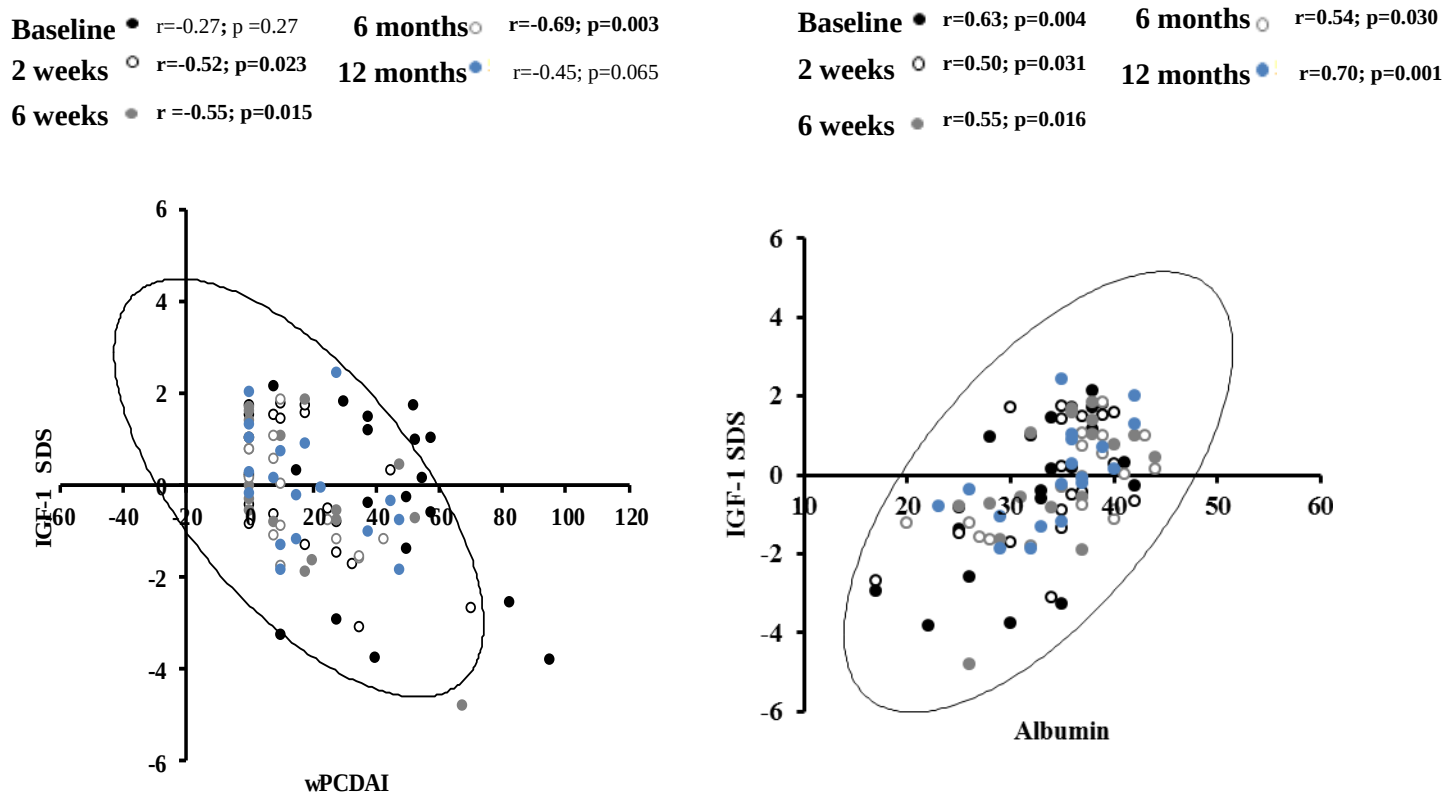

Figure 2-8: Correlation between IGF-1 SDS with wPCDAI (as indicator for disease activity) on the left and Albumin (as indicator for nutritional status) on the right.

Baseline (filled black circle), 2 weeks (empty black circle), 6 weeks (filled grey circle), 6 months (empty grey circle) and 12 months (filled blue circle) in 19 CD received anti-TNF-α for 12 month. Large black circle indicates confidence limit, Values in bold indicate statistical significance ($p<0.05$).

2.4.7.2 Correlation between IGFBP-3 and disease biomarkers

Figure 2-9 shows the correlation between IGFBP-3 SDS with wPCDAI and albumin at each time point in 19 CD receiving anti-TNF-α for 12 months. At baseline a significant negative correlation was observed between IGFBP-3 SDS and wPCDAI (r=–0.57; p=0.014) and a positive correlation with albumin (r=0.69; p=0.001). At 2 weeks a significant negative correlation was observed between IGFBP-3 SDS and CRP (r=–0.60; p=0.007), and a positive correlation with albumin (r=0.64; p=0.003). At 6 weeks a significant negative correlation was observed between IGFBP-3 SDS and CRP (r=–0.61; p=0.006) and a positive correlation with albumin (r=0.53; p=0.02). At 6 months a significant negative correlation was observed between IGFBP-3 SDS with CRP (r=–0.73; p=0.001) and wPCDAI (r=–0.60; p=0.015). At 12 months no significant correlation was observed between IGF-1 SDS and disease biomarkers.

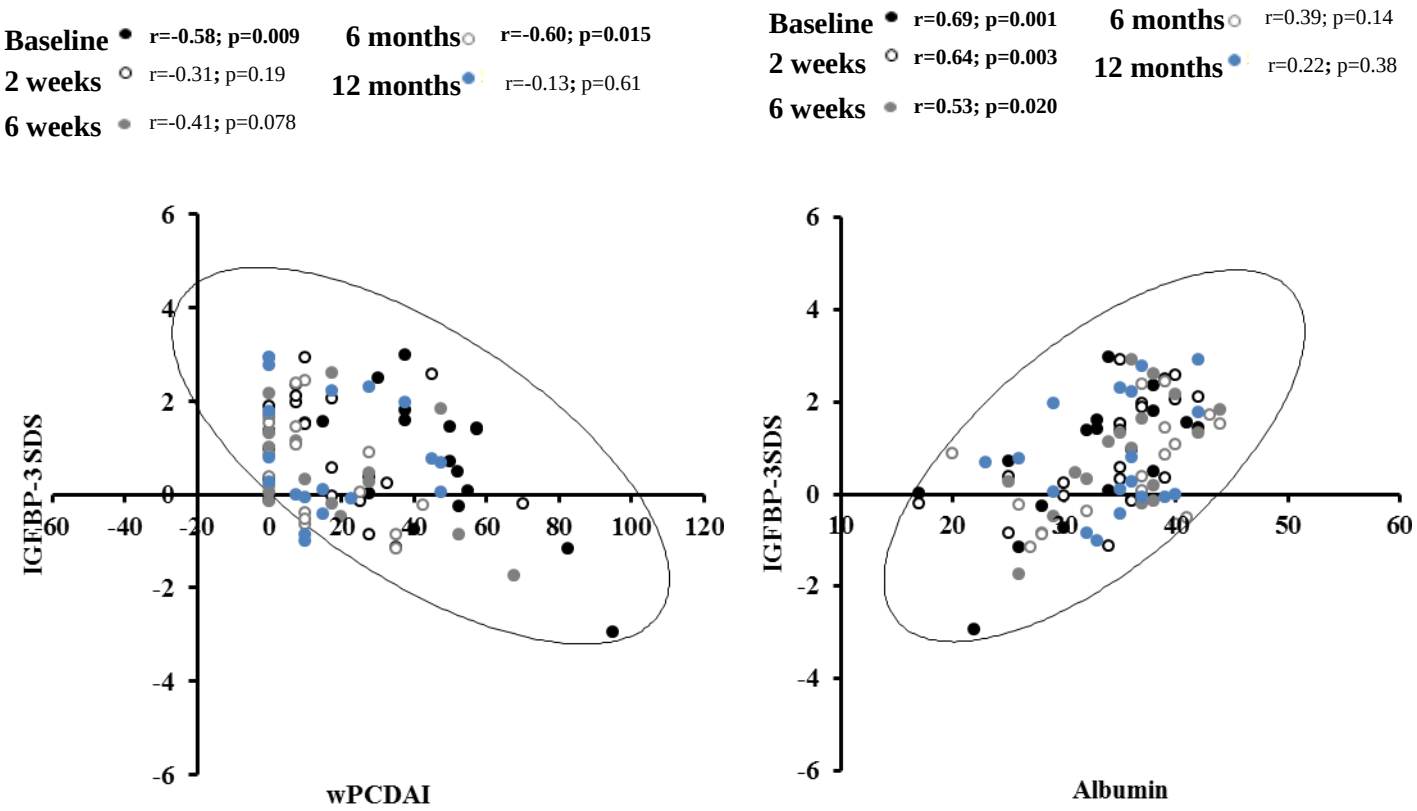

Figure 2-9: Correlation between IGFBP-3SDS with wPCDAI (as indicator for disease activity) on the left and Albumin (as indicator for nutritional status) on the right.

Baseline (filled black circle), 2 weeks (empty black circle), 6 weeks (filled grey circle), 6 months (empty grey circle) and 12 months (filled blue circle) in 19 CD received anti-TNF-α for 12 months. Large black circle indicates confidence limit, Values in bold indicate statistical significance ($p<0.05$).

2.4.7.3 Correlation between ALS and disease biomarkers

Figure 2-10 shows the correlation between ALS SDS with wPCDAI and albumin at each time point in 19 CD receiving anti-TNF-α for 12 months. At baseline a significant negative correlation was observed between ALS SDS with ESR (r=–0.48; p=0.040), CRP (r=–0.54; p=0.018), wPCDAI (r=–0.41; p=0.093) and a positive correlation with albumin (r=0.70; p=0.001). At 2 weeks no significant correlation was observed between ALS SDS and any of the disease biomarkers. At 6 weeks a significant negative correlation was observed between ALS SDS with CRP (r=–0.53; p=0.021) and a positive correlation with albumin (r=0.49; p=0.032). At 6 months a significant negative correlation was observed between ALS SDS with CRP (r=–0.57; p=0.023). At 12 months no significant correlation was observed between ALS SDS and any of the disease biomarkers.

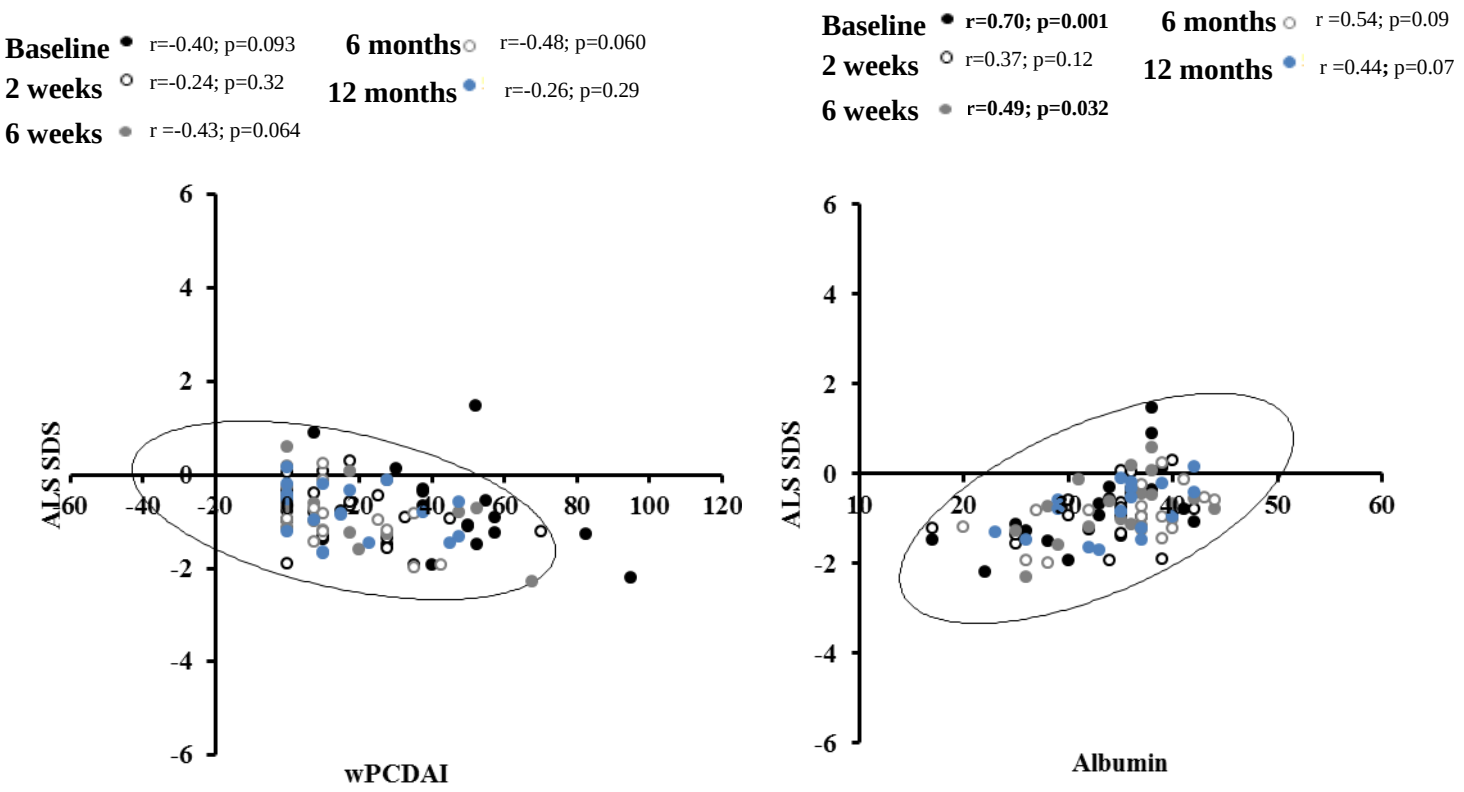

Figure 2-10: Correlation between ALS SDS with wPCDAI (as indicator for disease activity) on the left and Albumin (as indicator for nutritional status) on the right.

Baseline (filled black circle), 2 weeks (empty black circle), 6 weeks (filled grey circle), 6 months (empty grey circle) and 12 months (filled blue circle) in 19 CD received anti-TNF-α for 12 months. Large black circle indicates confidence limit, Values in bold indicate statistical significance ($p<0.05$).

2.4.7.4 Correlation between IGFBP-2 and disease biomarkers

Figure 2-11 shows the correlation between IGFBP-2 SDS with wPCDAI and albumin at each time point in 19 CD receiving anti-TNF-α for 12 months. At baseline a significant positive correlation was observed between IGFBP-2 SDS and ESR (r=0.48; p=0.036), CRP (r=0.54; p=0.017), and a negative correlation with albumin (r=-0.75; p=0.000). At 2 weeks a significant positive correlation was observed between IGFBP-2 SDS and wPCDAI (r=0.50; p=0.031) and a negative correlation with albumin (r=-0.59; p=0.008). At 6 weeks a positive correlation was observed between IGFBP-2 SDS and CRP (r=0.70; p=0.001) and wPCDAI (r=0.75; p=0.000) and a negative correlation with albumin (r=-0.66; p=0.002). At 6 months a significant positive correlation was observed between IGFBP-2 SDS and CRP (r=0.56; p=0.024) and wPCDAI (r=0.71; p=0.002) and a negative correlation with albumin (r=-0.67; p=0.004). At 12 months a significant positive correlation was observed between IGFBP-2 SDS and wPCDAI (r=0.72; p=0.001) and a negative correlation with albumin (r=-0.89; p=0.000). At 12 months, no significant correlation was observed between IGFBP-2 SDS and any of the disease biomarkers.

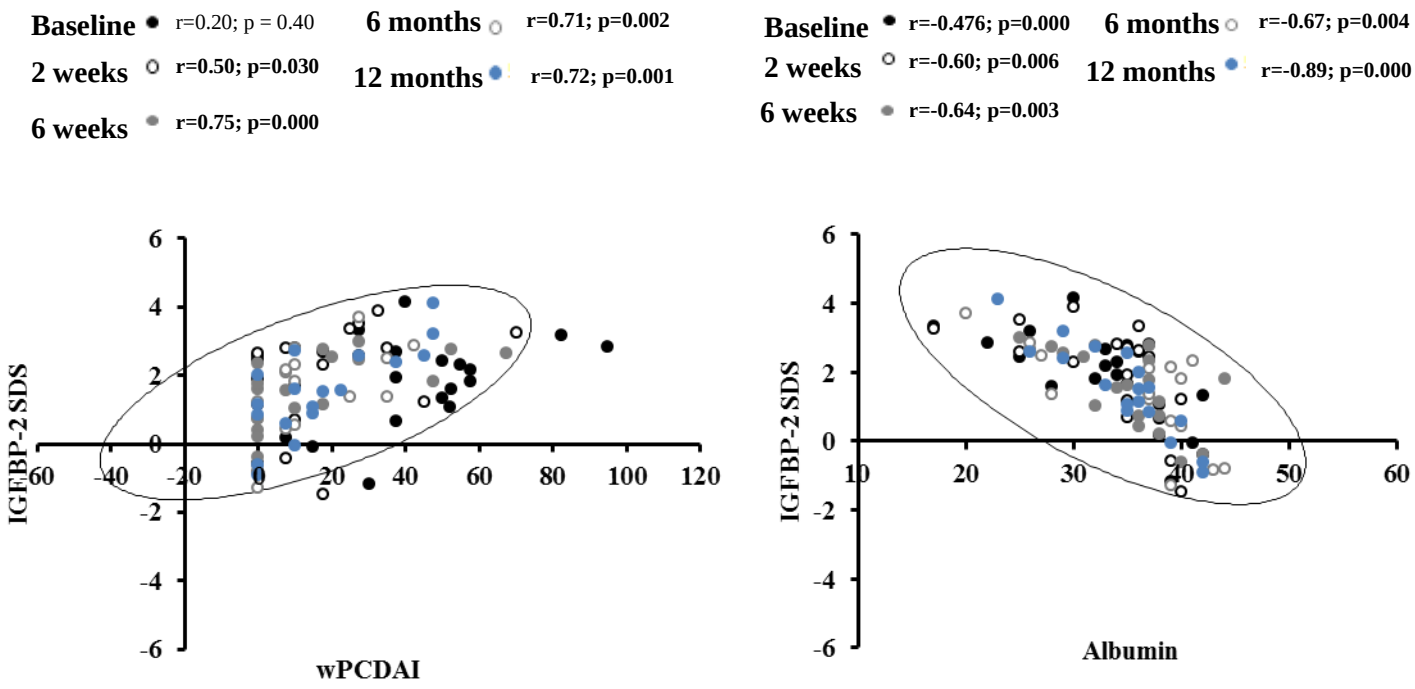

Figure 2-11: The Correlation between IGFBP-2 SDS with wPCDAI (as indicator for disease activity) on the left and Albumin (as indicator for nutritional status) on the right.

Baseline (filled black circle), 2 weeks (empty black circle), 6 weeks (filled grey circle), 6 months (empty grey circle) and 12 months (filled blue circle) in 19 CD received anti-TNF-α for 12 month. Large black circle indicates confidence limit, Values in bold indicate statistical significance ($p<0.05$).

2.4.7.5 Correlation between IGF-1/IGFBP-3 and disease biomarkers

At baseline, 2 weeks and 6 weeks no significant correlation was observed between IGF-1/IGFBP-3 and any of the disease biomarkers. At 6 months a significant negative correlation was observed between IGF-1/IGFBP-3 with CRP (r=–0.61; p=0.009) and wPCDAI (r=–0.51; p=0.035). At 12 months a significant correlation was observed between IGF-1/IGFBP-3 with albumin (r=0.57; p=0.012).

2.4.7.6 Correlation between growth biomarkers with cytokines and anthropometry

No correlation was observed between growth biomarkers and inflammatory cytokines measured at baseline. At 6wks IGFBP-3 SDS was inversely associated with IL-6 (r=–0.52; p=0.029); however, taking out the outliers the results were not significant (r=-0.46; p=0.072). No association was observed between other growth biomarkers and inflammatory cytokines measured at other time points. At baseline, 12 months no significant correlation was observed between Ht SDS and growth biomarkers in children with growth potential. ΔHt SDS and HV at 12 months in children with growth potential were not associated with a 12 month change in growth biomarkers.

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2.4.7.7 Mixed model

Table 2-10 displays the results of multivariate mixed-model regression analysis of the relationship of growth biomarkers SDS adjusted for gender and BA with albumin, wPCDAI, IL6 and GC exposure (yes/no) during the 12-month study interval. IGF-1 SDS and ALS SDS were significantly and positively associated with albumin independent of wPCDAI, IL6 and GC use. IGFBP-3 SDS was also significantly and positively associated with albumin and inversely associated with GC use independent of wPCDI and IL6. IGFB-2 SDS was inversely and significantly associated with albumin and GC use. Similar results were observed by running the same model but with CRP/ESR.

Table 2-10: Mixed model assessment of the effect of inflammation, nutrition and glucocorticoid use on growth biomarkers adjusted for bone age at each time points during 12 months study.

Independent variables	**Dependent variables**							
	IGF-1 SDS		**IGFBP-3 SDS**		**ALS SDS**		**IGFBP-2 SDS**	
	Estimate (SE)	**p-value (95%CI)**	**Estimate (SE)**	**p-value (95%CI)**	**Estimate (SE)**	**p-value (95%CI)**	**Estimate (SE)**	**p-value (95%CI)**
wPCDAI	-0.008 (0.009)	0.362 (-0.02, 0.01)	-0.008 (0.007)	0.250 (-0.02, 0.01)	-0.003 (0.004)	0.523 (-0.01, 0.00)	0.009 (0.006)	0.122 (-0.002, 0.02)
IL-6* (pg/ml)	-0.260 (0.714)	0.404 (-0.88, 0.36)	-0.509 (0.264)	0.058 (-1.04, 0.02)	-0.720 (0.145)	0.629 (-0.36, 0.22)	-0.179 (0.207)	0.389 (-0.59, 0.23)
Albumin (g/l)	0.138 (0.029)	**0.000** (0.08, 0.19)	0.079 (0.025)	**0.002** (0.03, 0.13)	0.056 (0.014)	**0.000** (0.03, 0.08)	-0.138 (0.019)	**0.000** (-0.18, -0.10)
GC exposure	0.263 (0.327)	0.425 (-0.39, 0.91)	-0.691 (0.279)	**0.016** (-1.25, -0.13)	0.113 (0.156)	0.417 (-0.20, -0.42)	-0.512 (0.219)	**0.022** (-0.95, -0.08)

IGF-1 SDS, serum insulin like growth factor-1SD score; IGFBP-3 SDS, serum insulin like growth factor binding protein-3 SD score; ALS SDS acid-labile subunit; IGFBP-2 SDS, serum Insulin like growth factor binding protein-2; wPCDAI, weighted paediatric disease activity index; IL-6, interleukin-6; GC, Glucocorticoid. Values in bold indicate statistical significance ($p<0.05$). *Natural log used to produce normality.

2.4.8 Bone biomarkers

Table 2-11 summarises the median (ranges) changes of bone biomarkers and Figure 2-12 shows individual data of bone biomarkers at baseline, 2 weeks, 6 weeks, 6 months and 12 months among 19 participants. At baseline, BSAP SDS was lower than zero (p=0.00) and significantly increased over 6 weeks. Whereas β-CTX SDS was significantly below zero (p=0.00) at baseline and there was a trend toward an increase over 6 weeks. The median percentage change of BSAP from baseline to 2 weeks was 24.2% (-86.5, 379.4) (p=0.09), from baseline to 6 weeks was 43.6% (-31.2, 299.6) (p=0.001), from baseline to 6 months was 69.3% (-39.5, 844.9) (p=0.001) and from baseline to 12 months was 52.3% (-67.2, 710.3) (p=0.01). The median percentage change of β-CTX from baseline to 2 weeks was 0.7% (-67.1, 189.7) (p=0.95), from baseline to 6 weeks was 14.3% (-51.9, 187.5) (p=0.16), from baseline to 6 months was 24.9% (-59.4, 1359.6) (p=0.03) and from baseline to 12 months was -0.3% (-53, 823.7) (p=0.34). The 12 month magnitude of increases in BSAP SDS levels was higher in participants who were in remission compared with those who did not enter remission (p=0.042) (Table 2-12, Figure 2-13). The magnitude of increases in bone biomarker levels was comparable in participants who were not treated with glucocorticoids throughout induction, compared with those in whom glucocorticoids were discontinued during the induction period (Table 2-13). At baseline, median (range) of serum 25(OH) vitamin D levels was 40.0 nmol/l with 6 (32%) participants having their level below 25 nmol/l and remaining unchanged at 12 months. All 6 participants with serum 25(OH) vitamin D levels below 25 nmol/l were fair skinned and measurements taken between December and January.

Table 2-11:.Changes in bone biomarkers following anti-TNF-α therapy.

n, 19	Baseline	2 Weeks	6 Weeks	6 Months	12 Months	p-values			
						Baseline vs. 2 weeks	Baseline vs. 6 weeks	Baseline vs. 6 months	Baseline vs. 12 months
BSAP SDS	-1.7 (-3.6, -1.0)	-1.5 (-3.6, -0.4)	-1.2 (-3.6, -0.5)	-0.5 (-2.4, 1.1)	-1.1 (-2.3, 2.3)	0.40	**0.013**	**0.00**	**0.004**
β-CTX SDS	-1.1 (-2.6, 0.4)	-1.1 (-2.7, 0.4)	-1.0 (-2.3, 0.5)	-0.6 (-2.1, 0.4)	-0.5(-1.9, 0.4)	0.69	0.30	0.17	0.31
25(OH)D (nmol/l)	40.0 (13.0, 91.0)				37.0 (8.0, 81.0)				0.73
<25 nmol/l, n (%)	6 (32)				6 (32)				

Data presented as median (range) and adjusted for bone age; BSAP SDS, bone-specific alkaline phosphatase SD score; β-CTX SDS, C-telopeptide of collagen cross-link SD score. Values in bold indicate statistical significance ($p<0.05$).

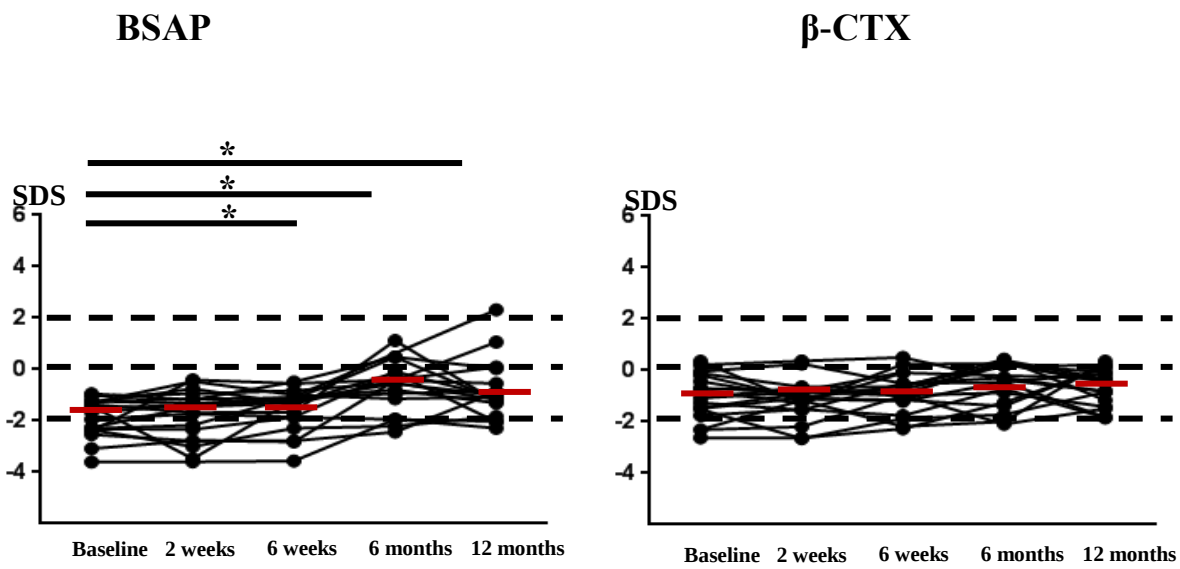

Figure 2-12: Individual change in bone biomarkers following 12 months of anti-TNF-α.
BSAP SDS, bone-specific alkaline phosphatase SD score; B-CTX SDS, C-telopeptide of collagen cross-link SD score. Red solid lines represent the median values. Black dotted lines represent the 50th centile (0SDS), 3rd (-2SDS) and 97th (2SDS) centile. *indicate statistical significance ($p<0.05$).

Table 2-12: Changes in bone biomarkers following anti-TNF-α. Participants divided ccording to wPCDAI at 12months.

n, 19	Baseline	2 Weeks	6 Weeks	6 Months	12 Months	p-values				
						Baseline vs. 2 weeks	Baseline vs. 6 weeks	Baseline vs. 6 months	Baseline vs. 12 months	between groups at 12 months
BSAP SDS										
Remission (n, 9)	-2.3 (-3.1, -1.0)	-1.8 (-2.8, -0.4)	-1.3 (-2.8, -0.5)	-0.4 (-2.6, 1.0)	-0.9 (-1.3, 2.3)	0.17	0.14	**0.048**	0.06	**0.042**
Non-remission (n, 10)	-1.6 (-3.6, -1.0)	-1.4 (-3.6, -1.2)	-1.2 (-3.6, -0.5)	-0.80 (-2.2, 0.5)	-1.3 (-2.3, 0.04)	0.96	**0.02**	**0.044**	0.08	
β-CTX SDS										
Remission (n, 9)	-0.8 (-2.6, 0.4)	-1.3 (-2.7, 0.4)	-0.6 (-2.3, 0.5)	-0.5 (-1.7, 0.3)	-0.5 (-1.8, 0.1)	0.20	0.37	0.26	0.68	**0.54**
Non-remission (n, 10)	-1.1 (-2.3, -0.04)	-0.8 (-1.5, 0.3)	-0.9 (-2.2, -0.1)	-0.3 (-2.1, 0.4)	-0.3 (-1.8, 0.4)	0.09	0.51	0.28	0.31	

Data presented as median (range) and adjusted for bone age; BSAP SDS, bone-specific alkaline phosphatase SD score; B-CTX SDS, C-telopeptide of collagen cross-link. Values in bold indicate statistical significance ($p<0.05$).

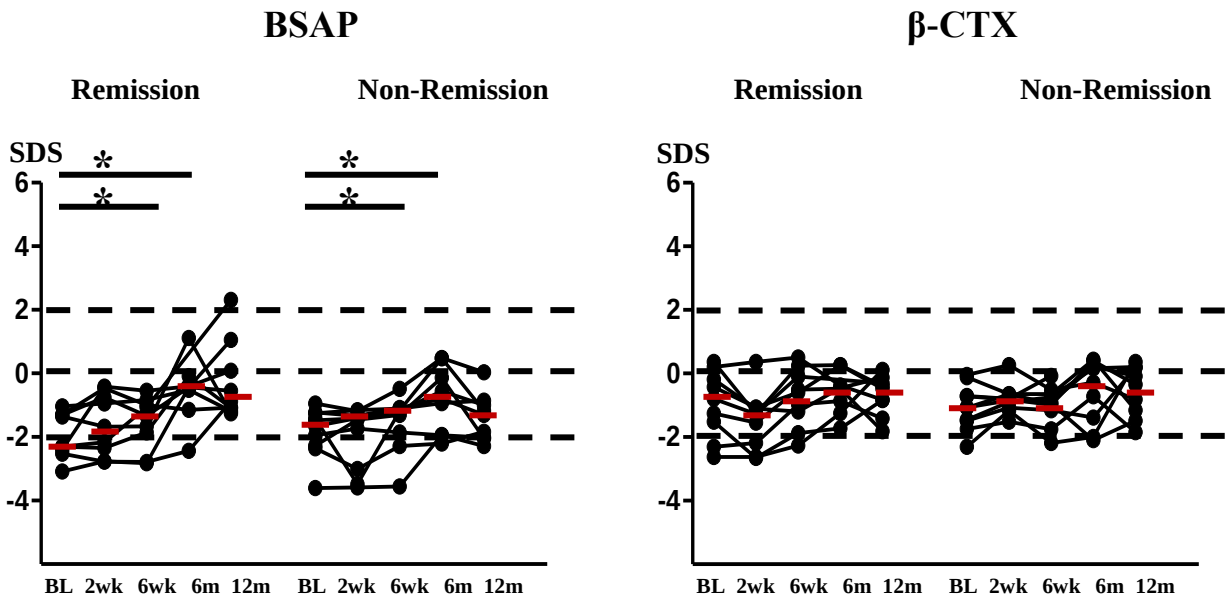

Figure 2-13: Individual change in bone biomarkers following 12 months of anti-TNF-α. Participants divided according to wPCDAI at 12 months (remission (left) and non-remission (right)).

BSAP SDS, bone-specific alkaline phosphatase SD score; B-CTX SDS, C-telopeptide of collagen cross-link SD score. BL, baseline; 2wks, 2weeks; 6wks, 6week; 6m, 6 months; 12m, 12months. Red solid lines represent the median values. Black dotted lines represent the 50th centile (0SDS), 3rd (-2SDS) and 97th (2SDS) centile. *indicate statistical significance ($p<0.05$).

Table 2-13:.Change in bone biomarkers following anti-TNF-α. Participants divided according to glucocorticoid use.

n, 19	Baseline	2 Weeks	6 Weeks	6 Months	12 Months	p-values				
						Baseline vs. 2 weeks	Baseline vs. 6 weeks	Baseline vs. 6 months	Baseline vs. 12 months	between groups at 12 months
BSAP SDS										
GC naïve (n, 10)	-1.5 (-2.4, -1.0)	-1.4 (-3.5, -0.4)	-1.2 (-1.9, -0.5)	-0.4 (-2.0, 0.5)	-1.2 (-2.0, 0.1)	0.66	**0.028**	**0.04**	0.20	0.59
GC +/- (n, 7)	-2.4 (-3.6, -1.3)	-2.8 (-3.6, -1.2)	-2.3 (-3.6, -1.1)	-1.4 (-2.4, -0.1)	-0.9 (-2.3, 2.3)	1.0	0.09	0.11	0.07	
GC +/+ (ID-18)	-1.4	-1.7	-1.7	1.1	-1.3					
GC +/+ (ID-19)	-1.3	-0.5	-1.0	-1.2	-1.1					
β-CTX SDS										
GC naïve (n, 10)	-0.6 (-1.5, 0.4)	-0.9 (-1.3, 0.3)	-0.7 (-1.2, 0.2)	0.2 (-2.1, 0.4)	-0.4 (-1.5, 0.2)	0.39	0.72	0.37	0.95	1.00
GC +/- (n, 7)	-1.7 (-2.6, -0.8)	-1.5 (-2.6, -0.9)	-1.8 (-2.3, -0.1)	-1.1 (-2.0, 0.2)	-0.3 (-1.8, 0.4)	0.61	0.13	0.18	0.11	
GC +/+ (ID-18)	-1.3	-1.5	-0.5	-0.5	-1.8					
GC +/+ (ID-18)	0.2	0.4	0.5	-0.4	-0.8					

Data presented as median (range) and adjusted for bone age; BSAP SDS, bone-specific alkaline phosphatase SD score; β-CTX SDS, C-telopeptide of collagen cross-link SD score. GC, glucocorticoid; +/-, glucocorticoid at baseline and stopped by 6 months; +/+, glucocorticoid throughout the study period. Values in bold indicate statistical significance ($p<0.05$).

2.4.8.1 Correlation between bone biomarkers with disease biomarkers and anthropometric

At baseline, 2 weeks, and 6 weeks no significant correlation was observed between BSAP SDS and β-CTX SDS with any of the disease biomarkers (ESR, CRP, wPCDAI, albumin) or cytokines. At 6 weeks, a significant negative correlation was observed between β-CTX SDS and IL-6 (r=-0.55; p =0.019); however, taking out the outliers the results were not significant (r=-0.44; p=0.090). At 6 months a significant negative correlation was observed between BSAP SDS and CRP (r=-0.50; p=0.048) and a positive correlation with albumin (r=0.65; p=0.007). At 12 months, a significant negative correlation was observed between BSAP SDS and wPCDAI (r=-0.67; p=0.002). The 12 month changes in bone biomarkers were not correlated with 12 month changes in disease biomarkers or 12 months changes in cytokines.

At baseline, 12 months no significant correlation was observed between Ht SDS and bone biomarkers in children with growth potential. ΔHt SDS and HV at 12 months were not associated with a 12 month change in bone biomarkers.

2.5 Discussion

In this chapter we have comprehensively evaluated linear growth, the IGF-1 axis and biomarkers of bone turnover following initiation of anti-TNF-α therapy in children and adolescents with CD. Anti-TNF therapy was associated with rapid improvements in disease activity, improvement in IGF-1 axis for those with low levels and increases in BSAP. The declines in wPCDAI scores and increases in bone formation biomarker levels were associated with subsequent improvements in height in those with growth potential, although no benefit was seen in more mature patients over 12 months follow up.

The rates of growth failure at time of diagnosis seen in our cohort are similar to what has already been published (84;88;89). In our cohort anti-TNF-α maintenance therapy for 12 months was associated with significant improvement in growth velocity and ΔHt SDS, in those who had potential to grow at time of anti-TNF-α induction, although no benefit was seen in more mature patients. These observations are consistent with previous data showing that anti-TNF-α therapy is associated with linear growth improvement in children with chronically active CD in early puberty (88;240). The available data propose that there is a window of opportunity for improvement in growth for those with potential to grow. However, inflammatory bowel disease tends to present in late childhood and adolescence (12) giving a narrow window for growth failure to be diagnosed and treated. In accordance with previous observation (88;240), our study's anti-TNF-α therapy was not associated with significant improvement in Ht SDS and may suggest that controlling the inflammation improves growth velocity, which is not sufficient to improve overall height but prevent further deterioration in height. This finding may suggest that if the anti-TNF-α therapy is given to patients with CD who still had potential to grow, attained height may still be sub-optimal as would be expected in a healthy population. The restoration of normal height distribution in childhood onset patients with CD has been only published recently in one study which showed that among tanner stage 1-2, the earlier initiation of anti-TNF-α (disease duration <18 months after diagnosis) was associated with restoration of normal height distribution by 3 years, whereas tanner stage 1-2 with duration of disease >18 months at initiation of infliximab remain shorter than healthy counterparts (248). The patients in our cohort had longer disease duration compared to the previous study; this may explain our finding.

The sub-optimal first year growth response is defined as minimal increase in height SDS of <0.3 or <0.5 compared with healthy children (283). In our study the net increase in Ht SDS in those with growth potential was 0.1. This observation is consistent with previous data

showing that anti-TNF-α therapy associated with net gain Ht SDS in children with CD ranged from 0.1 to 0.3 SDS with duration of follow up between 6 to 12 months (203;235;239;240;245;249) with one study reporting net increase in height of 2SDS after 1 year; however, the number of patients involved is not clear (238). It obvious from our finding and other previous evidence, net height gain, albeit statistically significant, was clinically modest and if growth is the concern then adjuvant therapy with other forms of growth promoting therapy may also need to be considered.

The intact GH-IGF1 pathway is a critical regulator of linear growth (284). Systemic GH-IGF-1 axis is frequently disturbed in children with CD and it was realised that the depressed concentration was a direct consequence of pro-inflammatory cytokines (112;114) and not merely the result of undernutrition. Thus, the best strategy to restore IGF-1 axis and therefore to improve growth and also improve bone impairment is the resolution of inflammation.

At the time of anti-TNFα induction, we found that patients with CD have a IGF-1 SDS within the average, and IGFBP-3 SDS relatively higher than average. Our finding of an alteration in the IGF-1:IGFBP3 ratio, normal IGF-1 and relatively high IGFBP3 in adolescents with CD suggests that the disease may have a disproportionate effect on these two GH-responsive proteins in favour of reduced IGF-1 bioavailability and is similar to that previously understood for patients with CD (98;115). Furthermore, there was a sub-group in the present study with low IGF-1 SDS which normalised by 12 months. The ability of TNF-α blockade to restore liver GH signalling was shown by DiFedele in a murine colitis model (250); however, a study by Vespasiani et al. (251) failed to demonstrate normalization of IGF-1 levels during maintenance therapy with infliximab. IGF-1 levels did increase during induction though they returned to low baseline levels during maintenance. The short-term effect of anti-TNF therapy on IGF-1 was supported by Eivindson et al. (252).

This is the first report in paediatrics to show low level of ALS SDS in patients with CD which remained low throughout the study period even though those with very low values normalised by 12 months. The finding of low ALS in CD patients is consistent with previous reports of an association of IL-1β with low plasma concentrations of ALS (285;286).

The finding of high IGFBP-2 SDS levels has previously been described in patients with CD diseases and may be caused by catabolic status induced by inflammation itself (117;252;287). Despite controlling disease status in the present study, there was partial normalisation of IGFBP-2 SDS by 12 months; this finding is consistent with adult data

(252). Furthermore, decrease in IGFBP-2 SDS level was more pronounced in subgroups of children who achieved remission compared to those who still had mild to moderate disease.

Multiple correlations have been reported between IGF-1 and IGFBP-3 and inflammatory markers in CD (117;251;252), as in our study univariate analysis demonstrated that IGF-1 SDS and IGFBP3 SDS, ALS SDS were lower in patients with greater disease activity at baseline as depicted by a significant negative correlation of IGF-1SDS, ALS SDS and IGFBP3 SDS with different markers of disease activities; this correlation disappeared by 12 months as disease status improved. However, this is not proof of causality. When these parameters were adjusted for a variety of confounders in a multivariate regression model, albumin was associated with IGF-1, with ALS independent of GC use, inflammatory markers and cytokines. The relative role of nutrition status on GH/IGF-1 axis is established (112). Based on the level of albumin and BMI SDS our study population seems to have a satisfactory nutritional status; this may explain the relatively normal IGF-1 level seen in this cohort. The multivariate regression model showed that albumin and GC use are associated with IGFBP-3 axis independent of inflammatory markers and cytokines. Forty seven percent of our cohort were on GC during the first six weeks of anti-TNF-α therapy and these drugs may also be associated with an alteration in the GH/IGF-1 axis (124). The growth biomarkers based on GC exposure were not different in those who did versus those who did not use GC during the study interval. Nevertheless, it is impossible to isolate the effect of GC therapy on GH-IGF-1 axis weighing its anti-inflammatory properties against its described impact on the GH-IGF-1 axis.

Our data is the first study to explore the improvement in growth in CD following anti-TNF-α in relation to IGF-1 axis. We did not find a relationship between systemic markers of growth and height in those with growth potential. However, the liver is not the only source of IGF-1 as IGF-1 is produced locally in the growth plate. In the mouse, the targeted disruption of IGF-1 synthesis in the liver results in relatively normal-sized mice with a 75% reduction in circulating IGF-1 levels, suggesting that local IGF-1 may be more important for body growth than liver IGF-1 (69) and may in part explain the relatively modest improvement in growth that we see in our cohort.

At the time of starting anti-TNF-α, indirect markers of bone cell function, including BSAP for osteoblasts and β-CTX for osteoclasts reduced suggest that participants have decreased bone turnover and in turn reduced both bone modelling and remodelling at different sites. This state of low bone turnover closely mirrors previously reported results (149;150;178). Comparable to the previous evidence, the current study demonstrates marked increases in

bone formation biomarkers and moderate increases in bone resorption markers following biological therapy (237;243). As cited before, CTX is subjected to diurnal variation and a fasting sample is required (58;60). In the current study blood was taken coincident with time of anti-TNF-α; we did not use fasting blood samples. However, the sample was collected at the same time of day to minimise diurnal variability. In contrast to previous evidence, the biomarkers of bone formation and resorption were not associated with height (205;237).

2.6 Limitations and strengths of study

This study had numerous limitations. First, the number of patients included was small; therefore, meaningful analyses of various subgroups were restricted. However, we increase the obtained association through the application of mixed model regression analysis. A second limitation is the large number of studies outcomes and repeated measures increase the potential for type 1 error; however, we adjust for multiple comparisons by using Bonferroni correction. An additional limitation is we did not have a comparable control group to examine the protective role of anti-TNF-α; however, it was not ethically possible to enrol CD patients with comparable disease severity for untreated control as anti-TNF-α is a standard therapy for children and adolescents with moderate to severe disease. Moreover, we relied on a validated survey of child self-assessment of puberty instead of investigator examination, potentially leading to misclassification of pubertal stage. However, we overcome this issue by adjusting for bone age and subdividing the group according to bone age. Notwithstanding these limitations, this study has important strengths. To our knowledge this is the first study to have examined the effect of anti-TNF-α on IGF-1 and binding protein in the context of chronic inflammation in paediatrics even if these must be regarded as preliminary data owing to the small number of patients enrolled in the study.

2.7 Conclusion

In summary, there is a modest increase in growth in those children who have the potential to grow; there is some evidence of increased bone formation. Our data suggest that targeted therapy with either recombinant human GH or, perhaps, in combination with recombinant human IGF-1 during critical periods of growth needs further exploration.

Chapter Three

Persistence of Musculoskeletal Deficits in Paediatric Crohn's Disease Following Anti-Tumour Necrosis Factor Therapy

3.1 Abstract

Background: CD is associated with deficits in bone mineral density (BMD) and bone structure that are potentially related to tumour necrosis factor α.

Aims: (1) To assess change in bone density and structure in a cohort of children and adolescents with CD at time and 12 month after initiation of treatment with anti-TNF-α. (2) To explore association of IGF-1 axis, cytokines and muscle with bone density in children with CD.

Design and participants: Prospective longitudinal study of 16 (11 male) participants with CD, aged 11.2, 17.2 (median 15.1) years, completing a 12 months study.

Main outcomes: Assessment of areal bone mineral density (aBMD) by DXA (total body (TB), lumbar spine (LS) and volumetric BMD and geometry at tibia and radius were performed using pQCT and obtained at baseline, 6 months and 12 months. DXA bone outcomes expressed as sex, race, bone age or height age specific standard deviation scores (SDS) and compared to reference data. pQCT bone outcomes were expressed as sex, race and age specific standard deviation scores (SDS) and compared to reference data. Muscle measurement by DXA and pQCT and pQCT parameters for thickness were adjusted for sex and height. Maximal isometric grip force (MIGF) of the non-dominant hand was determined by an adjustable-handle Jamar Dynamometer and transformed to sex and height-dependent SDS.

Results: wPCDAI decreased significantly during 2 weeks of induction (p=0.006) with subsequent gains in bone specific alkaline phosphatase level from -1.5 (-3.1, -1.0) at baseline to -0.5 (-2.4, 1.1) at 6 months (p=0.002) and to -1 (-2.0, 2.3) at 12 months (p=0.008). At baseline, DXA BMD SDS (TB-BMD SDS and LS-BMD SDS), radius and tibia pQCT (trabecular BMD SDS and cortical thickness SDS) and muscle mass and function were in substantial deficit and remained unchanged over 12 months. Mixed model regression analysis of tibia pQCT bone outcomes showed that ALS SDS (p=0.007) and muscle area (p=0.003) were positively associated with trabecular BMD; muscle area (p<0.0001) and IGFBP3 SDS (p=0.004) were associated with cortical thickness positively and negatively respectively.

Conclusion: Musculoskeletal health as assessed by imaging in children starting anti-TNF-α therapy for CD showed a deficit in bone and muscle mass at baseline as well as 12 months. Thus, although anti-TNF-α therapy was associated in an improvement in bone

formation, there was insufficient evidence to show a change in bone health as assessed by imaging.

3.2 Introduction

CD is a chronic inflammatory condition that is characterized by impaired bone density and alteration in bone geometry in children (149;150;153;155;165;199;288). The clinical relevance of CD related bone disease can be immediate and results in increased fracture risk (159) or delayed with suboptimal peak bone mass accrual by end of adolescence (165) and therefore may cause significant increase in fracture risk in adulthood. Although the underlying pathogenesis for this poor bone health may be multifactorial (289), the inflammatory process mediated by pro-inflammatory cytokines such a TNF-α and interleukins (IL) may play an important role (150;178;179). The monoclonal anti TNF-α antibody induces and maintains remission in moderate to severe disease and is first line medication in refractory CD (227;249). However, the effect of anti TNF-α therapy on bone health in paediatrics has not yet been established (186;237;241;243). DXA is advised by The International Society for Clinical Densitometry (ISCD) (161) to monitor of BMD in children with CD. However, areal BMD (g/cm2) does not separate bone compartments (cortical/ trabecular) and is confounded by differences in body size (146;290). Furthermore, monitoring areal BMD in young individuals with CD may not entirely account for all the bone abnormalities of a CD related bone disease. Investigating other parameters of bone (volumetric trabecular/cortical BMD, bone geometry such as cortical thickness) and muscle, which is possible pQCT, may provide additional relevant information for skeletal health (146).

The current prospective study was, therefore, performed to evaluate bone health and muscle function using both DXA and pQCT in a cohort of children and adolescents with CD at the time and after initiation of treatment with anti-TNF-α and to explore the association of IGF-1 axis, cytokines and muscle with bone density.

3.3 Methods

3.3.1 Study population

Of 19 participants who were diagnosed with CD and who participated in prospective study of effect of anti-TNF-α on linear growth (Chapter-2), 16 had their bone imaging and 19 had muscle function were included for the purpose of current study. These participants were either newly diagnosed or patients with long-lasting disease in clinical relapse who failed to reach inactive disease despite conventional therapy. Participants were excluded for prior anti-TNF-α therapy in the previous 6 months; pregnancy; and medical illnesses or therapies unrelated to CD that could potentially affect bone , nutrition, or growth such as kidney disease, seizure disorder, diabetes, or liver disease. Disease phenotype was classified using the Montreal criteria (5). None of the participants were on growth or puberty promoting treatments during the period of study. The diagnosis reached in each participant was according to standard criteria after small bowel imaging, endoscopy and colonoscopy with multiple biopsies, and other investigations, as indicated(3).
The study protocol was approved by the local research ethics committee (LREC) Reference Number: 09/S0703/58 and the research and development office. Informed consent was obtained from patients and the parents or guardians.

3.3.2 Concomitant medication, anthropometry and biochemical markers

Detailed of concomitant medication, anthropometry data collection and biochemical markers of inflammation, biochemical markers of growth and bone turnover were described in chapter 2. Cut-off points for categorised the participants according to disease status, GC exposure or growth potential were detailed in chapter 2 and adapted in this chapter to categorise DXA and pQCT and muscle function outcomes.

3.3.3 DXA

DXA scans were performed in 15 participants at the baseline, 6 and 12 months and performed with a narrow fan beam Lunar Prodigy densitometer (GE Medical Systems, Waukesha, Wisconsin, U.S.A) and phantoms analysed using the Encore software (Version 8.80.001). The measurements were performed at total body (TB) and anteroposterior lumbar spine (LS) (L2-L4) with standard positioning techniques were analysed to generate estimates of BMD (g/cm^2) and TB lean mass (TB-LM, kg) and TB fat mass (TB-FM, kg).

Each scan took approximately 10 min to complete. All these scans were acquired by Dr Sheila Shepherd at RHC, Glasgow. To minimise the size effect, we applied height age (Ht-age) adjustment for TB and LS using GE Lunar paediatric reference data of over 2000 US children (5-19 years). To adjust for skeletal maturation, we employed bone age (BA) for TB and LS using GE Lunar paediatric reference data of over 2000 US children (5-19 years). Body composition was adjusted for sex and height using our local reference data of 201(140 male) children aged between 5-19 years. The LS and TB phantom scan was performed daily in our hospital and the coefficient of variation (CV) was 1.315% for spine-BMD for children; precision data for TB measurements are not available for children.

3.3.4 pQCT

Bone measures for density and geometry in non-dominant radius (n,16) and tibia (n,15) were performed by pQCT (XCT-2000scanner; Stratec, Pforzheim, Germany) with voxel size of 0.4 mm and slice thickness of 2 mm. Image acquisition, processing and the calculation of numerical values were performed using the manufacturer's software package (XCT 5.50). The phantom was scanned weekly and no precision data is available in our centre.

All scans were performed and analysed by Dr Sheila Shepherd at RHC, Glasgow to ensure consistency of reference line placement. The pQCT scans were performed at similar time points to DXA. Radius limb length was defined as the distance between the ulnar styloid process and the olecranon. The reference line was placed at the distal part of the horizontal part of the radius end plate. Tibia length was defined as the distance between the medial malleolus and the superior margin of the medial condyle. Limb length was measured manually using a non-stretch retractable anthropometric tape. The reference line was placed through the middle of the horizontal part of the tibia endplate. The measurement sites for the radius were located proximal to a reference line by a distance corresponding to 4% (distal radius, metaphysis) and 66% (diaphyseal radius) of the forearm length. The measurement sites for the tibia were located proximal to a reference line by a distance corresponding to 4% (distal tibia, metaphysis) and 38% (diaphyseal tibia) of the tibia length. For the upper limb the muscle and subcutaneous fat were measured at 66% of the length of the radius. At the 4% site of the radius and tibia trabecular volumetric BMD were evaluated. At the 66% site of the radius and 38% site of the tibia cortical volumetric BMD and cortical thickness were measured. As growth retardation is common in children with CD, the bone size-dependent parameters (muscle and fat cross sectional area and cortical thickness) were corrected for height. Radius reference data were software derived (n, 478)

(291;292) and published data were used to adjust tibia parameters (n, 432) (293). Four participants were excluded from analysis of radius cortical thickness due to movement and poor scan.

3.3.5 Maximal isometric grip force (MIGF)

Maximal isometric grip force (MIGF) of the non-dominant (ND) hand was determined in 19 participants by an adjustable-handle Jamar Dynamometer (Preston, Jackson, Mich., USA). The test was performed in a sitting position with elbow flexed at 90° and the children asked to squeeze as hard as possible. MIGF measured in triplicate with 1 min rest between each test and the highest value was recorded; the result for each participant transformed to height-dependent standard deviation scores (SDS) based on published reference data (n, 315 (148 mal)) (294).

3.3.6 Statistics

Analyses were performed using SPSS software version 22 (New York, USA). Non-parametric data were presented with medians and ranges. Changes between parameters were assessed at different time points and analysed using repeated measures with Wilcoxon signed-rank tests and subsequently adjusted for multiple comparison using a Bonferroni correction and $p<0.05$ considered as statistically significant. Mann Whitney U test was used for continuous and Chi-square test for categorical variable to assess inter-group differences divided according to disease, GC exposure or growth potential. The univariate correlation between variables was assessed using the Spearman's correlation. Mixed-model regression analysis was used to assess the independence of the relationship of bone mass with IGF-1 axis (SDS adjusted for gender and BA), wPCDAI/IL-6, GC exposure and muscle over 3 study visits. The statistical analysis was only done for those who had complete data. All graphs were prepared by GraphPad Prism software version 7 (San Diego California, USA) and XSTAT software version 2016.02.27970 (Addinsoft, New York, USA).

3.4 Results

3.4.1 Participant characteristics

Table 3-1 summarises the characteristics of the 16 CD participants at the time of starting anti-TNF-α. The median (range) age at diagnosis of CD and age at anti-TNF-α initiation was 12.0 years (5.5, 15.5) and 15.1 years (11.2-17.2) with median interval since diagnosis of 3.1 years (0.2, 10.7); only 3 participants had the disease duration less than 18 months.

Table 3-1: Participant characteristics at time of starting anti-TNF-α.

	CD (n, 16)
Male, n (%)	11 (69%)
CA at Diagnosis (year)	12.0 (5.5, 15.5)
CA at starting anti-TNF-α (year)	15.1 (11.2, 17.2)
Duration of disease	3.1 (0.20, 10.7)
BA at starting anti-TNF-α (year)	14.4 (9.3, 16.9)
BA delay (year)	0.5 (-0.8, 2.3)
Tanner stage, n (%)	
Pre-puberty (tanner stage-1)	1 (6)
Mid-puberty (tanner stage 2-3)	7 (44)
Late puberty (tanner stage 4-5)	8 (50)
Ht SDS at starting anti-TNF-α	-0.7 (-2.7, 1.7)
BMI SDS at starting anti-TNF-α	-0.4 (-2.7,3 .2)
Disease location, n (%)	
Ileal (L1)	1 (6)
Colonic (L2)	8 (50)
Ileocolonic (L3)	7 (44)
Isolated upper disease(L4)	4 (25)
Behaviour (non-stricturing, non-penetrating)	16 (100)
Perianal	4 (25)

Data presented as number, n; percentage, %, median (range). CA, chronological age; BA, bone age; Ht SDS, height SDS score; BMI SDS, body mass index SDS score.

3.4.2 Disease, laboratory, treatment characteristics and anthropometry

Table 3-2 summarises disease, laboratory and treatment characteristic in 16 CD participants at each visit. At baseline, 8 (50%) participants had moderate to severe disease activity based on wPCDAI with marked improvement to two (12%) at 6 weeks and three (19%) at 12 months. Serum level of growth biomarkers and 25(OH) vitamin D levels did not change during 12 months. 5/16 (31%) participants have their 25(OH) vitamin D level below 25 nmol/l and remained unchanged at 12 months. At baseline, BSAP SDS was lower than zero (p=0.00) and significantly increased over 6 months and this increase was sustained at 12 months. Whereas β-CTX SDS was significantly below zero (p=0.00) at baseline with a trend toward an increase at 6 months. 8/16 (50%) participants were treated with GC at baseline and corresponding data were 8/16 (50%) at 2 weeks, 8/16 (50%) at 6 weeks, 2/16 (12%) at 6 months and 2/16 (12%) at 12 months respectively. None of the participants had surgery during the 12 months of the study interval.

Table 3-2: Changes in disease activity, laboratory results, and treatment over the study interval.

n, 16	Baseline	2 Weeks	6 Weeks	6 Months	12 Months	p-values			
						Baseline vs. 2 weeks	Baseline vs. 6 weeks	Baseline vs. 6 months	Baseline vs. 12 months
wPCDAI	45.0 (0.0, 95.0)	17.5 (0.0, 70.0)	3.7 (0.0, 67.0)	10.0 (0.0, 42.0)	10.0 (0.0, 47.0)	**0.006**	**0.012**	**0.008**	**0.002**
wPCDAI, n (%)									
Not active (<12.5)	2 (12)	7 (44)	10 (58)	10 (63)	9 (56)				
Mild (12.5-40)	6 (38)	8 (50)	4 (25)	4 (25)	4 (25)				
Moderate-sever (>40)	8 (50)	1 (6)	2 (12)	1 (6)	3 (19)	**0.006**	**0.022**	**0.006**	**0.06**
Albumin (g/l)	33.0 (17.0, 41.0)	35.0 (17.0, 42.0)	36.0 (25.0, 42.0)	37.0 (20.0, 44.0)	36.0 (23.0, 42.0)	0.12	0.06	**0.036**	**0.044**
ESR (mm/hr)	28.0 (5.0, 70.0)	12.0 (4.0, 70.0)	13.0 (3.0, 86.0)	13.0 (4.0, 56.0)	12.0 (2.0, 48.0)	**0.004**	0.11	0.092	0.12
CRP (mg/l)	11.0 (3.0, 95.0)	7.0 (3.0, 98.0)	7.0 (3.0, 51.0)	3.0 (3.0, 39.0)	4.0 (3.0, 23.0)	0.19	0.07	0.11	0.11
Il-6 (pg/ml)	53.0 (4.0, 153.0)	-	59.3 (23.8, 155.5)	54.0 (21.0, 151.5)	43.3 (26, 116.0)	-	0.61	0.73	**0.022**
TNF-α (pg/ml)	14.0 (10.0, 48.5)	-	17.5 (9.0, 52.5)	18.8 (8.8, 118.5)	14.5 (9.5, 91.8)	-	0.13	0.09	0.33
IGF-1 SDS	0.2 (-3.8, 2.1)	-0.6 (-3.1, 1.7)	-0.3 (-4.8, 1.8)	-0.8 (-2.6, 1.9)	-0.1 (-1.9, 2.0)	0.82	0.72	0.57	0.68
IGFBP-3 SDS	0.9 (-2.9, 3.0)	0.3 (-1.1, 2.9)	0.3 (-1.7, 2.9)	0.4 (-1.2, 2.4)	0.2 (-1.0, 2.9)	0.49	0.22	0.11	0.72
ALS SDS	-0.9 (-2.2, 1.5)	-0.9 (-1.9, 0.3)	-0.7 (-2.3, 0.6)	-1.0 (-2.0, 0.2)	-0.9 (-1.7, 0.2)	0.96	0.88	0.39	0.76
IGFBP-2 SDS	2.2 (-1.2, 4.1)	2.4 (-1.5, 3.9)	1.7 (-0.6, 3.0)	1.8 (-1.3, 3.7)	1.3 (-0.9, 4.1)	0.57	0.18	0.13	0.25
25(OH) vitamin D	38.0 (13.0, 91.0)	-	-	-	38.0 (8.0, 81.0)	-	-	-	1.00
BALP SDS	-1.5 (-3.1, -1.0)	-1.5 (-3.5, -0.4)	-1.2 (-2.8, -0.5)	-0.5 (-2.4, 1.1)	-1 (-2.0, 2.3)	0.79	.052	**0.002**	**0.008**
β-CTX SDS	-0.9 (-2.6, 0.4)	-1.1 (-2.7, 0.4)	-0.8 (-2.3, 0.5)	-0.4 (-2.1, 0.4)	-0.6 (-1.8, 0.2)	0.30	0.61	0.19	0.76
Medication, n (%)									
Glucocorticoid	8 (50)	8 (50)	8 (50)	2 (12)	2 (12)				**0.022**
Aminosalicylate	8 (50)	7 (44)	8 (50)	7 (44)	4 (25)				0.14
Methotrexate	10 (63)	10 (63)	10 (63)	10 (63)	10 (63)				1.0
Mercaptopurine/Azathioprine	3 (19)	4 (25)	4 (25)	5 (31)	5 (31)				0.41
Exclusive enteral nutrition	5 (31)	4 (25)	3 (19)	2 (12)	2 (12)				0.20
Anthropometry									
Ht SDS	-0.7 (-2.7, 1.7)	-	-	-0.5 (-3.0, 1.7)	-0.4 (-3.1, 1.8)	-	-	0.72	0.84
BMI SDS	-0.4 (-2.7, 3.3)			-0.2 (-2.7, 2.9)	0.2 (-2.7, 2.7)	-	-	0.10	0.28

Data presented as number, n; percentage, %, median (range). wPCDAI, weighted paediatric disease activity index; ESR, erythrocyte sedimentation rate; CRP, C-reactive protein; IL-6, interleukin-6; TNF-α, tumour necrosis factor-α; IGF-1 SDS, serum insulin like growth factor-1SD score; IGFBP-3 SDS, serum Insulin like growth factor binding protein-3 SD score; ALS SDS acid-labile Subunit SD score; IGFBP-2 SDS, serum insulin like growth factor binding protein-2 SD score; BSAP SDS, bone-specific alkaline phosphatase SD score; β-CTX SDS, C-telopeptide of collagen cross-link SD score; HtS DS, height SDS score; BMI SDS, body mass index SDS score. Values in bold indicate statistical significance ($p<0.05$).

3.4.3 DXA bone outcomes

Table 3-3 summarise the median (ranges) changes of the DXA bone outcomes and Figure 3-1 shows individual data at baseline, 6 months and 12 months among 15 participants with results at each visit limited to those with a 12 month visit to facilitate comparisons across the visits. At baseline, overall TB-BMD SDS $_{\text{Ht-age}}$ (p=0.004), TB-BMD SDS $_{\text{BA}}$ (p=0.011), LS-BMD SDS $_{\text{Ht-age}}$ (p=0.00) and LS-BMD SDS $_{\text{BA}}$ (p=0.00) were below zero. The values remained unchanged over the study interval. The categorised DXA bone parameters based on disease status, or GC exposure or growth potential yield the same results (Table 3-4 and Figure 3-2; Table 3-5, Table 3-6 and Figure 3-3).

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Table 3-3: DXA bone outcomes at baseline, 6 months and 12 months following anti-TNF-α.

	Baseline	6 Months	12 Months	p-values	
				Baseline vs. 6 months	Baseline vs. 12 months
DXA	**(n, 15)**	**(n, 14)**	**(n, 15)**		
TB-BMD SDS $_{Ht\text{-}age}$	-0.9 (-2.3, 0.5)	-0.7 (-1.6, 1.1)	-0.9 (-2.1, 1.1)	0.56	0.90
TB-BMD SDS $_{BA}$	-1.2 (-3.0, 0.6)	-1.4 (-2.9, 0.1)	-1.3 (-3.0, 0.2)	0.21	0.12
LS-BMD SDS $_{Ht\text{-}age}$	-1.1 (-2.9, 0.4)	-1.1 (-3.5, 0.4)	-1.0 (-3.2, 0.8)	0.22	0.27
LS-BMD SDS $_{BA}$	-1.5 (-3.4, 0.6)	-1.7 (-2.9, -0.1)	-0.8 (-3.0, -0.2)	0.10	0.18

Data presented as median (range). TB, total body; BMD, areal bone mineral density; SDS $_{Ht\text{-}age}$, adjusted for height age and sex; SDS $_{BA}$, adjusted for bone age and sex; LS, lumbar spine.

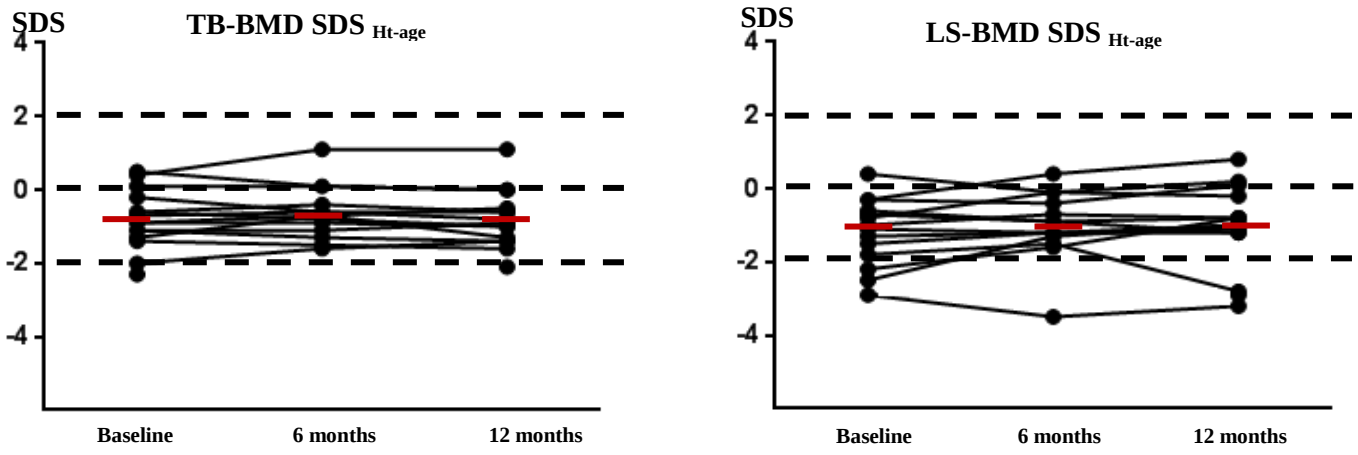

Figure 3-1: Individual changes in DXA bone outcomes at baseline, 6 months and 12 months following anti-TNF-α.

TB, total body; BMD, areal bone mineral density; SDS $_{Ht-age}$, adjusted for height age and sex; SDS $_{BA}$, adjusted for bone age and sex; LS, lumbar spine. Red solid lines represent the median values. Black dotted lines represent the 50th centile (0SDS), 3rd (-2SDS) and 97th (2SDS) centile.

Table 3-4: DXA bone outcomes at baseline, 6 months and 12 months following anti-TNF-α. Participants divided according to wPCDAI at 12 months.

n, 15	Baseline	6 Months	12 Months	p-values		
				Baseline vs. 6 months	Baseline vs. 12 months	Between groups at 12 months
TB-BMD SDS $_{Ht\text{-}age}$						
Remission (n, 9)	-0.6 (-1.3, 0.5)	-0.7 (-1.3, 0.1)	-0.8 (-1.4, 0. 0)	0.59	0.33	0.22
Non-remission (n, 6)	-1.2 (-2.3, 0.4)	-0.70 (-1.6, 1.1)	-1.4 (-2.1, 1.1)	0.10	0.40	
TB-BMD SDS $_{BA}$						
Remission (n, 9)	-0.8 (-3.0, 0.6)	-1.3 (-2.9, 0.10)	-0.9 (-3.0, 0.0)	**0.042**	0.23	0.52
Non-remission (n, 6)	-1.3 (-2.3, -0.5)	-1.3 (-2.0, -0.3)	-1.4 (-2.2, 0.2)	0.14	0.89	
LS-BMD SDS $_{Ht\text{-}age}$						
Remission (n, 9)	-0.8 (-2.9, 0.4)	-0.9 (-3.5, -0.1)	-1.0 (-3.2, 0.2)	0.68	0.77	0.86
Non-remission (n, 6)	-1.7 (-2.9, -0.3)	-1.2 (-1.6, 0.4)	-1.0 (-2.9, 0.8)	0.08	0.23	
LS-BMD SDS $_{BA}$						
Remission (n, 9)	-1.5 (-3.4, 0.6)	-1.9 (-2.9, -0.1)	-0.6 (-2.8, -0.2)	0.09	0.26	0.29
Non-remission (n, 6)	-1.6 (-2.9, -0.5)	-1.6 (-2.3, -0.6)	-1.3 (-3.0, -0.5)	0.71	0.42	

Data presented as median (range). TB, total body; BMD, areal bone mineral density; SDS $_{Ht\text{-}age,}$ adjusted for height age and sex; SDS $_{BA}$, adjusted for bone age and sex; LS, lumbar spine. Values in bold indicate statistical significance ($p<0.05$)

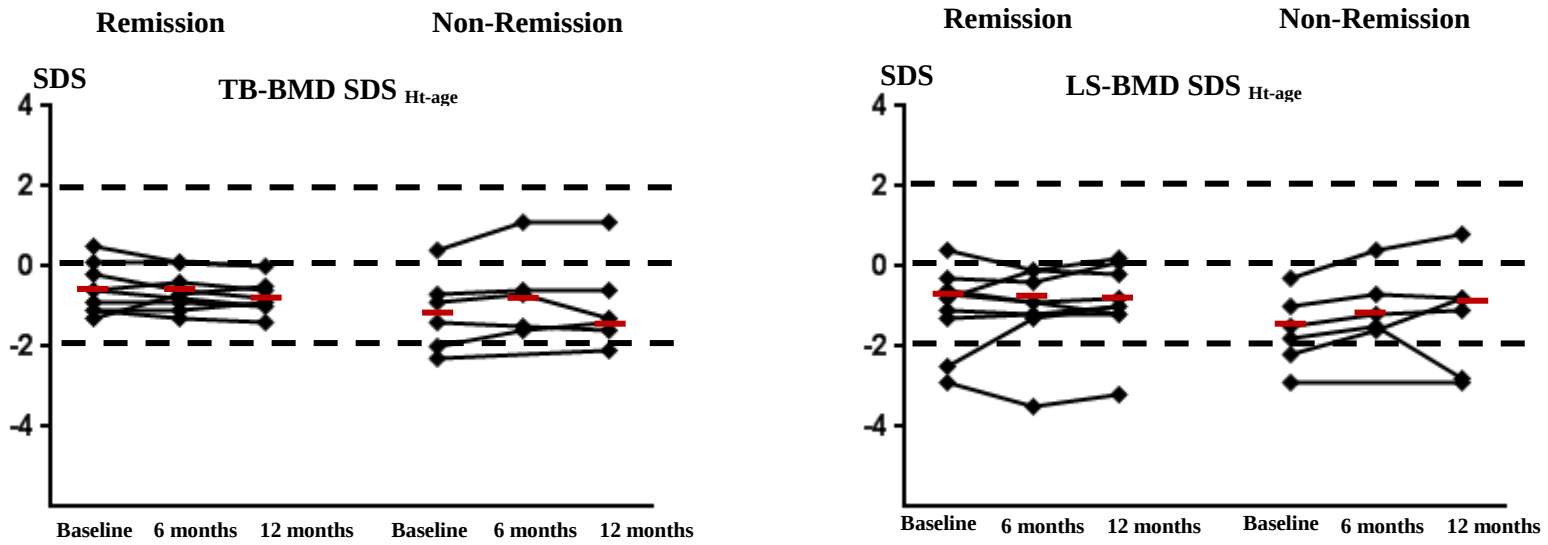

Figure 3-2: Individual changes in DXA bone outcomes at baseline, 6 months and 12 months following anti-TNF-α. Participants divided according to wPCDAI at 12 months (remission (left) and non-remission (right)).

TB, total body; BMD, areal bone mineral density; SDS $_{Ht\text{-}age,}$ adjusted for height age and sex; SDS $_{BA,}$ adjusted for bone age and sex; LS, lumbar spine. Red solid lines represent the median values. Black dotted lines represent the 50th centile (0SDS), 3rd (-2SDS) and 97th (2SDS) centile.

Table 3-5: DXA bone outcomes at baseline, 6 months and 12 months following anti-TNF-α. Participants divided according to glucocorticoid use.

n, 15	Baseline	6 Months	12 Months	p-values		
				Baseline vs. 6 months	Baseline vs. 12 months	Between groups at 12 months
			TB-BMD SDS $_{Ht\text{-}age}$			
GC naïve (n, 7)	-0.7 (-2.3, - 0.2)	-0.7 (-1.6, -0.4)	-1.0 (-2.1, -0.6)	0.67	0.67	0.25
GC +/- (n, 6)	-1.0 (-1.4, 0.4)	-0.8 (-1.5, 1.10)	-0.7 (-1.6, 1.1)	0.28	0.41	
GC+/+ (ID-18)	0.5	0.1	0.0			
GC+/+ (ID-19)	-1.1	-1.3	-1.4			
			TB-BMD SDS $_{BA}$			
GC naïve (n, 7)	-0.8 (-2.3, 0.3)	-1.1 (-2.0, -0.3)	-1.4 (-2.2, -0.2)	0.05	0.12	0.39
GC +/- (n, 6)	-1.5 (-1.7, 0.30)	-1.4 (-1.9, -0.30)	-1.1 (-1.4, 0.2)	0.28	0.28	
GC+/+ (ID-18)	0.6	0.1	0.0			
GC+/+ (ID-19)	-3.0	-2.9	-3.0			
			LS-BMD SDS $_{Ht\text{-}age}$			
GC naïve (n, 7)	-1.8 (-2.9, -0.6)	-1.4 (-3.5, -0.9)	-1.2 (-3.2, -0.8)	0.67	1.00	**0.03**
GC +/- (n, 6)	-0.5 (-1.4, -0.3)	-0.3 (-0.3, 0.4)	-0.3 (-1.2, 0.8)	0.11	0.09	
GC+/+ (ID-18)	0.4	-0.1	-0.2			
GC+/+ (ID-19)	-0.7	-0.9	-0.8			
			LS-BMD SDS $_{BA}$			
GC naïve (n, 7)	-1.8 (-2.9, -0.1)	-2.0 (-2.9, -0.7)	-1.8 (-3.0, -0.2)	0.07	0.60	0.15
GC +/- (n, 6)	-1.0 (-2.7, -0.5)	-1.1 (-2.5, -0.6)	-0.6 (-1.5, -0.4)	0.69	0.22	
GC+/+ (ID-18)	0.6	-0.1	-0.2			
GC+/+ (ID-19)	-3.4	-2.9	-2.8			

Data presented as median (range).TB, total body; BMD, areal bone mineral density; SDS Ht-age, adjusted for height age and sex; SDS BA, adjusted for bone age and sex; LS, lumbarspine; GC, glucocorticoid; +/-, glucocorticoid at baseline and stopped by 6 months; GC+/+, glucocorticoid throughout the study period. Values in bold indicate statistical significance ($p<0.05$).

Table 3-6: DXA bone outcomes at baseline, 6 months and 12 months following anti-TNF-α. Participants divided into two groups on whether they have potential to grow at time of starting anti-TNF-α based on bone age.

n, 15	Baseline (n, 15)	6 Months (n, 14)	12 Months (n, 15)	p-Value		
				Baseline vs. 6 months	Baseline vs. 12 months	Between groups at 12 month
TB-BMD SDS $_{Ht\text{-}age}$						
Group A (n, 7)	-0.9 (-1.4, 0.1)	-0.7 (-1.5, 0.1)	-0.9 (-1.6, 0.0)	0.72	0.55	1.0
Group B (n, 8)	-0.7 (-2.3, 0.5)	-0.6 (-1.6, 1.1)	-0.8 (-2.1, 1.1)	0.67	0.74	
TB-BMD SDS $_{BA}$						
Group A (n, 7)	-1.4 (-1.7, 0.3)	-1.4 (-1.9, -0.4)	-0.90 (-1.4, -0.3)	0.09	0.92	0.52
Group B (n, 8)	-1.0 (-3.0, 0.6)	-1.3 (-2.9, 0.1)	-1.5 (-3.0, 0.2)	0.06	0.24	
LS-BMD SDS $_{Ht\text{--}age}$						
Group A (n, 7)	-1.0 (-2.5, -0.3)	-0.9 (-1.5, -0.1)	-1.0 (-2.8, 0.2)	0.23	0.55	1.0
Group B (n, 8)	-1.4 (-2.9, 0.4)	-1.2 (-3.5, 0.4)	-0.9 (-3.2, 0.8)	0.67	0.35	
LS-BMD SDS $_{BA}$						
Group A (n, 7)	-1.0 (-2.7, -0.1)	-1.2 (-2.5, -0.6)	-0.6 (-1.8, -0.4)	0.53	0.50	0.52
Group B (n, 8)	-2.1 (-3.4, 0.6)	-2.2 (-2.9, -0.1)	-1.5 (-3.0, -0.2)	0.14	0.25	

Data presented as median (range). 15 participants; 7 (47%) had growth potential (BA<13.5yrs in girls and <14.5yrs in boys) (Group (A)); 8 (53%) patients had little/no growth potential (BA≥13.5yrs in girls and ≥14.5yrs in boys) (Group (B)). TB, total body; BMD, areal bone mineral density; SDS $_{Ht\text{-}age}$, adjusted for height age and sex; SDS $_{BA}$, adjusted for bone age and sex; LS, lumbar spine. Values in bold indicate statistical significance (p<0.05).

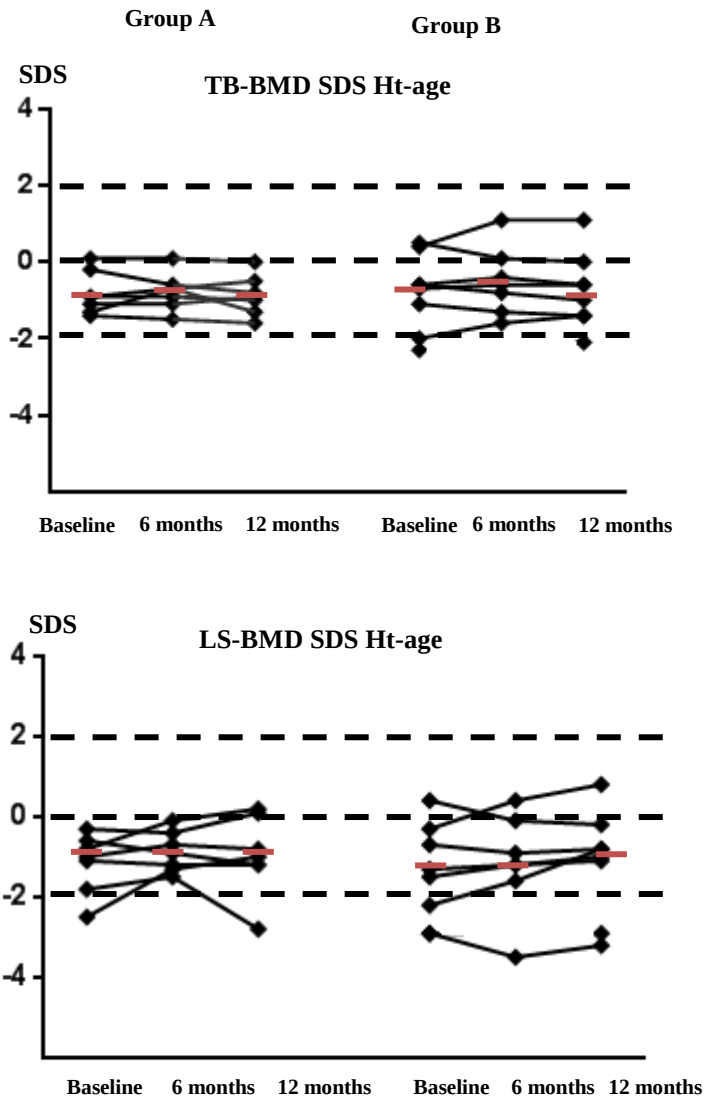

Figure 3-3: Individual changes in DXA bone outcomes at baseline, 6 months and 12 months following anti-TNF-α. Participants divided into two groups on whether they have potential to grow at time of starting anti-TNF-α based on bone age.

Growth potential on the left and had little/no growth potential on the right. TB, total body; BMD, areal bone mineral density; SDS $_{Ht\text{-}age,}$ adjusted for height age and sex; SDS $_{BA,}$ adjusted for bone age and sex; LS, lumbar spine. Red solid lines represent the median values. Black dotted lines represent the 50th centile (0SDS), 3rd (-2SDS) and 97th (2SDS) centile.

3.4.3.1 Correlation of DXA bone outcomes with disease and anthropometry

At baseline a significant negative correlation was observed between TB-BMD SDS $_{Ht\text{-}age}$ and disease duration (r=-0.71, p=0.003) (Figure 3-4) but not with age, disease biomarkers (wPCDAI, CRP, ESR) or cytokines. A significant negative correlation was observed between TB-BMD SDS $_{BA}$ with disease duration (r=-0.73, p=0.002) but not with age disease biomarkers (wPCDAI, CRP, ESR) or cytokines. At baseline LS-BMD SDS $_{Ht\text{-}age}$ and LS-BMD SDS $_{BA}$ were not associated with disease duration or age disease biomarkers (wPCDAI, CRP, ESR) or cytokines. At 6 months, no significant correlation was observed between any of the DXA bone outcomes and disease biomarkers or cytokines. At 12 months a significant negative correlation was observed between TB-BMD SDS $_{Ht\text{-}age}$ and CRP (r=-0.53, p=0.040).

At baseline a significant positive correlation was observed between TB-BMD SDS $_{BA}$ with height SDS (r=0.53, p=0.04). At 6 months no significant correlation was observed between any of the DXA bone outcomes and Ht SDS. At 12 months no significant correlation was observed between any of the DXA bone outcomes and Ht SDS. Repeated analysis for sub-group with growth potential showed no correlation with any of the DXA bone outcomes and Ht SDS at each time point.

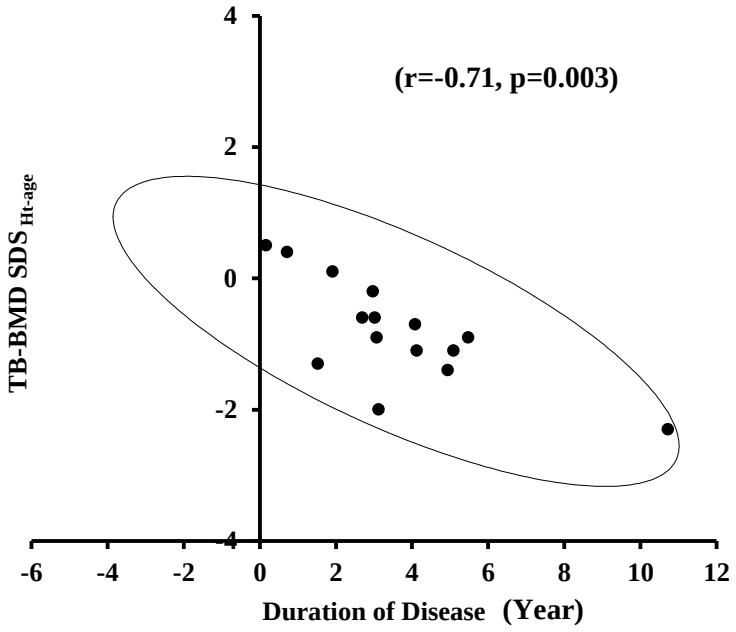

Figure 3-4: Correlation between TB-BMD SDS $_{Ht\text{-}age}$ at baseline with disease duration in 15 CD received anti-TNF-α for 12 months. Large black circle indicates confidence limit

3.4.3.2 Correlation of DXA bone outcomes with biomarkers of growth and bone turnover

Figure 3-5 shows a correlation between TB-BMD SDS $_{Ht\text{-}age}$ with growth biomarkers at each time point in 15 CD receiving anti-TNF-α for 12 months. At baseline a significant positive correlation was observed between TB-BMD SDS $_{BA}$ and IGF-1 SDS (r=0.56, p=0.024), IGFBP-3 SDS (r=0.51, p=0.052), ALS SDS (r=0.65, p=0.009) and a negative correlation with IGFBP-2 SDS (r=-0.55, p=0.036). At 6 months a significant positive correlation was observed between TB-BMD SDS $_{Ht\text{-}age}$ and IGF-1 SDS (r=0.59, p=0.034). At 6 months no significant correlation was observed between DXA bone outcomes and growth biomarkers. At 12, a significant positive correlation was observed between TB-BMD SDS $_{Ht\text{-}age}$ and IGF-1 SDS (r=0.56, p=0.030) and a negative correlation with IGFBP-2 SDS (r=-0.60, p=0.019). At 12, a significant positive correlation was observed between TB-BMD SDS $_{BA}$ and IGF-1 SDS (r=0.55, p=0.034) and a significant negative correlation was observed between LS-BMD SDS $_{BA}$ and IGFBP-2 SDS (r=-0.56, p=0.032). At baseline, 6 months and 12 months no correlation was observed between LS-BMD SDS $_{Ht\text{-}age}$ and growth biomarkers.

At baseline, 6 months and 12 months no significant correlation was observed between DXA bone outcomes with bone biomarkers or 25(OH) vitamins D. No association was observed between 12 months change in BSAP with 12 month change in DXA bone parameters.

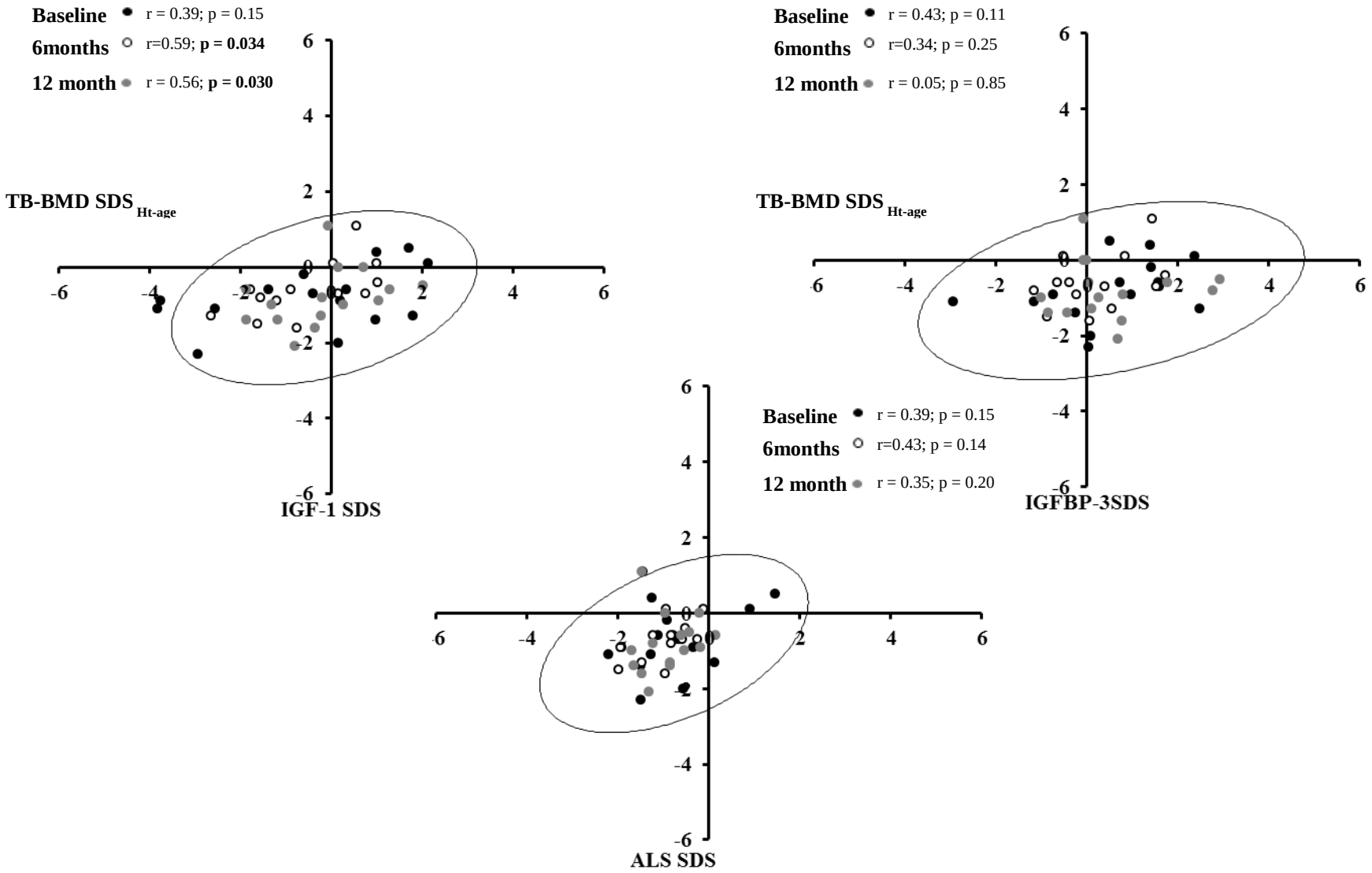

Figure 3-5: Correlation between TB-BMD SDS $_{\text{Ht-age}}$ with growth biomarkers.
Baseline (filled black circle), 6 months (empty black circle) and 12 months (filled grey circle) in 15 CD received anti-TNF-α for 12 months. IGF-1 SDS, serum insulin like growth factor-1SD score; IGFBP-3 SDS, serum insulin like growth factor binding protein-3 SD score; ALS SDS, acid-labile subunit SD score. Large black circle indicates confidence limit, Values in bold indicate statistical significance ($p<0.05$).

3.4.4 pQCT bone outcomes

Table 3-7 summarise radius (n, 16) and tibia (n, 15) pQCT outcomes and Figures 3-6 shows individual data at each visit limited to those with a 12 month visit to facilitate comparisons across the visits.

3.4.4.1 Trabecular BMD

At baseline, both median radius (p=0.001) and tibia (p=0.000) trabecular BMD SDS were significantly below zero and significant deficits remained between 6 and 12 months. Similar observations were seen by stratifying the participants according to the disease conditions or GC exposure or growth potential (Table 3-8 and Figure 3-7; Table 3-9; Table 3-10 and Figure 3-8).

3.4.4.2 Cortical BMD

At baseline, radius cortical BMD SDS was not different from zero (p=0.56) and stayed unchanged over the study period. In contrast at baseline, the tibia cortical BMD was above zero (p=0.004) and there was a trend towards a decline over the study period but it stayed above zero (p=0.029). Consistent with trabecular BMD SDS, no difference in results was seen by stratifying the participants according to the disease conditions or GC exposure or growth potential (Table 3-8 and Figure 3-7; Table 3-9; Table 3-10 and Figure 3-8). However, when dividing the group according to growth potential, those with growth potential had a lower radius cortical BMD SDS at 12 months compared with those who completed their growth and there was a trend towards declined tibia cortical BMD in both groups, even though it was more pronounced in those who still had potential to grow.

3.4.4.3 Cortical Dimension

At baseline the median radius cortical thickness SDS was below zero (p=0.00) in the participants and deficiency persisted at 12 months. At the tibia, cortical thickness SDS was not different from zero (p=0.13) and did not change over the study period or when analysed according to disease status or GC use or growth potential (Table 3-8 and Figure 3-7; Table 3-9; Table 3-10 and Figure 3-8).

Table 3-7: pQCT Bone outcomes at baseline, 6 months and 12 months following Anti-TNF-α.

	Baseline	6 Months	12 Months	p-values	
				Baseline vs. 6 months	Baseline vs. 12 months
Radius pQCT	**(n, 16)**	**(n, 14)**	**(n, 16)**		
Trabecular BMD SDS	-1.8 (3.5, 3.5)	-1.8 (-3.5, 1.0)	-1.8 (-3.0, 2.0)	0.78	0.83
Cortical BMD SDS	-0.2 (-3.3, 2.3)	0.1 (-2.2, 2.5)	-0.4 (-4.8, 2.0)	0.43	0.29
Cortical Thickness SDS (n, 12)	-1.8 (-3.7, 0.8)	-1.8 (-3.3, -1.1)	-1.9 (-2.6, 0.46)	0.72	0.64
Tibia pQCT	**(n, 15)**	**(n, 12)**	**(n, 15)**		
Trabecular BMD SDS	-1.6 (-3.2, 1.1)	-0.9 (-2.1, 0.7)	-1.3 (-2.6, 1.2)	0.12	0.73
Cortical BMD SDS	1.2 (-0.6, 2.8)	0.9 (-1.2, 2.6)	0.5 (-2.5, 2.1)	0.18	0.05
Cortical Thickness SDS	-0.1 (-2.1, 1.0)	-0.3 (-1.9, 0.3)	-0.3 (-2.0, 0.7)	0.43	0.53

Data presented as median (range). BMD, volumetric bone mineral density.

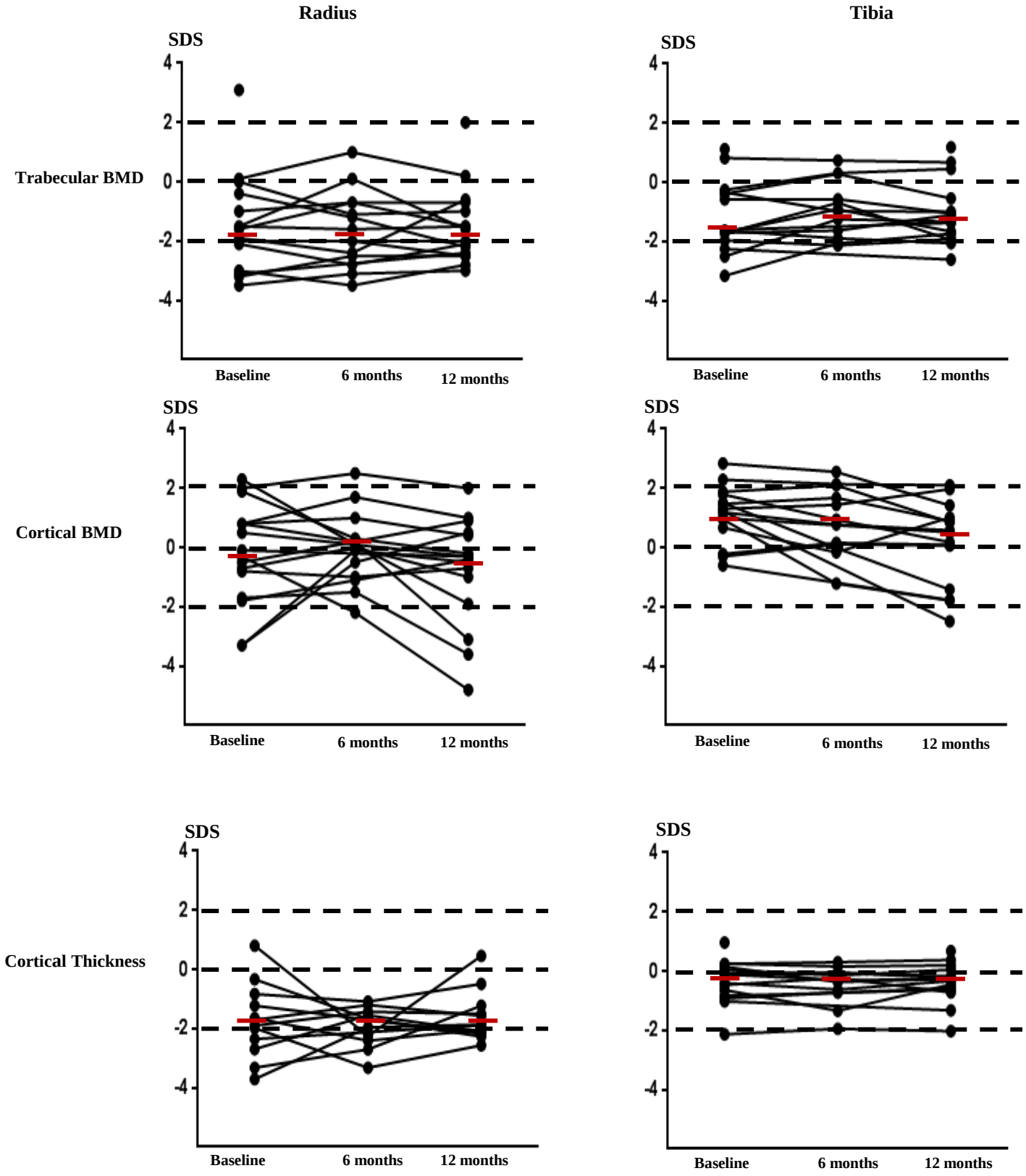

Figure 3-6: Individual changes in pQCT (Radius& Tibia) bone outcomes at baseline, 6 months and 12 months following anti-TNF-α.
BMD, volumetric bone mineral density. Red solid lines represent the median values. Black dotted lines represent the 50th centile (0SDS), 3rd (-2SDS) and 97th (2SDS) centile.

Table 3-8: pQCT bone outcomes at baseline, 6 months and 12 months following anti-TNF-α. Participants divided according to wPCDAI at 12months.

	Baseline	6 Months	12 Months	p-values		
				Baseline vs. 6 months	Baseline vs. 12 months	Between groups at 12 months
Radius pQCT	**(n, 16)**	**(n, 14)**	**(n, 16)**			
			Trabecular BMD SDS			
Remission (n,9)	-1.9 (-3.5, 0.0)	-2.0 (-3.5, -0.7)	-1.5 (-3.0, -0.6)	0.40	0.53	0.79
Non-remission (n,7)	-1.5 (-3.2, 3.1)	-1.2 (-2.5, 1.0)	-2.0 (-2.5, 2.0)	0.27	0.67	
			Cortical BMD SDS			
Remission (n,9)	-0.3 (-3.3, 2.0)	0.0 (-2.2, 2.5)	-0.5 (-4.8, 2.0)	0.51	0.32	0.53
Non-remission (n,7)	-0.1 (-1.8, 2.3)	0.1 (-1.1, 1.7)	-0.3 (-1.9, 1.0)	0.68	0.93	
			Cortical Thickness SDS (n, 12)			
Remission (n,6)	-1.8 (-3.3, -0.5)	-1.6 (-2.7, -1.1)	-1.8 (-2.3, -0. 3)	0.92	0.60	0.94
Non-remission (n,6)	-1.9 (-3.7, 0.8)	-2.1 (-3.3, -1.4)	-1.9 (-2.6, 0.5)	0.72	0.92	
Tibia pQCT	**(n, 15)**	**(n, 12)**	**(n, 15)**			
			Trabecular BMD SDS			
Remission (n,9)	-1.9 (-3.5, 0.0)	-2.0 (-3.5, -0.7)	-1.5 (-3.0, -0.6)	0.40	0.53	
Non-remission (n,7)	-1.5 (-3.2, 3.1)	-1.2 (-2.5, 1.0)	-2.0 (-2.5, 2.0)	0.27	0.67	0.18
			Cortical BMD SDS			
Remission (n,8)	1.2 (-0.3, 2.3)	0.2 (-1.2, 2.1)	0.1 (-2.5, 2.1)	0.50	0.07	0.22
Non-remission (n,7)	1.3 (-0.6, 2.8)	1.4 (-0.2, 2.6)	0.9 (-1.8, 2.0)	0.23	0.11	
			Cortical thickness SDS			
Remission (n,8)	-0.4 (-2.1, 0.3)	-0.4 (-1.9, 0.2)	-0.4 (-2.0, 0.2)	0.40	0.67	0.33
Non-remission (n,7)	-0.1 (-0.8, 1.0)	-0.2 (-0.6, 0.3)	-0.2 (-0.7, 0.7)	0.89	0.74	

Data presented as median (range). BMD, volumetric bone mineral density.

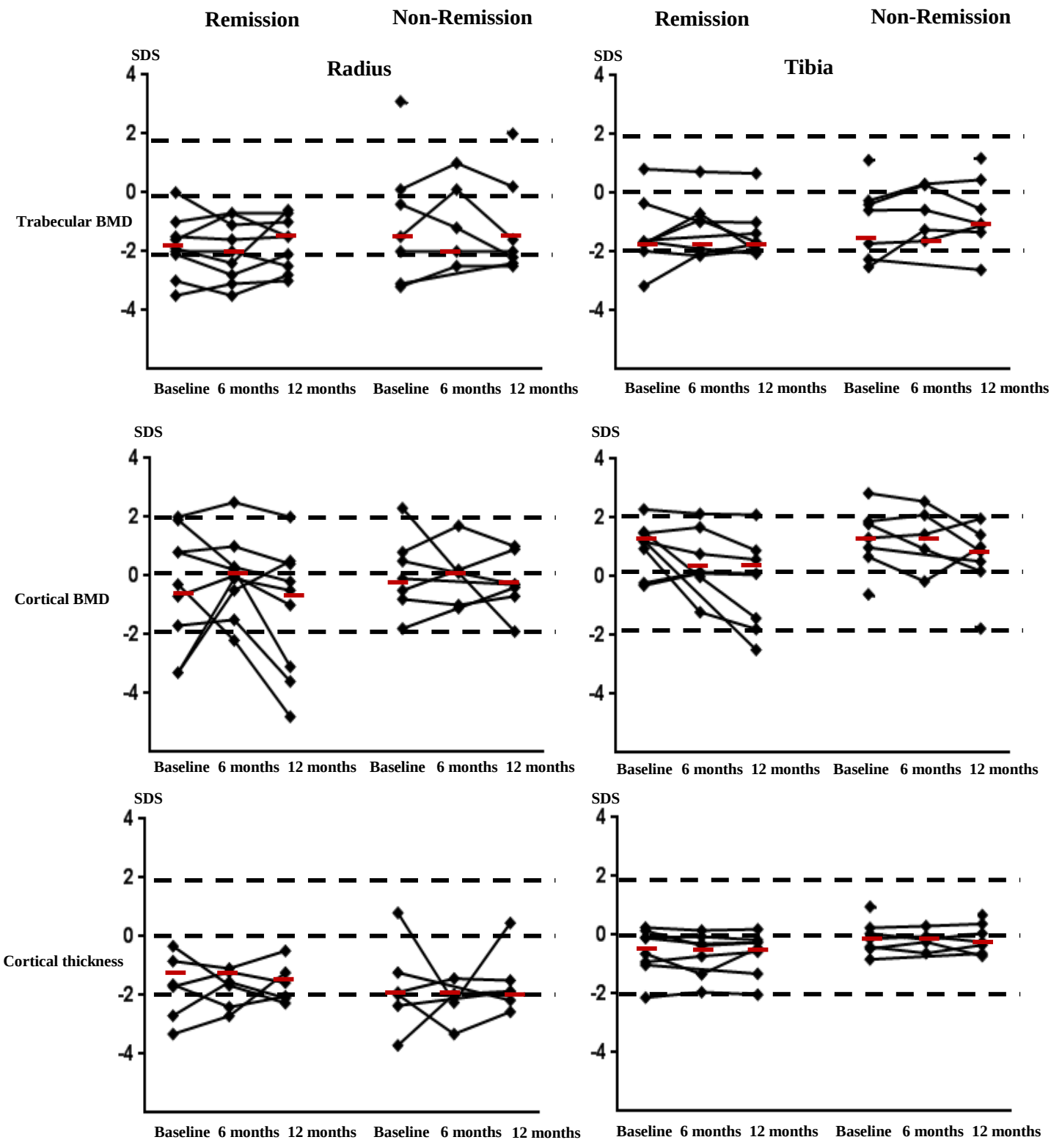

Figure 3-7: Individual changes in pQCT (Radius& Tibia) bone outcomes at baseline, 6 months and 12 months following anti-TNF-α. Participants divided according to wPCDAI at 12 months (remission (left) and non-remission (right)).

BMD, volumetric bone mineral density. Red solid lines represent the median values. Black dotted lines represent the 50th centile (0SDS), 3rd (-2SDS) and 97th (2SDS) centile.

Table 3-9: pQCT bone outcomes at baseline, 6 months and 12 month following anti-TNF-α. Participants divided according to glucocorticoid use.

	Baseline	6 Months	12 Months	p-values		
				Baseline vs. 6 months	Baseline vs. 12 months	Between groups at 12 months
Radius pQCT	**(n, 16)**	**(n, 14)**	**(n, 16)**			
			Trabecular BMD SDS			
GC naïve (n, 8)	-2.0 (-3.2, 3.1)	-2.2 (-2.8, -0.7)	-2.1 (-2.5, 2.0)	1.00	0.92	1.0
GC +/- (n, 6)	-1.3 (-3.5, 0.1)	-0.9 (-3.1, 1.0)	-1.3 (-3.0, 0.2)	0.35	0.75	
GC +/+ (ID-18)	-1.5	-1.6	-1.5			
GC +/+ (ID-19)	-3.0	-3.5	-2.8			
			Cortical BMD SDS			
GC naïve (n, 8)	0.7 (-1.8, 1.9)	0.3 (-1.1, 1.7)	-0.3 (-3.1, 1.0)	0.61	0.44	0.06
GC +/- (n, 6)	-0.8 (-3.3, 2.3)	-0.8 (-2.2, 0.1)	-1.5 (-4.8, 0.5)	1.00	0.25	
GC+/+ (ID-18)	2.0	2.5	2			
GC+/+ (ID-19)	-3.3	-0.1	-0.5			
			Cortical thickness SDS(n, 12)			
GC naïve (n, 7)	-1.9 (-3.7, -0.3)	-2.0 (-3.3, -1.4)	-2.0 (-2.6, -1.5)	0.69	0.61	0.17
GC +/- (n, 3)	-2.7 (-3.3, 0.8)	-2.3 (-2.7, -1.6)	-1.2 (-2.1, 0.5)	1.00	0.29	
GC+/+ (ID-18)	-0.8	-1.1	-0.5			
GC+/+ (ID-19)	-1.7	-1.2	-1.6			
Tibia pQCT	**(n, 15)**	**(n, 12)**	**(n, 15)**			
			Trabecular BMD SDS			
GC naïve (n, 7)	-1.6 (-2.3, 1.1)	-1.1 (-2.1, 0.3)	-1.1 (-2.6, 1.2)	0.47	1.00	0.83
GC +/- (n, 6)	-1.7 (-3.2, -0.3)	-0.9 (-2.1, 0.3)	-1.5 (-2.0, 0.5)	0.16	0.25	
GC+/+ (ID-18)	0.8	0.7	0.7			
GC+/+ (ID-19)	-1.6	-1.9	-2.1			
			Cortical BMD SDS			
GC naïve (n, 7)	1.2 (-0.6, 2.8)	1.6 (-0.2, 2.6)	0.9 (-2.5, 2.0)	0.46	0.13	0.72
GC +/- (n, 6)	1.7 (1.0, 2.3)	0.9 (-1.2, 2.1)	0.4 (-1.8, 2.1)	0.16	0.06	
GC+/+ (ID-18)	-0.2	0.2	0.1			
GC+/+(ID-19)	-0.03	0.1	0.1			
			Cortical thickness SDS			
GC naïve (n, 7)	-0.1 (-1.0, 1.0)	-0.1 (-0.2, -0.1)	-0.2 (-1.3, -0.7)	0.47	0.13	1.00
GC +/- (n, 6)	-0.5 (-2.1, 0.3)	-0.7 (-1.9, 0.3)	-0.4 (-2.0, 0.4)	0.60	0.09	
GC+/+ (ID-18)	0.2	-0.4	-0.3			
GC+/+(ID-19)	-0.1	-0.3	-0.3			

Data presented as median (range). BMD, volumetric bone mineral density; GC, glucocorticoid; +/-, glucocorticoid at baseline and stopped by 6 months; GC+/+, glucocorticoid throughout the study period.

Table 3-10: pQCT Bone outcomes at baseline, 6 months and 12 months following anti-TNF-α. Participants divided into two groups on whether they have potential to grow at time of starting anti-TNF-α based on bone age.

	Baseline	6 Months	12 Months	p-values		
				Baseline vs. 6 months	Baseline vs. 12 months	Between groups at 12 months
Radius pQCT	**(n, 16)**	**(n, 14)**	**(n, 16)**			
			Trabecular BMD SDS			
Group (A) (n, 7)	-1.5 (-3.5, 0.0)	-1.1 (-3.1, 0.1)	-1.6 (-3.0,- 0.7)	0.60	0.41	0.67
Group (B) (n, 9)	-2.0 (-3.2, 3.1)	-2.4 (-3.5, 1.0)	-2.0 (-2.8, 2.0)	1.00	0.25	
			Cortical BMD SDS			
Group (A) (n, 7)	-0.8 (-3.3, 0.8)	-1.0 (-2.2, 0.2)	-1.0 (-4.8, 0.5)	0.55	0.41	**0.026**
Group (B) (n, 9)	0.8 (-3.3, 2.3)	0.3 (-0.1, 2.5)	-0.2 (-1.9, 2.0)	0.61	0.53	
			Cortical thickness SDS (n, 12)			
Group (A) (n, 3)	-2.7 (-3.3, -2.0)	-2.7 (-3.3, -1.6)	-2.1 (-2.6, -1.2)	1.00	0.29	
Group (B) (n, 9)	-1.6 (-3.7, 0.8)	-1.7 (-2.4, -1.1)	-1.9 (-2.3, 0.5)	0.61	0.95	0.46
Tibia pQCT	**(n, 15)**	**(n, 12)**	**(n, 15)**			
			Trabecular BMD SDS			
Group (A) (n, 7)	-1.7 (-3.2, -0.3)	-1.0 (-2.1, 0.3)	-1.4 (-2.0, -0.5)	0.09	0.51	0.69
Group (B) (n, 8)	-1.1 (-2.3, 1.1)	-1.1 (-2.1, 0.7)	-1.1 (-2.6, 1.2)	0.75	1.0	
			Cortical BMD SDS			
Group (A) (n, 7)	1.2 (0.7, 1.8)	0.4 (-1.2, 2.1)	0.2 (-2.5, 2.1)	**0.04**	0.09	0.52
Group (B) (n, 8)	1.1 (-0.6, 2.8)	1.6 (0.1, 2.6)	0.7 (-1.8, 1.9)	0.17	0.16	
			Cortical thickness SDS			
Group (A) (n, 7)	-0.6 (-2.1, 0.3)	-0.8 (-1.9, 0.2)	-0.5 (-2.0, 0.1)	0.35	0.31`	0.27
Group (B) (n, 8)	-0.1 (-0.8, 1.0)	-0.2 (-0.4, 0.3)	-0.3 (-0.7, 0.8)	0.92	0.16	

Data presented as median (range). BMD, volumetric bone mineral density. Values in bold indicate statistical significance ($p<0.05$)

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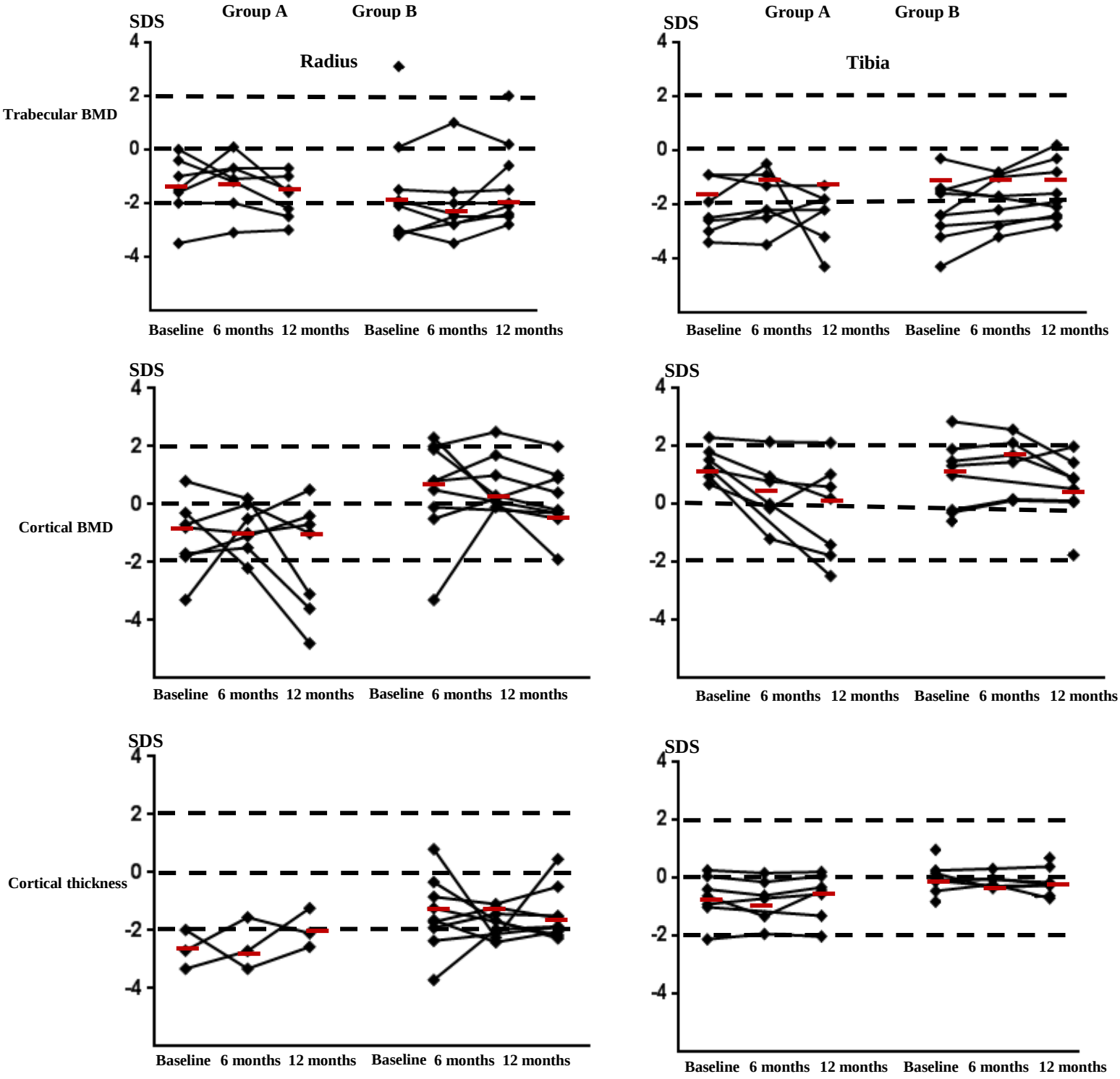

Figure 3-8: Individual changes in pQCT (Radius & Tibia) bone outcomes at baseline, 6 months and 12 months following anti-TNF-α. Participants divided into two groups on whether they have potential to grow at time of starting anti-TNF-α based on bone age.
Growth potential on the left and had little/no growth potential on the right. BMD, volumetric bone mineral density. Red solid lines represent the median values. Black dotted lines represent the 50th centile (0SDS), 3rd (-2SDS) and 97th (2SDS) centile.

3.4.4.4 Correlation of pQCT bone with disease and anthropometry

At baseline, 6 months and 12 months no significant correlation observed between pQCT bone outcomes (at radius /tibia) with disease duration, age, disease biomarkers (wPCDAI, CRP, ESR), cytokines (IL-6, TNF-α). At baseline, 6 months and 12 months no significant correlation was observed between pQCT bone outcomes (at radius /tibia) with Ht SDS. Repeating the analysis for sub-group with growth potential; at 6 months, a significant negative correlation was observed between cortical BMD SDS (Radius) and Ht SDS (r=-0.79, p=0.036). No correlation was observed with other pQCT bone outcomes and Ht SDS at each time point.

3.4.4.5 Correlation of pQCT bone with biomarkers of growth and bone turnover

Figure 3-9; Figure 3-10; Figure 3-11 show correlation between tibia pQCT bone outcomes with growth biomarkers at each time point. At baseline and 6 months no significant correlation was observed between pQCT bone outcomes (at radius /tibia) with growth biomarkers. At 12 months, a significant negative correlation was observed between tibia cortical BMD SDS with IGFBP-3 SDS (r=-0.55, p=0.033).

At baseline, no significant correlation was observed between pQCT bone outcomes (at radius /tibia) with bone biomarkers. At 6 months, a significant positive correlation was observed between radius cortical BMD SDS and BSAP SDS (r=0.69, p=0.01). At 12 months no significant correlation was observed between pQCT bone outcomes (at radius /tibia) with bone biomarkers. Twelve month change in tibia cortical BMD SDS was significantly and negatively correlated with 12 months change in BSAP SDS (r=-0.63, p=0.011). At baseline, 6 months and 12 months no significant correlation was observed between pQCT bone outcomes (radius /tibia) with 25(OH) vitamin D.

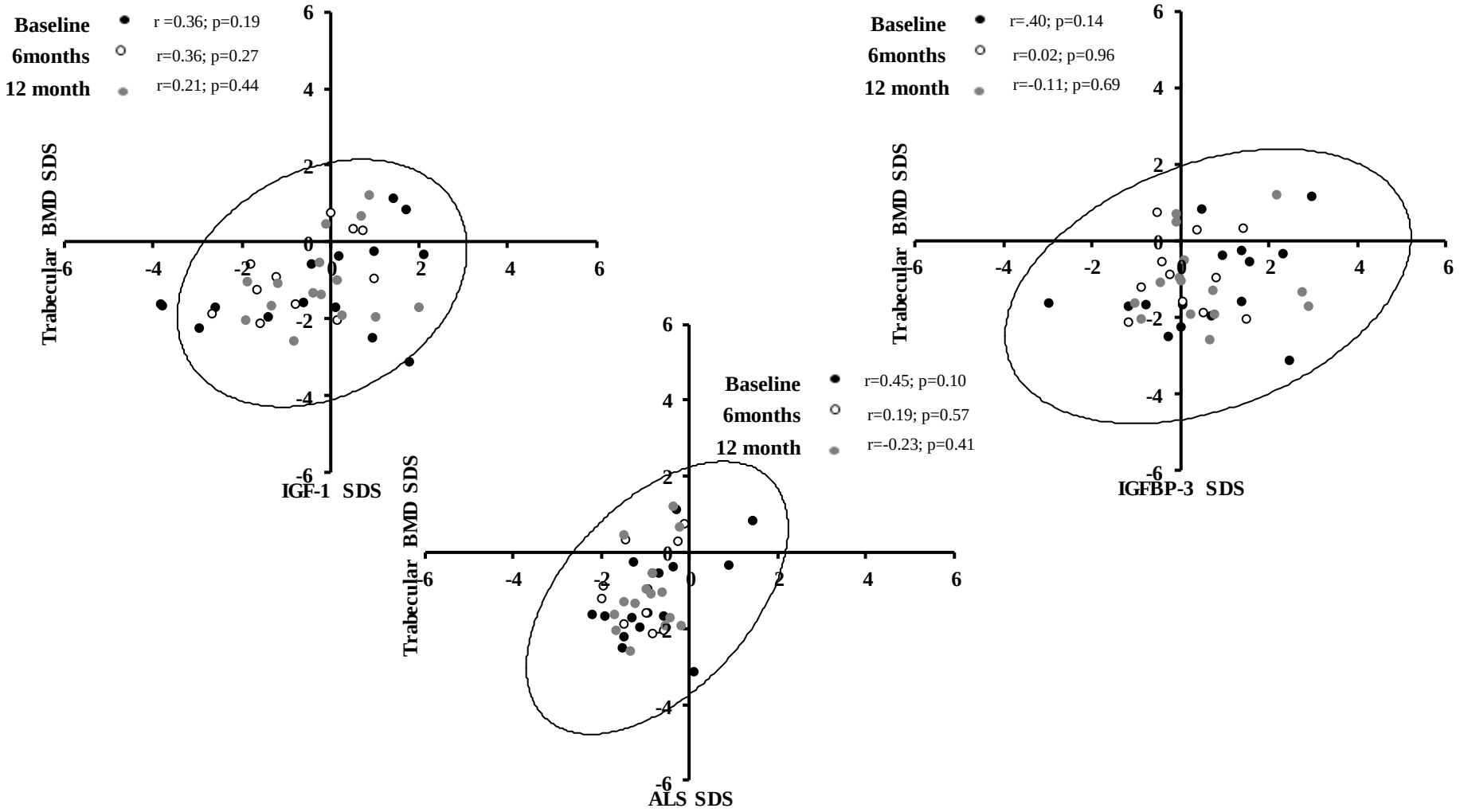

Figure 3-9: Correlation between tibia trabecular BMD SDS with growth biomarkers.

Baseline (filled black circle), 6 months (empty black circle) and 12 months (filled grey circle) in 16 CD received anti-TNF-α for 12 month. IGF-1 SDS, serum insulin like growth factor-1SD score; IGFBP-3 SDS, serum insulin like growth factor binding protein-3 SD score; ALS SDS, acid-labile subunit SD score. Large black circle indicates confidence limit, Values in bold indicate statistical significance ($p<0.05$).

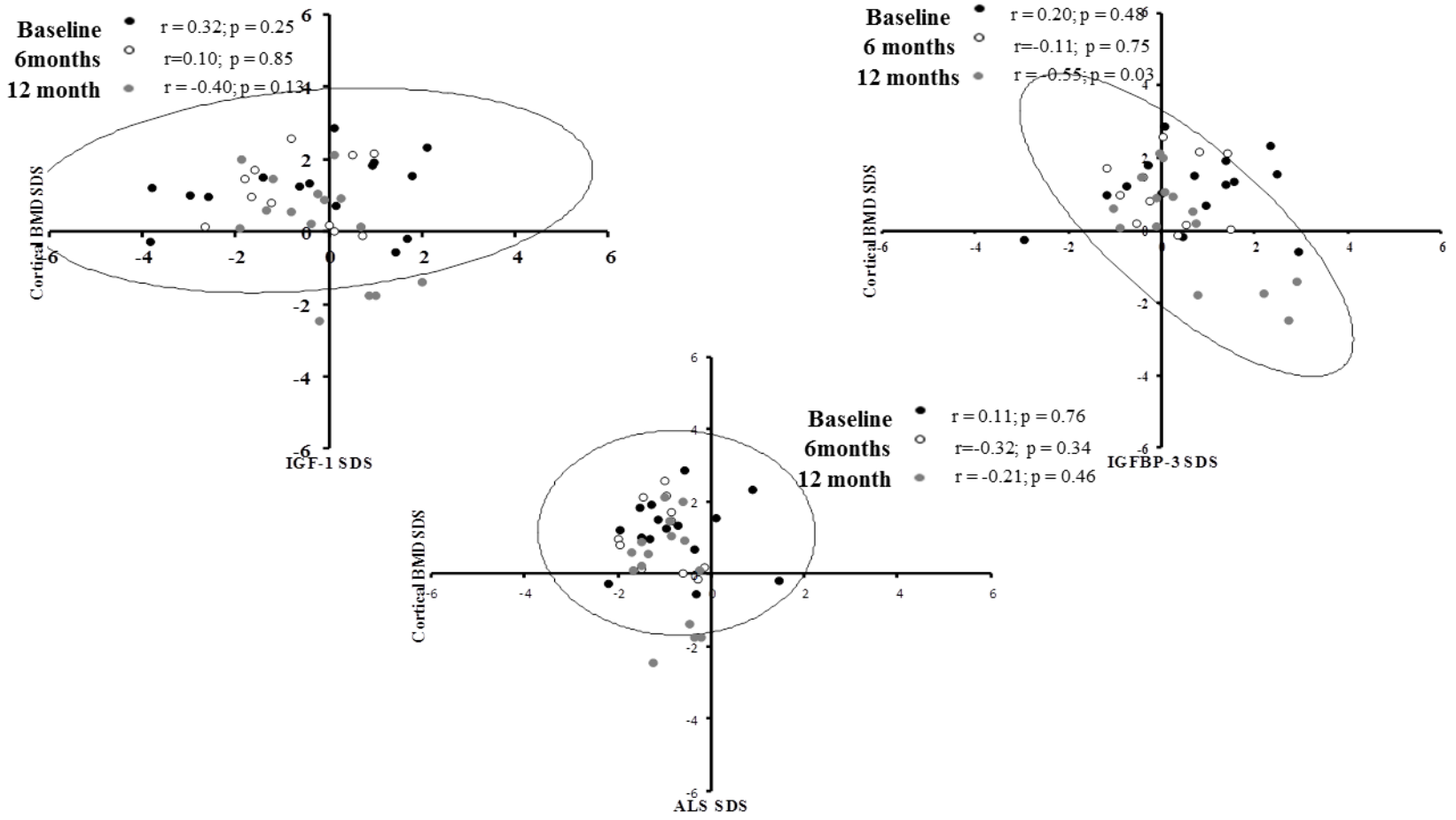

Figure 3-10: Correlation between tibia cortical BMD SDS with growth biomarkers.
Baseline (filled black circle), 6 months (empty black circle) and 12 months (filled grey circle) in 16 CD received anti-TNF-α for 12 month. IGF-1 SDS, serum insulin like growth factor-1SD score; IGFBP-3 SDS, serum insulin like growth factor binding protein-3 SD score; ALS SDS, acid-labile subunit SD score. Large black circle indicates confidence limit, Values in bold indicate statistical significance ($p<0.05$).

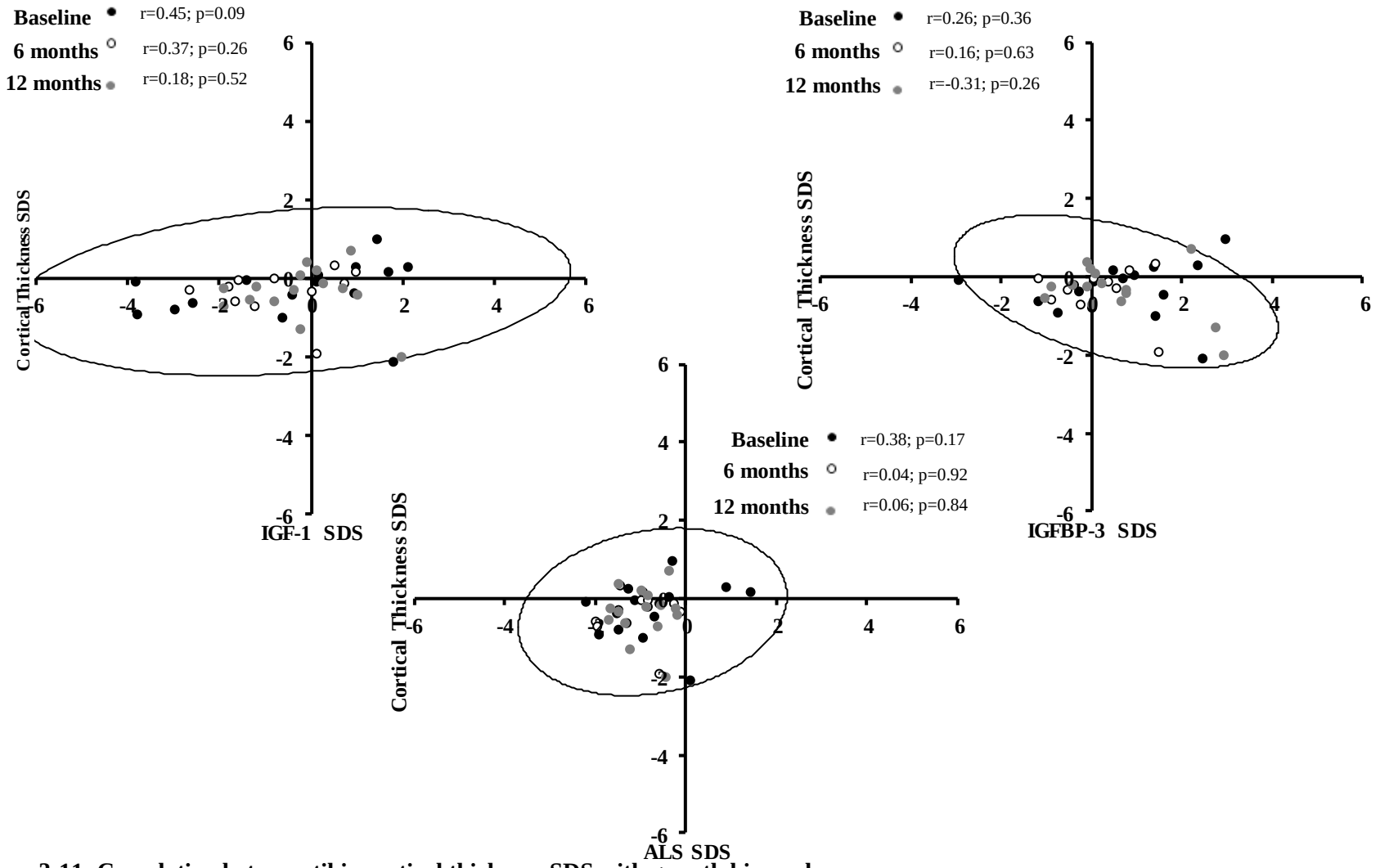

Figure 3-11: Correlation between tibia cortical thickness SDS with growth biomarkers.

Baseline (filled black circle), 6months (empty black circle) and 12 months (filled grey circle) in 16 CD received anti-TNF-α for 12 month. IGF-1 SDS, serum insulin like growth factor-1SD score; IGFBP-3 SDS, serum insulin like growth factor binding protein-3 SD score; ALS SDS, acid-labile subunit SD score. Large black circle indicates confidence limit, Values in bold indicate statistical significance ($p<0.05$).

3.4.5 Change in body composition and ND-MIGF

Table 3-11 summarises DXA (n, 15) and radius pQCT (n, 16) body composition outcomes and MIGF in CD participants at each visit, limited to those with a 12 months visit to facilitate comparisons across the visits.

Table 3-11: Change in body composition by DXA, radius pQCT and MIGF at baseline, 6 months and 12 months following anti-TNF-α.

	Baseline	6 Months	12 Months	p-values	
				Baseline vs. 6 months	Baseline vs. 12 months
DXA	**(n, 15)**	**(n, 14)**	**(n, 15)**		
TB-LM SDS	-1.3 (-3.1, 0.1)	-1.0 (-2.8, 1.8)	-1.4 (-3.7, 0.1)	0.07	0.139
TB-FM SDS	-0.2 (-0.6, 4.3)	-0.1 (-0.7, 3.5)	0.1 (-0.8, 3.0)	0.57	0.38
Radia pQCT	**(n, 16)**	**(n, 14)**	**(n, 16)**		
Muscle CSA SDS	-2.4 (-4.3, -0.3)	-2.0 (-3.5, -0.5)	-2.0 (-4.3,0.2)	0.12	0.21
Fat CSA SDS	-0.4 (-1.5, 4.3)	-0.2 (-1.0, 4.2)	-0.2 (-0.9, 3.6)	0.53	0.73
Grip strength	**(n, 19)**	**(n, 19)**	**(n, 19)**		
ND-hand MIGF SDS	-1.5 (-4.5, 0.5)	-0.6 (-5.3, 1.1)	-1.2 (-5.8, 0.5)	0.33	0.57

Data presented as median (range). LM SDS, lean mass adjusted for height and sex. FM, fat mass adjusted for height and gender; Muscle CSA, muscle cross sectional area adjusted for height and sex; Fat CSA, Fat cross sectional area adjusted for height and sex; ND-hand MIGF, non-dominant hand maximal isometric grip force adjusted for height and sex.

3.4.6 Whole body composition outcomes by DXA

At baseline, participants had TB-LM SDS significantly lower than zero (p=0.00) and this persisted over the 12 months study. At baseline, median TB-FM SDS was not different from zero (p=0.78) and stayed unchanged (Figure 3-12). Categorising the participants according to disease status or GC exposure or growth potential did not show any significant results (Table 3-12).

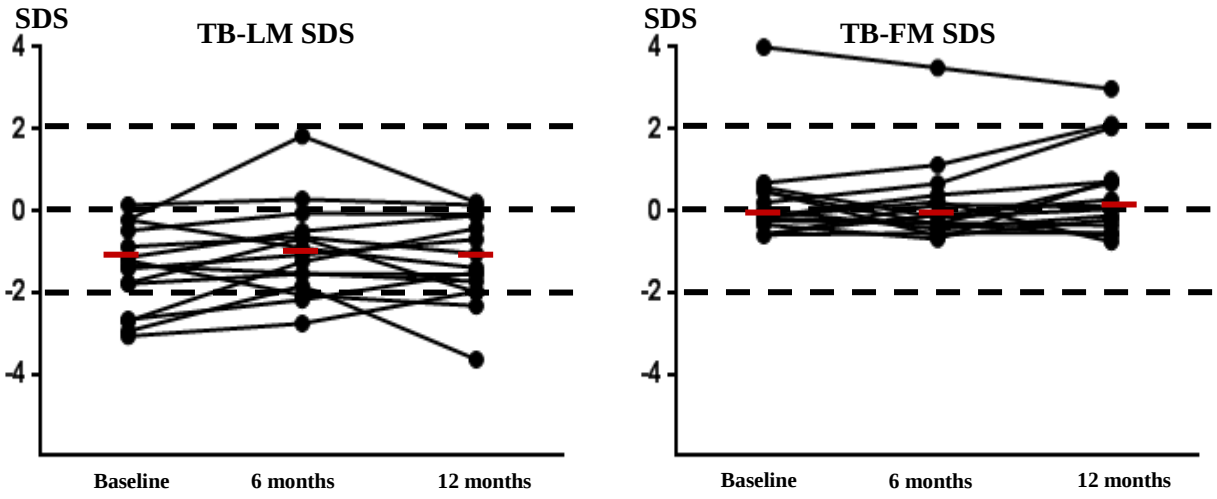

Figure 3-12: Individual changes in body composition by DXA at baseline, 6 months and 12 months following anti-TNF-*α*.
TB-LM SDS, total body lean mass adjusted for height and sex. TB-FM SDS, total body fat mass adjusted for height and sex. Red solid lines represent the median values. Black dotted lines represent the 50th centile (0SDS), 3rd (-2SDS) and 97th (2SDS) centile.

Table 3-12: Change in body composition in 15 CD participants by DXA at baseline, 6 months and 12 months following anti-TNF-α. Participants divided according to disease status, GC use and growth potential.

	Baseline (n, 15)	6 Months (n, 14)	12 Months (n, 15)	p-values		
				Baseline vs. 6 months	Baseline vs. 12 months	Between groups at 12 months
			TB-LM SDS			
Remission (n, 9)	-1.4 (-3.1, 0.1)	-1.1 (-2.8, 1.8)	-0.7 (-3.7, 0.2)	**0.016**	0.17	0.26
Non-remission (n, 6)	-1.3 (-2.7, -0.2)	-0.9 (-2.2, -0.1)	-1.6 (-2.3, -0.1)	0.89	0.60	
GC naïve (n, 7)	-1.3 (-2.9, 0.1)	-1.5 (-2.2, 0.3)	-1.5 (-3.7, 0.1)	0.46	0.75	0.17
GC +/- (n, 6)	-1.0 (-1.8, -0.2)	-0.6 (-1.1, 1.8)	-0.4 (-2.0, 0.2)	0.06	0.12	
GC+/+ (ID-18)	-1.8	-1.5	-1.6			
GC+/+ (ID-19)	-3.1	-2.8	-2.0			
Group A (n, 7)	-1.1 (-2.9, -0.2)	-0.7 (-1.8, 1.8)	-1.1 (-3.7, 0.2)	0.09	1.00	0.86
Group B (n, 8)	-1.6 (-3.1, 0.1)	-1.5 (-2.8, 0.3)	-1.5 (-2.3, 0.1)	0.18	0.26	
			TB-FM SDS			
Remission (n, 9)	0.02 (-0.6, 4.3)	-0.1 (-0.6, 3.5)	0.1 (-0.5, 3.0)	0.59	0.52	0.61
Non-remission (n, 6)	-0.2 (-0.4, 0.7)	-0.3 (-0.7, 1.1)	0.2 (-0.8, 2.1)	0.89	0.46	
GC naïve (n, 7)	-0.2 (-0.6, 4.3)	0.3 (-0.3, 3.5)	0.7 (-0.6, 3.0)	0.75	0.74	0.43
GC +/- (n, 6)	-0.1 (-0.6, 0.5)	-0.5 (-0.7, 0.7)	-0.2 (-0.8, 2.0)	0.60	0.75	
GC+/+ (ID-18)	-0.6	-0.1	0.3			
GC+/+ (ID-19)	0.6	-0.2	0.04			
Group A (n, 7)	-0.2 (-0.6, 0.5)	-0.3 (-0.6, 0.7)	-0.1 (-0.8, 2.0)	0.89	0.40	0.27
Group B (n, 8)	0.2 (-0.6, 4.3)	-0.1 (-0.7, 3.5)	0.5 (-0.6, 3.0)	0.40	0.67	

Data presented as median (range); TB; total body; LM SDS, lean mass adjusted for height and sex; FM, fat mass adjusted for height and sex.

3.4.6.1 Correlation of whole body composition outcomes by DXA with disease and bone outcomes by DXA

At baseline, 6 months and 12 months, no significant correlation was observed between TB-LM SDS and disease biomarkers (wPCDAI, ESR, CRP, albumin), cytokines (TNF, IL-6) or duration of disease. At baseline, 6 months and 12 months, no significant correlation was observed between TB-FM SDS and disease biomarkers (wPCDAI, ESR, CRP, albumin), cytokines (TNF, IL-6) or duration of disease. At baseline, 6 months and 12 months, no significant correlation observed between TB-LM SDS and bone parameters by DXA.

3.4.7 Body composition outcomes by radius pQCT

At baseline, participants had muscle CSA SDS significantly lower than zero (p=0.00) and this persisted over the 12 months study. At baseline, median fat CSA SDS was not different from zero (p=0.24) and stayed unchanged (Figure 3-13). Categorising the participants according to disease status or GC exposure or growth potential did not show any significant results (Table 3-13).

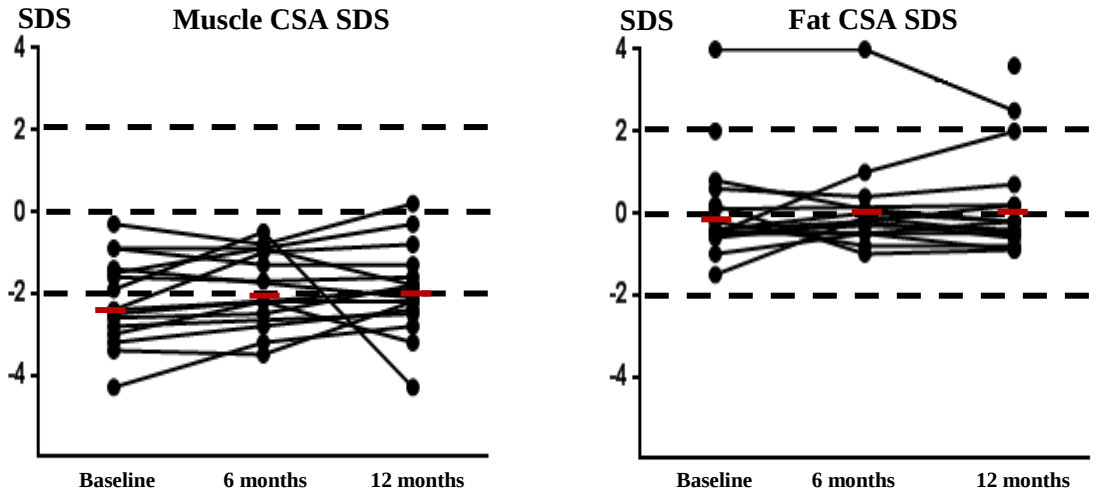

Figure 3-13: Individual changes in body composition by radius pQCT at baseline, 6 months and 12 months following anti-TNF-α.
Muscle CSA, muscle cross sectional area adjusted for height and sex; Fat CSA, Fat cross sectional area adjusted for height and sex. Red solid lines represent the median values. Black dotted lines represent the 50th centile (0SDS), 3rd (-2SDS) and 97th (2SDS) centile.

Table 3-13: Change in body composition in 16 CD participants by radius pQCT at baseline, 6 months and 12 months following anti-TNF-α. Participants divided according to disease status, GC use and growth potential.

	Baseline (n, 16)	6 Months (n, 14)	12 Month (n, 16)	p-values		
				Baseline vs. 6 months	Baseline vs. 12 months	Between groups at 12 months
Muscle CSA SDS						
Remission (n, 9)	-2.4 (-4.3, -0.3)	-2.2 (-3.5, -0.5)	-1.9 (-4.3, 0.2)	0.022	0.07	0.92
Non-remission (n, 7)	-1.6 (-3.2, -0.9)	-1.7 (-2.8, -0.9)	-2.1 (-2.5, -0.3)	0.14	0.46	
GC naïve (n, 8)	-2.0 (-3.2, -0.3)	-1.4 (-2.8, -0.8)	-2.0 (-3.2, 0.2)	0.50	0.87	1.0
GC +/- (n, 6)	-2.3 (-3.4, -0.9)	-1.8 (-3.5, -0.5)	-2.0 (-4.3, -0.3)	0.21	0.46	
GC+/+ (ID-18)	-4.3	-3.2	-2.8			
GC+/+ (ID-19)	-2.4	-2.2	-1.9			
Group (A) (n, 7)	-2.5 (-3.4, -0.9)	-2.2 (-3.5, -0.5)	-2.2 (-4.3, -1.3)	0.29	0.87	0.34
Group(B) (n, 9)	-2.4 (-4.3, -0.3)	-1.7 (-3.2, -0.8)	-1.9 (-2.8, 0.2)	0.13	0.10	
Fat CSA SDS						
Remission (n, 9)	-0.4 (-1.5, 4.3)	-0.1 (-1.0, 4.2)	-0.2 (-0.9, 2.5)	0.57	0.95	0.63
Non-remission (n, 7)	-0.4 (-0.6, 2.0)	-0.2 (-0.5, 0.1)	-0.2 (-0.6, 3.6)	0.71	0.59	
GC naïve (n, 8)	-0.2 (-1.0, 4.3)	-0.1 (-0.5, 4.2)	0.00 (-0.4, 3.6)	0.46	0.57	**0.02**
GC +/- (n, 6)	-0.5 (-1.5, 0.6)	-0.4 (-0.8, 0.4)	-0.6 (-0.9, 0.7)	0.85	0.69	
GC+/+ (ID-18)	-0.50	1.0	2.0			
GC+/+ (ID-19)	0.20	-1.0	-0.9			
Group (A) (n, 7)	-0.5 (-1.5, 0.6)	-0.3 (-0.8, 0.4)	-0.5 (-0.9, 0.7)	0.46	0.74	0.09
Group (B) (n, 9)	0.1 (-0.6, 4.3)	0.0 (-1.0, 4.2)	0.2 (-0.9, 3.6)	0.92	0.78	

Data presented as median (range). Muscle CSA, muscle cross sectional area adjusted for height and sex; Fat CSA, Fat cross sectional area adjusted for height and sex. Participants divided according to disease status, GC use and growth potential. Values in bold indicate statistical significance ($p<0.05$).

3.4.7.1 Correlation of body composition outcomes by pQCT with disease and bone outcomes by pQCT

At baseline, 6 months and 12 months, no significant correlation was observed between muscle CSA SDS or fat CSA SDS and disease biomarkers (wPCDAI, ESR, CRP, albumin), or duration of disease. At baseline, 6 months and 12 months, no significant correlation was observed between muscle CSA SDS and cytokines (TNF, IL-6). At baseline and 6 months, no significant correlation was observed between fat CSA SDS and cytokines (TNF, IL-6). At 12 months, a significant negative correlation was observed between fat CSA SDS with TNF-α ($r=-0.66$, $p=0.007$).

Figure 3-14 and Figure 3-15 show correlation between pQCT (radius& tibia) bone outcomes and muscle CSA SDS at each time point. At baseline, a significant positive correlation was observed between muscle CSA SDS with radius trabecular BMD SDS ($r=0.52$, $p=0.041$), tibia trabecular BMD SDS ($r=0.53$, $p=0.042$) and tibia cortical thickness SDS ($r=0.53$, $p=0.041$). At 6 months, no correlation was observed between muscles CSA SDS with radius or tibia pQCT bone outcomes. At 12 months, a significant positive correlation was observed between muscle CSA SDS and tibia cortical BMD SDS ($r=0.67$, $p=0.006$).

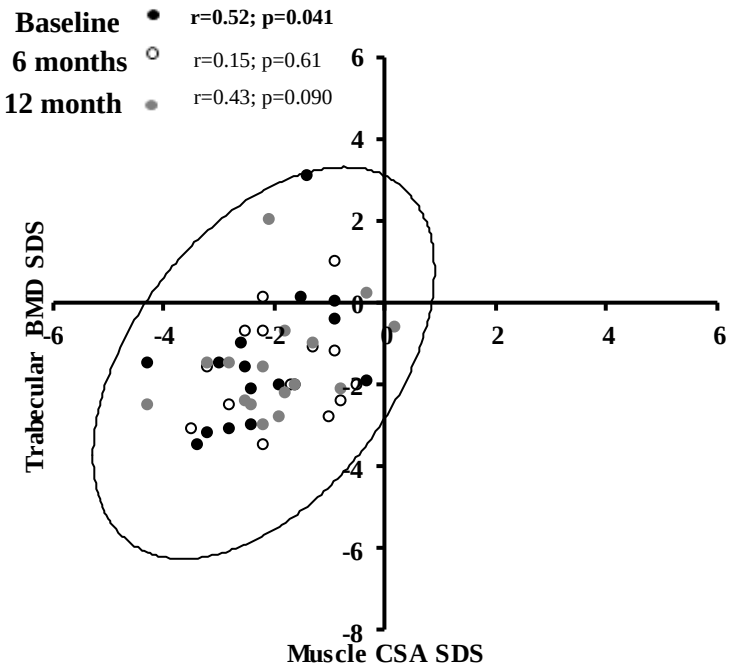

Figure 3-14: Correlation between radius trabecular BMD SDS with radius muscle CSA SDS. Baseline (filled black circle), 6 months (empty black circle) and 12 months (filled grey circle) in 16 CD received anti-TNF-α for 12 month. Large black circle indicates confidence limit, Values in bold indicate statistical significance ($p<0.05$).

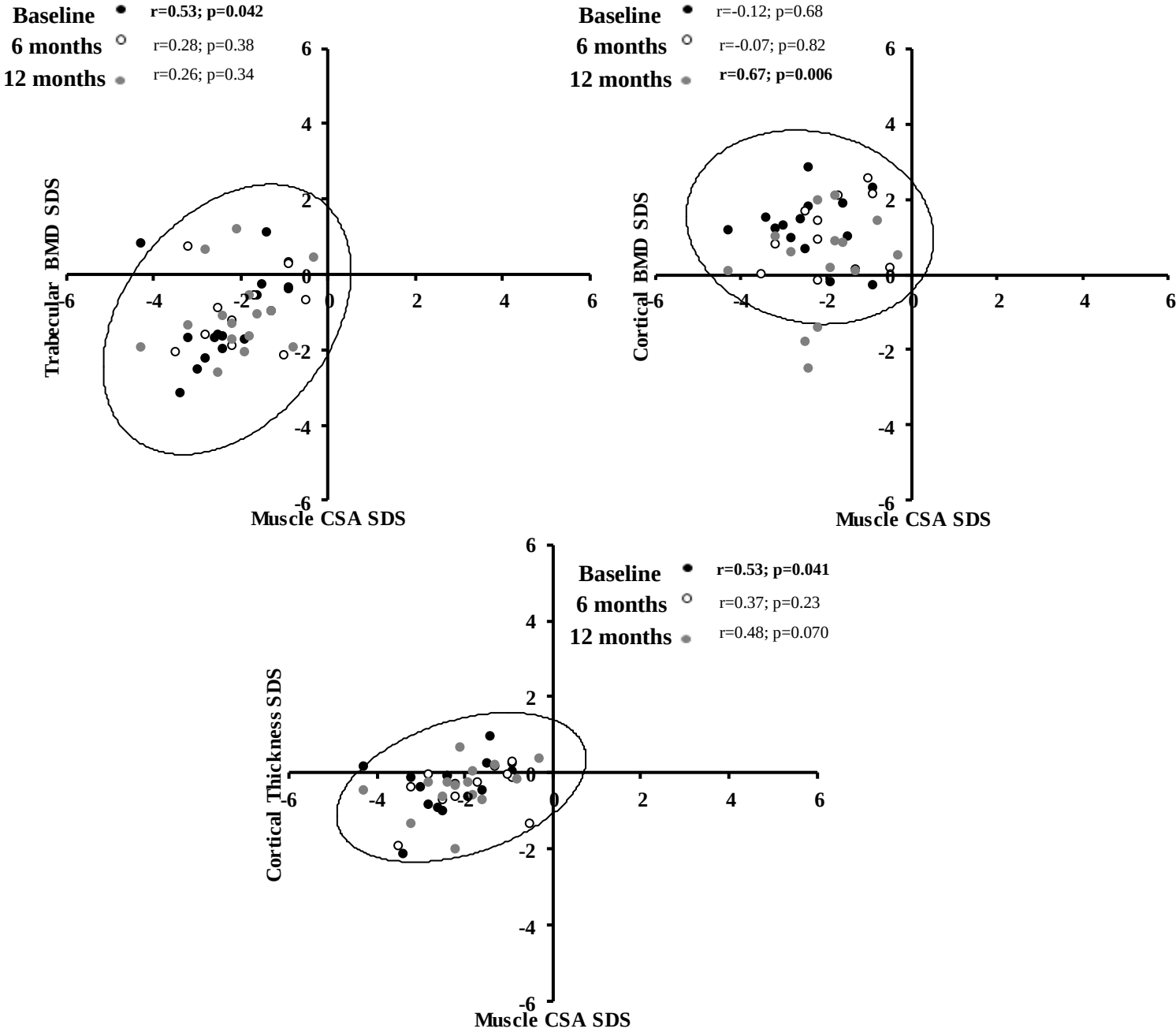

Figure 3-15: Correlation between tibia pQCT bone outcomes with radius muscle CSA SDS
Baseline (filled black circle), 6 months (empty black circle) and 12 months (filled grey circle) in 16 CD received anti-TNF-α for 12 months. Large black circle indicates confidence limit, Values in bold indicate statistical significance ($p<0.05$).

3.4.8 ND-MIGF

ND-MIGF SDS was reduced at baseline (p=0.00 vs zero) with no improvement noticed during the study interval (Figures 3-16). The result was not different when the participants were categorised according to disease condition or GC exposure or growth potential (Table 3-14).

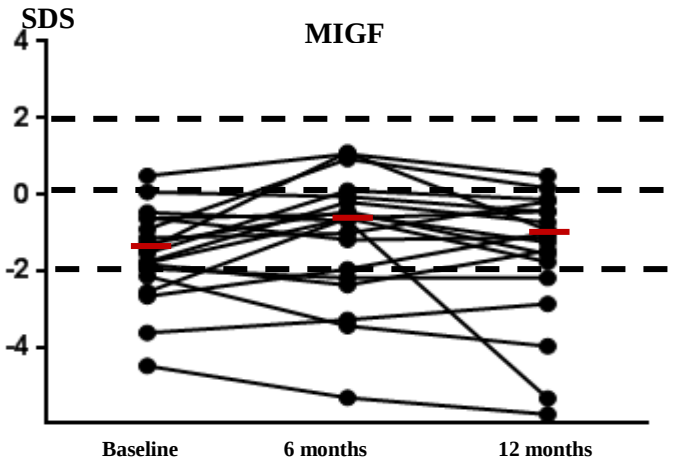

Figure 3-16: Individual changes in MIGF at baseline, 6 months and 12 months following anti-TNF-α.
MIGF, maximum isometric grip force adjusted for height and sex. Red solid lines represent the median values. Black dotted lines represent the 50th centile (0SDS), 3rd (-2SDS) and 97th (2SDS) centile.

Table 3-14: Change in ND-MIGF at baseline, 6 months and 12 months following anti-TNF-α. Participants divided according to disease status, GC use or growth potential.

ND-MIGF SDS (n, 19)	Baseline	6 Months	12 Months	p-values		
				Baseline vs. 6 months	Baseline vs. 6 months	Between groups at 12 months
Remission (n, 9)	-1.4 (-4.5, 0.5)	-0.6 (-5.3, 1.1)	-0.7 (-5.8, 0.5)	0.17	0.31	0.13
Non-remission (n, 10)	-1.7 (-3.6, 0.1)	-0.7 (-3.4, 0.1)	-1.5 (-5.3, -0.1)	0.39	0.80	
GC naïve (n, 10)	-1.7 (-3.6, -0.5)	-0.7 (-3.3, 0.1)	-1.1 (-5.3, -0.1)	0.047	0.17	1.00
GC +/- (n, 7)	-0.9 (-4.5, 0.5)	-0.6 (-5.3, 1.1)	-1.1 (-5.8, 0.5)	1.00	0.31	
GC+/+ (ID-18)	-1.4	1.09	-0.94			
GC+/+ (ID-19)	-1.9	-2.2	-2.2			
Group(A) (n, 9)	-1.8 (-4.5, 0.5)	-0.6 (-5.3, 1.1)	-1.1 (-5.8, 0.5)	0.31	0.77	0.71
Group(B) (n, 10)	-1.4 (-3.6, 0.1)	-0.7 (-3.3, 1.1)	-1.1 (-5.3, -0.2)	0.09	0.58	

Data presented as median (range); ND-hand MIGF SDS, non-dominant hand maximal isometric grip force adjusted for height and sex.

3.4.8.1 Correlation of ND-MIGF and disease

At baseline and 6 months, no significant correlation was observed between ND-MIGF SDS with disease biomarkers (wPCDAI, ESR, CRP, albumin), duration of disease, cytokines (TNF, IL-6). At 12 months, a significant negative correlation was observed between ND-MIGF SDS with wPCDAI (r=-0.47, p=0.41). At baseline, 6 months and 12 months no significant correlation was observed between ND-MIGF SDS with cytokines (TNF, IL-6).

3.4.8.2 Correlation of MIGF and muscle

Figure 3-17 shows the correlation between muscle CSA SDS and ND-MIGF SDS at each time point. At baseline, a significant positive correlation was observed between ND-MIGF SDS with radius muscle CSA SDS (r=0.57, p=0.020). At 6 months, a significant positive correlation was observed between ND-MIGF SDS with radius muscle CSA SDS (r=0.65, p=0.011). No correlation was observed between ND-MIGF SDS and TB-LM SDS by DXA.

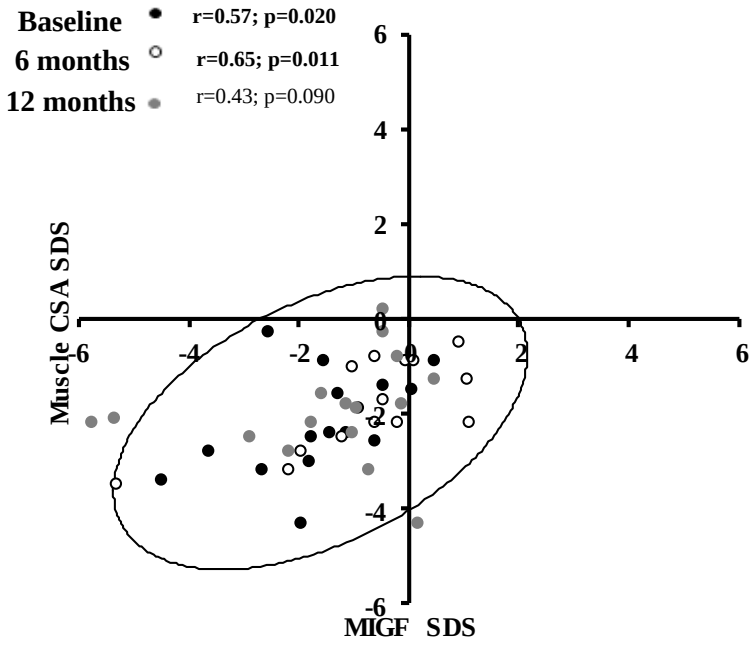

Figure 3-17: Correlation between muscle CSA SDS and ND-MIGF SDS.
Baseline (filled black circle), 6 months (empty black circle) and 12 months (filled grey circle) in 16 CD received anti-TNF-α for 12 months. Large black circle indicates confidence limit, Values in bold indicate statistical significance ($p<0.05$).

3.4.8.3 Correlation of MIGF and bone

At baseline, a significant positive correlation was observed between ND-MIGF SDS with radius trabecular BMD SDS (r=0.69, p=0.003) (figure 3-18), tibia trabecular BMD SDS (r=0.54, p=0.037) and tibia cortical thickness SDS (r=0.53, p=0.041) (figure 3-19). At 6 months and 12 months no significant correlation was observed between ND-MIGF SDS and pQCT bone parameters (radius/tibia). No correlation was observed between ND-MIGF SDS and bone outcome by DXA.

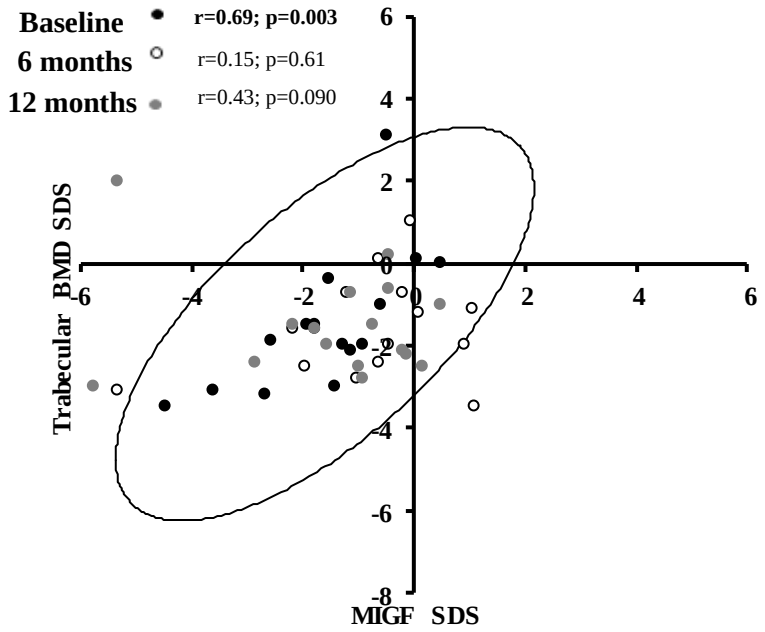

Figure 3-18: Correlation between radius trabecular BMD SDS and ND-MIGF SDS.
Baseline (filled black circle), 6 months (empty black circle) and 12 months (filled grey circle) in 16 CD received anti-TNF-α for 12 months. Large black circle indicates confidence limit, Values in bold indicate statistical significance ($p<0.05$).

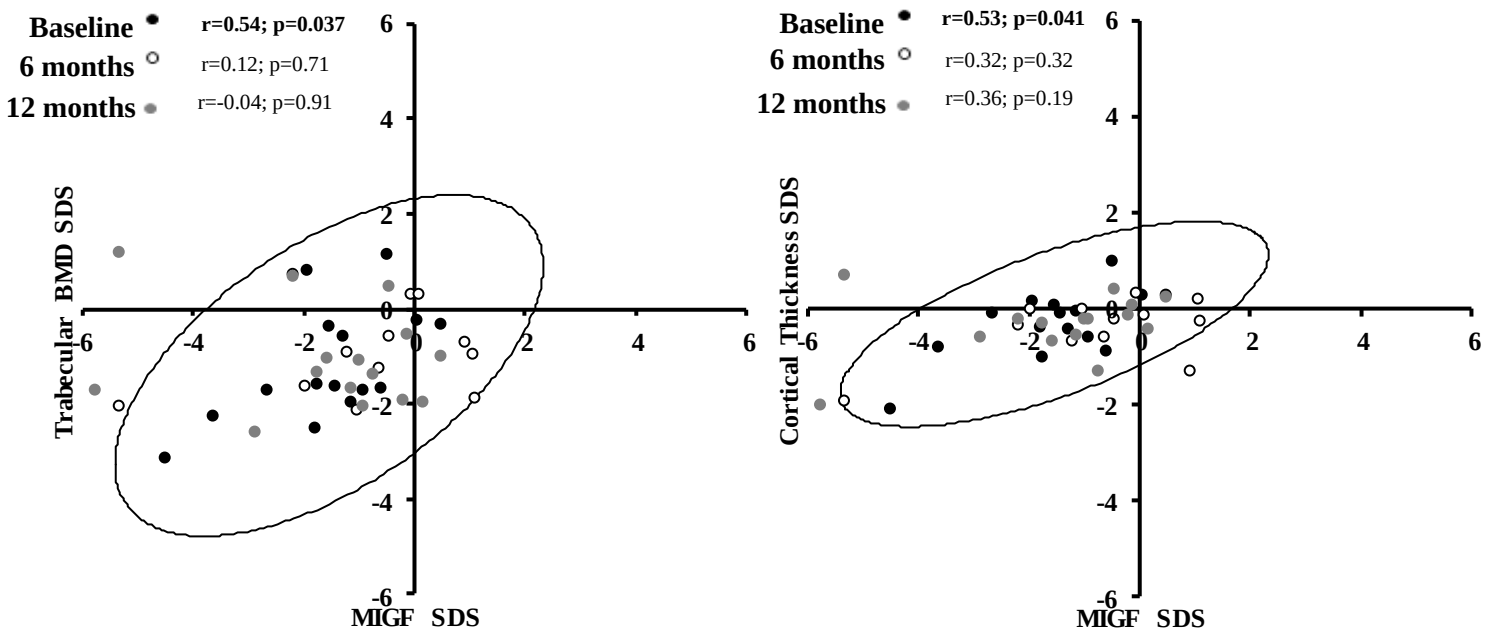

Figure 3-19: Correlation between Tibia trabecular BMD SDS (on the left), tibia cortical thickness SDS (on the right) and ND-MIGF SDS
Baseline (filled black circle), 6 months (empty black circle) and 12 months (filled grey circle) in 16 CD received anti-TNF-α for 12 month. Large black circle indicates confidence limit, Values in bold indicate statistical significance ($p<0.05$).

3.4.9 Multivariate mixed-model regression analysis for DXA bone outcomes

Table 3-15 displays the results of multivariate mixed-model regression analysis of the relationship of DXA bone outcomes with IL6, GC (Yes/No) growth biomarkers SDS and TB-LM SDS during the 12-months study interval. TB-BMD SDS BA and LS-BMD SDS BA, were significantly and positively associated with IGF-1 SDS independent of IL6, GC use, other growth biomarkers and TB-LM SDS. Similar results were observed by running the same model but using wPCDAI.

Table 3-15: Mixed model assessment of the effect of inflammation, glucocorticoid use, and growth biomarkers adjusted for bone age on DXA bone outcomes at each time points during 12 months study.

Independent variables	Dependent Variables							
	TB-BMDSDS $_{Ht\text{-}age}$		**TB-BMDSDS $_{BA}$**		**LS-BMDSDS $_{Ht\text{-}age}$**		**LS-BMDSDS $_{BA}$**	
	Estimate (SE)	**p-value (95%CI)**	**Estimate (SE)**	**p-value (95%CI)**	**Estimate (SE)**	**p-value (95%CI)**	**Estimate (SE)**	**p-value (95%CI)**
IL-6*	-0.219 (0.226)	0.337 (-0.67, 0.23)	0.206 (0.252)	0.417 (-0.31, 0.71)	-0.554 (0.322)	0.089 (-1.20, 0.09)	-0.157 (0.298)	0.600 (-0.75, 0.44)
GC	0.068 (0.233)	0.772 (-0.41, 0.534)	-0.114 (0.261)	0.661 (-0.63, 0.40)	-0.151 (0.332)	0.653 (-0.81, 0.51)	-0.255 (0.307)	0.410 (-0.88, 0.36)
IGF-1SDS	0.143 (0.121)	0.239 (-0.11, 0.38)	0.396 (0.134)	**0.004** (0.13, 0.66)	0.243 (0.171)	0.160 (-0.11, 0.58)	0.439 (0.159)	**0.007 (0.12, 0.76)**
IGFBP-3 SDS	-0.012 (0.118)	0.918 (-0.25, 0.22)	-0.164 (0.131)	0.217 (-0.43, 0.11)	-0.241 (0.168)	0.156 (-0.58, 0.09)	-0.276 (0.156)	0.08 (-0.59, 0.03)
ALS SDS	0.095 (0.185)	0.609 (-0.27, 0.46)	0.111 (0.206)	0.599 (-0.30, 0.52)	-0.244 (0.263)	0.357 (-0.77, 0.28)	-0.072 (0.244)	0.769 (-0.56, 0.41)
TB-LM SDS	0.122 (0.102)	0.236 (-0.08, 0.32)	-0.043 (0.113)	0.702 (-0.27, 0.18)	0.104 (0.144)	0.472 (-0.18, 0.39)	0.028 (0.134)	0.835 (-0.24, 0.29)

TB, total body; BMD, areal bone mineral density; SDS $_{Ht\text{-}age,}$ adjusted for height age and sex; SDS $_{BA}$, adjusted for bone age and sex; AP, anteroposterior; LS, lumbar spine; LM, lean mass; IGF-1 SDS, serum insulin like growth factor-1SD score; IGFBP-3 SDS, serum insulin like growth factor binding protein-3 SD score; ALS SDS acid-labile subunit; IL-6, interleukin-6; GC, glucocorticoid. Values in bold indicate statistical significance ($p<0.05$). *Natural log used to produce normality.

3.4.10 Multivariate mixed-model regression analysis for radius pQCT bone outcomes

Table 3-16 displays the results of multivariate mixed-model regression analysis of the relationship of radius trabecular BMD SDS (We have excluded cortical BMD and cortical thickness as the measurement seems to be influenced by partial volume effect compared to tibia) with, IL6, GC (yes/no), growth biomarkers SDS and radius muscle CSA SDS during the 12-months study interval. Results of mixed model regression analysis showed that IL-6 (p=0.005) was negatively and muscle CSA (p=0.035) positively and independently associated with trabecular BMD. Repeating the same model using wPCDAI instead of IL-6, only muscle CSA (p=0.005) was positively and independently associated with trabecular BMD SDS.

Table 3-16: Mixed-model assessment of the effect of inflammation, glucocorticoid use, growth biomarkers adjusted for bone age on radius trabecular BMD SDS at each time points during 12 months study.

Independent variables	Dependent Variables	
	Trabecular BMDSDS	
	Estimate (SE)	**p-value (95%CI)**
IL-6*	-1.234 (0.417)	**0.005** (-2.08, -0.39)
GC	-0.444 (0.451)	0.330 (-1.35, 0.47)
IGF-1 SDS	0.393 (0.214)	0.074 (-0.04, 0.83)
IGFBP-3 SDS	-0.009 (0.214)	0.967 (-0.44, 0.42)
ALS SDS	-0.467 (0.355)	0.195 (-1.18, 0.25)
Radius Muscle CSA SDS	0.386 (0.176)	**0.035** (0.03, 0.74)

BMD , volumetric bone mineral density; SDS, adjusted for age and sex; muscle CSA SDS, muscle cross sectional area adjusted for sex and height; IGF-1 SDS, serum insulin like growth factor-1SD score; IGFBP-3 SDS, serum insulin like growth factor binding protein-3 SD score; ALS SDS acid-labile subunit; IL-6, interleukin-6; GC, glucocorticoid. Values in bold indicate statistical significance ($p<0.05$). *Natural log used to produce normality.

3.4.11 Multivariate mixed-model regression analysis for tibia pQCT bone outcomes

Table 3-17 displays the results of multivariate mixed-model regression analysis of the relationship of tibia pQCT bone outcomes with, IL6, GC (yes/no), growth biomarkers SDS and radius muscle CSA SDS during the 12-months study interval. Results of mixed model regression analysis showed that ALS SDS (p=0.024) and muscle CSA (p=0.015) were positively and independently associated with trabecular BMD; muscle CSA was independently associated with cortical BMD SDS (p=0.049). Muscle CSA (p=0.003) and IGFBP3 SDS (p=0.024) were independently associated with cortical thickness positively and negatively respectively. Similar results were observed by running the same model but using wPCDAI.

Table 3-17: Mixed-model assessment of the effect of inflammation, glucocorticoid use, growth biomarkers adjusted for bone age on tibia pQCT bone outcomes at each time points during 12 months study.

Independent variables	Dependent Variables					
	Trabecular BMDSDS		Cortical BMD SDS		Cortical Thickness SDS	
	Estimate (SE)	p-value (95%CI)	Estimate (SE)	p-value (95%CI)	Estimate (SE)	p-value (95%CI)
IL-6*	-0.621 (0.322)	0.066 (-1.29, 0.05)	0.676 (0.359)	0.067 (-0.05, 1.40)	-0.311 (0.211)	0.129 (-0.71, 0.10)
GC	-0.294 (0.350)	0.409 (-1.02, 0.43)	-0.076 (0.388)	0.845 (-0.86, 0.71)	-0.162 (0.216)	0.460 (-0.60, 0.28)
IGF-1 SDS	0.135 (0.171)	0.435 (-0.21, 0.48)	0.166 (0.182)	0.368 (-0.20, 0.54)	0.111 (0.103)	0.285 (-0.11, 0.32)
IGFBP-3 SDS	-0.157 (0.172)	0.369 (-0.51, 0.19)	-0.372 (0.185)	0.052 (-0.75, 0.00)	-0.246 (0.104)	**0.024** (-0.46, -0.03)
ALS SDS	0.691 (0.292)	**0.024** (0.11, 1.28)	-0.177 (0.308)	0.569 (-0.80, 0.45)	0.185 (0.174)	0.295 (-0.17, 0.54)
Radius Muscle CSA SDS	0.427 (0.167)	**0.015** (0.09, 0.77)	0.358 (0.176)	**0.049** (0.00, 0.71)	0.319 (0.111)	**0.003** (0.12, 0.52)

BMD , volumetric bone mineral density; SDS, adjusted for age and sex; muscle CSA SDS, muscle cross sectional area adjusted for sex and height; IGF-1 SDS, serum insulin like growth factor-1SD score; IGFBP-3 SDS, serum insulin like growth factor binding protein-3 SD score; ALS SDS acid-labile subunit; IL-6, interleukin-6; GC, glucocorticoid. Values in bold indicate statistical significance ($p<0.05$). *Natural log used to produce normality

3.4.12 Correlations between DXA and radius pQCT outcomes

At baseline, a positive correlation was observed between radius pQCT trabecular volumetric BMD and DXA LS-BMD SDS Ht-age (r=0.60, p=0.017) and with LS-BMD SDS_{BA} (r=0.800, p=0.00). At 6 months, a positive correlation was observed between radius pQCT trabecular volumetric BMD and DXA LS-BMD $_{Ht\text{-}age}$ (r=0.61, p=0.035) and with LS-BMD SDS_{BA} (r=0.77, p=0.000). At 12, a positive correlation was observed between radius pQCT trabecular volumetric BMD and DXA LS-BMD SDS $_{BA}$ (r=0.54, p=0.037) and no correlation was observed between radius pQCT trabecular volumetric BMD and DXA LS-BMD SDS $_{Ht\text{-}age}$ (figure 3-20). No correlation was observed between radius pQCT cortical volumetric BMD and DXA TB-BMD SDS.

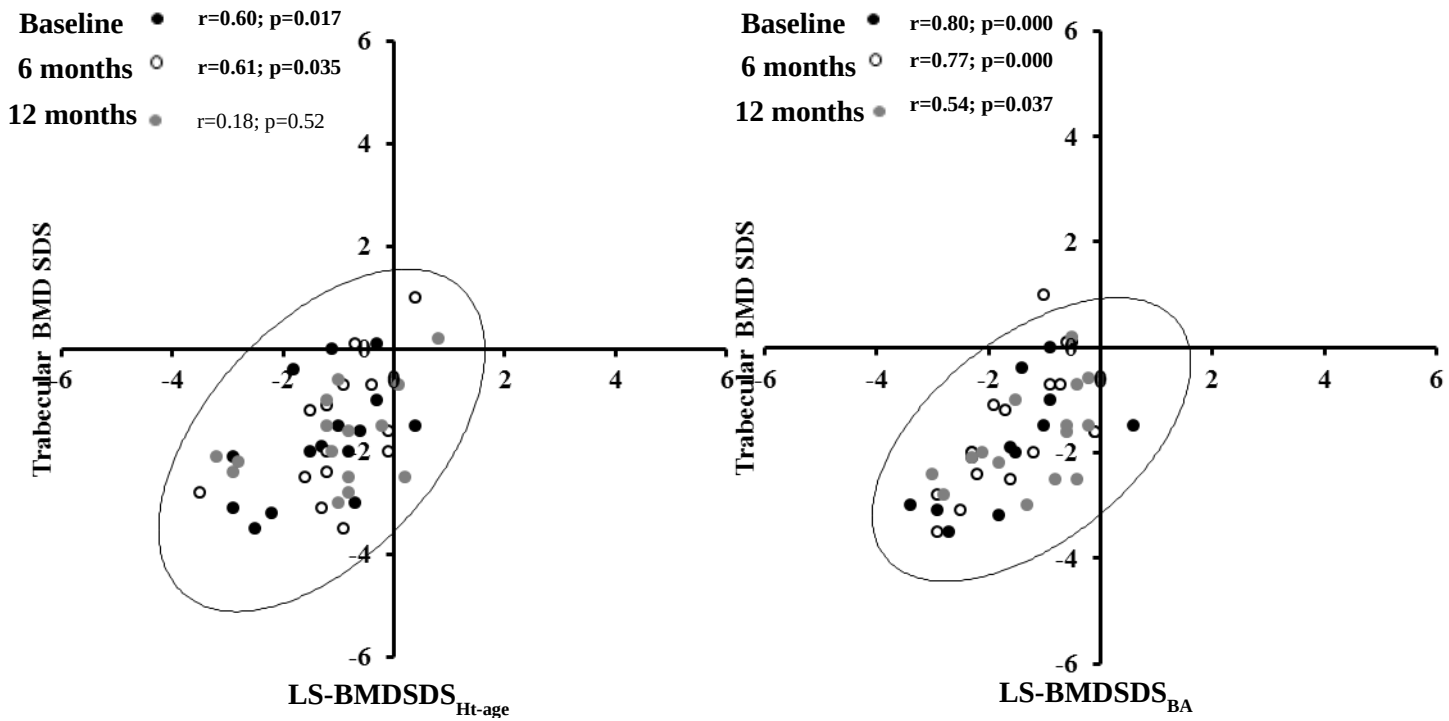

Figure 3-20: Correlation between radius trabecular BMD SDS and LS-BMD SDS $_{\text{Ht-age}}$ (on the left) and LS-BMD SDS$_{\text{BA}}$(on the right).

Baseline (filled black circle), 6months (empty black circle) and 12 months (filled grey circle) in 16 CD received anti-TNF-α for 12 month. Large black circle indicates confidence limit, Values in bold indicate statistical significance ($p<0.05$).

3.4.13 Correlations between DXA and tibia pQCT outcomes

At baseline, a positive was correlation observed between tibia pQCT trabecular volumetric BMD and DXA LS-BMD SDS $_{\text{Ht-age}}$ (r=0.61, p=0.025) and with LS-BMD SDS$_{\text{BA}}$ (r=0.61, p=0.021). At 6 months, a positive correlation was observed between tibia pQCT trabecular volumetric BMD and DXA LS-BMD SDS $_{\text{Ht-age}}$ (r=0.62, p=0.031) and with LS-BMD SDS$_{\text{BA}}$ (r=0.64, p=0.026) (figure 3-21). At 12 months, no correlation was observed between radius pQCT trabecular volumetric BMD and DXA LS-BMD SDS. No correlation was observed between tibia pQCT cortical volumetric BMD and DXA TB-BMD SDS.

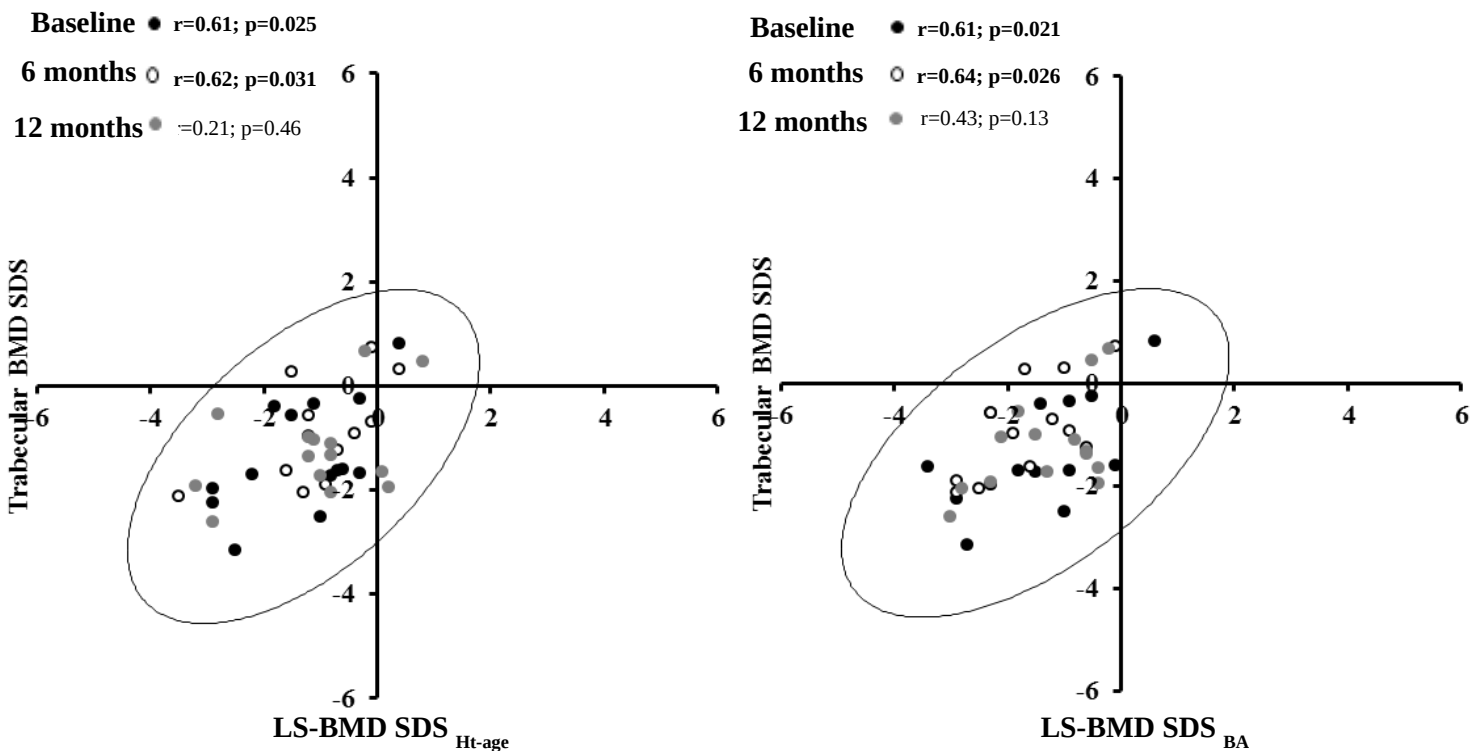

Figure 3-21: Correlation between tibia trabecular BMD SDS and LS-BMD SDS $_{Ht\text{-}age}$ (on the left) and LS-BMD SDS $_{BA}$(on the right).
Baseline (filled black circle), 6 months (empty black circle) and 12 months (filled grey circle) in 16 CD received anti-TNF-α for 12 month. Large black circle indicates confidence limit, Values in bold indicate statistical significance ($p<0.05$).

3.5 Discussion

The current study was primarily aimed at assessing the extent of change that may occur in bone health (by DXA and pQCT), bone geometry and muscle mass and function following initiation of anti-TNF-α therapy in children and adolescents with CD. In addition, this study aimed to study the association of IGF-1 axis, cytokines and muscle with bone density.

Anti-TNF-α therapy was associated with rapid improvements in disease activity and increases in BSAP. However, this improvement in markers of disease and bone turnover was not translated into meaningful improvements in bone mineral density or geometry. It is possible that this lack of improvement may have been related to poor muscle mass and function which tended to remain persistently low despite an improvement in disease status. Our cohort showed a high proportion of vitamin D deficiency according to the National Osteoporosis Society guidelines (295). However, in keeping with other reports in healthy children (171) as well as children with IBD (147-149;172;173), vitamin D in our cohort was not associated with any of the bone parameters. Our finding of no changes in vitamin D level following anti-TNF-α is consistent with recent studies (241;247).

This prospective study involved assessment of bone health at axial and appendicular skeleton by using DXA (TB, LS) and bone parameters at both weight- and non-weight bearing limbs by pQCT with evaluation of muscle health by DXA and pQCT and muscle function by grip strength. At baseline, both bone parameters and muscle mass were impaired and persist despite marked improvements in disease, bone biomarkers and reduction in corticosteroid use 12 months following anti-TNF-α therapy. Our data confirm the previous evidence that CD was associated with significant deficits in trabecular volumetric BMD and cortical thickness (150;154;155;199). Previous data suggest that younger age and concurrent growth provide a window of opportunity for skeletal recovery (153;156;237); we failed to confirm this finding as we did not find any improvement in bone or muscle health by categorised group according to their growth potential. However, the high percentage younger group were on GC at the time of starting anti-TNF-α and may have a more severe form of the disease.

Detrimental effect of glucocorticoids on bone health is well established (296) also there is some evidence showing that less glucocorticoid exposure was associated with greater recovery of the cortical area, especially in the setting of linear growth in children with CD (153). In our study 50% of the participants were on glucocorticoids during the first 6 weeks

of therapy and they had potential to grow and musculoskeletal deficiency did not differ in those who did versus did not use GC over the study interval.

At baseline, we found tibia cortical BMD above average in our patients. Assimilating all available data, we postulated that bone impairment in children with CD may include impairment of intracortical bone remodelling (154;155;199). Similar to the data of Griffin et al (237), we found a drop in cortical BMD at the tibia. This decline in cortical bone density may be transient and could be explained by the fact that bone mass accrual lags behind increase in linear growth by approximately 6 to 12 months in both boys and girls (297). The current study found a discrepancy between cortical BMD and cortical geometry measured at radius and tibia with the one measured at radius being significantly reduced. Our finding in line with the recommendation that tibia is the preferred site for measuring cortical BMD and thickness as it is less subjected to underestimation of cortical measurement (298). However, lack of reference data for tibia hamper it is use in paediatric (146;290). Indeed, there was no available reference data for muscle and fat measured at the tibia matched for our cohort; for that reason we have not included these parameters in our results.

CD children who participated in this study had reduced muscle mass measured by both DXA and radius pQCT. Reduced muscle mass has already been reported in CD children (115;153-155;199;200). Our cohort showed persistent muscle deficiency following anti-TNF-α regardless of disease status and GC use. Chronic diseases may be associated with lower muscle strength relative to muscle mass that is not captured by DXA or pQCT. Accordingly, direct measures of muscle force may more fully explain bone deficits in chronic disease (201). Our present data showed that the reduced muscle mass was reflected in the significant reduction in muscle strength in the non-dominant hand measured by grip strength and persisted at 12 months despite improvement of the disease status.

To gain insights into the possible underlying mechanisms that may lead to abnormality of skeletal development in our CD cohort, multivariate mixed-model regression analysis was used. The possible reason for the abnormality in trabecular bone mineral density and cortical geometry in this cohort of children with CD could be partially related to persistent deficiency in muscle mass and function. The relationship between muscle or lean mass deficits and bone mass or structure deficits has been documented in cross-sectional (199) and longitudinal studies in children with IBD (155;205;237). Our study emphasizes the importance of muscle mass on bone mass. We found a strong independent positive association between muscle and bone density and geometry. In accordance with the concept of bone muscle unit (muscle strength strongly modulates bone strength) (299), the

persistent muscle deficits and reduced muscle force may give a plausible explanation to persistent bone deficiency seen in our group despite improvement of disease and biomarkers of bone metabolism; this finding may lead to speculation on the beneficial role of physical activity after controlling the inflammation.

It is evident that systemic IGF-1 maintains cortical bone structure and geometry, whereas locally produced IGF-1 serves to maintain trabecular bone structure (76-79). The inability to form ternary complex, with the marked reduction in circulating IGF-1, and the reduction in trabecular BMD and cortical bone volume were present in ALS-Knock mice (77;77;300). The association between ALS SDS and trabecular BMD SDS and negative correlation between IGFBP-3 and tibia cortical thickness SDS raise the possibility that the IGF axis may contribute to trabecular and cortical geometry impairment seen in those groups of children. The finding in the current study of the positive association between ALS SDS and trabecular BMD SDS in agreement with recent reports from our group showed a significant negative relationship between ALS and trabecular bone separation in women with childhood onset type 1 diabetes mellitus (301). Taking our group finding together suggests that ALS could play a role in the regulation of trabecular bone, and this might be exerted via IGF-1 independent mechanisms. However, more in depth preclinical studies are needed to improve our understanding about the role of IGF ternary complex, not only on bone density, but also on the architectural structure of both trabecular and cortical bone in children with CD.

Even though the wPCDAI,or IL-6 was not independently associated with bone parameter in the regression model, persistent trabecular deficiency over 12 months after anti-TNF-α even in sub-groups with complete remission could be partly explained by persisting low-grade mucosal inflammation, even in clinical remission, similar to Sylvester et al (149) already described.

Another possibility is that improvement in bone mass may lag behind the improvement in inflammation and a 12 month study was insufficient; a longer period of follow up may be required(302). Our finding also could be explained by the fact that our cohort had longer (3.1 years) compared to 2.1 years reported by Griffin et al (237); also we found a negative relationship between disease duration and TB-BMD measured by DXA. Disease duration in our study was similar to two previous studies in paediatrics which showed a negative result (241;254). Available evidence about growth recovery and normalisation of height has been linked to duration of cytokines exposure and duration of disease (120;248). Integrating our finding with previous studies, we may speculate that there is a certain window of reversible recovery similar to the growth, after which the detrimental effect of

inflammation on bone may be irreversible. However, this needs to be further explored before a final conclusion can be made.

Finally, the Griffin group (237) showed that in the younger age group, greater decline in PCDAI was independently associated with greater increase in trabecular BMD. Our cohort may miss the narrow window of opportunity for intervention to improve bone health. It is clear that there is no possible individual mechanism for lack of musculoskeletal recovery in our cohort and most likely it is a combination of them all.

Irrespective of method adjustment of LS-BMD SDS, trabecular BMD measured at tibia and radius showed a significant association. This finding highlighted that reductions in aBMD, specifically at the LS regions of interest, may suggest reductions in trabecular BMD based on the understanding of the higher amount of trabecular bone located in LS (31;32). However, such results may still not be synonymous with true trabecular BMD at spine. The overlaid cortical bone may have obscured trabecular deficits in the DXA scans. The observation that the decreases in pQCT trabecular BMD SDS were significantly greater than the declines in LS BMD SDS in this study is consistent with this concept.

This study provides a broad view of skeletal health at different sites of the body by the use of DXA and pQCT. Furthermore, this is the first study that charts the effect of anti-TNF-α therapy on changes in upper limb muscle function in paediatric patients with CD. Finally, this study provides insight that may enable future clinical and research strategies to optimise bone health in children with CD. However, a number of important limitations need to be considered. The most important limitation lies in the fact that our study group was relatively small and therefore conclusions must be drawn with some caution. An additional limitation is by using DXA and pQCT we examine only the BMD and bone geometry, insufficient to characterise the microarchitecture of trabecular and cortical bone which are crucial components of bone strength; thus future studies need to use other techniques such as measuring trabecular bone score by DXA, high resolution pQCT and micro MRI for accurate measurement of bone microstructure. Lack of data on sex steroids and their relation with change in bone density is an additional limitation. pQCT geometry was adjusted only for height without taking age into consideration so it may yield in comparing the same height with different age and pubertal maturation, ignoring the influence of sexual hormones on bone and muscle development.

3.6 Conclusion

In summary, there is some evidence of bone formation but this is not translated into an improvement in bone mass or density over a period of one year of anti-TNF-α and this is

probably due to two factors. Firstly, there is probably a lag between growth and bone formation and secondly these patients have poor muscle mass and function and a sustained deficit in these may hinder skeletal acquisition. Given that our results suggest that inflammation, the insult to the skeleton is not completely removed even with anti TNF therapy, the role of adjuvant therapy to improve musculoskeletal development in children with CD such as nutritional, exercise or manipulation of the GH-IGF axis requires further exploration. Our result also highlighted that there may be perhaps limited window of intervention which may be augmented by improved nutritional status to improve muscle mass and limited window of bone recovery; further investigations are needed to verify our assumptions.

Chapter Four

The Sustained Effects of Recombinant Human Growth Hormone Therapy on Linear Growth in Children with Crohn's Disease

4.1 Abstract

Background: It is unclear whether the beneficial effect of rhGH on short-term growth in children with CD is sustained over a longer period

Aims: To investigate the effects of prolonged rhGH therapy on linear growth and insulin sensitivity in children with CD.

Design and participants: Fourteen cases of CD (9 male) who received rhGH (67mcg/kg/day) for 24 months were compared to another 14 children with CD matched for age, gender and duration of disease. Study time points included baseline, 12 months and 24 months of follow-up. Results were reported as median (range).

Results: Median change in Ht SDS was -0.3 (-0.7, -0.3) at baseline, 0.6 (0.2, 1.0) at 12 months (p=0.003 vs. baseline,) and 0.5 (-0.05, 0.9) at 24 months (p=0.003 vs. baseline) in the rhGH group, whereas in the control group it was 0.01 (-0.2, 0.7), 0.05 (-0.5,0.9) (p=0.88 vs. baseline) and -0.01 (-0.6, 0.5) (p=0.69 vs. baseline), respectively. In the rhGH group, median plasma IGF-1SDS adjusted for bone age increased from -2.9 (-6.0, +2.0) at baseline to 0.6 (-3.9, 3.0) (p=0.008 vs. baseline) at 12 months and -0.4 (-3.9, 2.0) (p=0.2 vs. baseline) at 24 months. HOMA-IR increased from 0.8 (0.4, 4.6) at baseline to 1.5 (0.6, 3.5) at 12 months (p=0.045 vs. baseline) and 1.7 (0.5, 6.1) at 24 months (p=0.03 vs. baseline). One patient developed impaired glucose tolerance at 12 months but normalised at 24 months with no reduction in rhGH dose.

Conclusion: This report showed that improved growth with rhGH therapy in children with CD is sustained over a two year period. However, the use of rhGH needs careful monitoring of glucose homeostasis.

4.2 Introduction

Despite improvements in the management of CD, growth retardation is still commonly encountered (84) with significant reduction in final adult height in approximately 20% treated with contemporary treatment regimens (91). Poorly growing children with CD may exhibit combined abnormalities of functional GH insufficiency as well as GH resistance (101). There may, therefore, be a rationale for considering treatment with high dose rhGH in children with CD and ongoing growth retardation. Therapy with rhGH in children with inflammatory diseases such as JIA and cystic fibrosis lead to short term catch up growth (256;267;303;304). Current studies of short term treatment with rhGH over 6-12 months in children with CD have reported significant improvement in linear growth (259;260;262) but it is unclear if longer term therapy is associated with continued catch up growth. Chronic inflammation maybe associated with a degree of insulin resistance (305) and this is evident in children with CD (262). Treatment with rhGH in such children may be therefore lead to deterioration in insulin sensitivity and this may be more notable in those who are on concomitant GC (259;264). The impact of longer term rhGH treatment in CD and insulin sensitivity is currently unknown.

The goal of this study is therefore to assess linear growth over 2 years of rhGH treatment in children with CD and a comparison of this growth to that in a contemporary group of children with CD who were matched for age, gender and duration of disease but who did not receive rhGH. The secondary objective is to evaluate insulin sensitivity over 2 years of rhGH treatment.

4.3 Methods

4.3.1 Study population

Fourteen children with CD who received rhGH (NorditropinSimplexx®; NovoNordisk, Crawley, UK), 67 mcg/kg/day as part of a six-month RCT (262) and who subsequently continued rhGH for 24 months as part of the study protocol were compared to a contemporary group of 14 CD children (84)who did not receive rhGH but were matched for gender (5female rhGH; 5female control), disease duration: median 3.3 years (0.6, 12.4) and 3.0 years (0.6, 7.3) and age 14.5 years (9.0, 16.4) and 13.0 years (8.6, 15.8) for rhGH and control group, respectively. The two groups also turned out comparable for their MPH SDS. rhGH dose was adjusted for weight at each visit.

Of the 22 children included in the initial RCT (262), rhGH was discontinued due to completion of linear growth (n, 2), poor compliance (n, 2), lost to follow-up (n, 2), needle phobia (n, 1) and post-colectomy in a child with UC (n, 1). Disease phenotype was classified using the Montreal classification (5). Data on therapeutic interventions including EEN, CD medication and surgery were collected for both groups at baseline, 12 months and 24 months. Figure 4-1 summarise the available study data for two groups.

The original clinical trial was approved by the UK multicentre research ethics committee, and its conduct was approved by the Health Boards. Written informed consent was obtained from all parents and patients as appropriate for those patients on rhGH. Data from control patients were collected as part of routine evaluation of the clinical care.

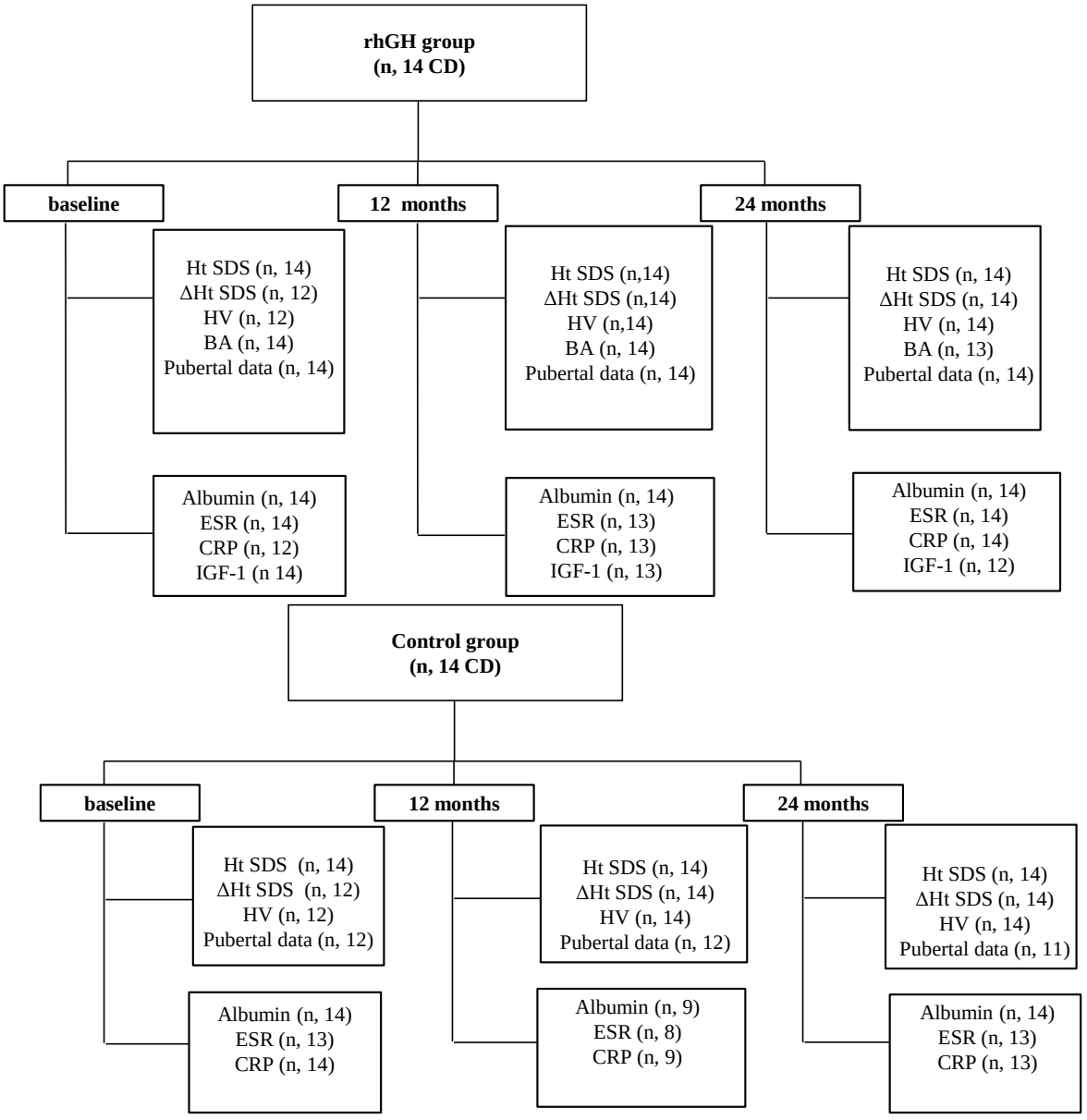

Figure 4-1: Flow chart of available data at each study visit for rhGH group and the control
n, number; Ht SDS, height SD score; ΔHt SDS, change in height SD score; HV, height velocity ;ESR, erythrocyte sedimentation rate; CRP, c-reactive protein.

4.3.2 Study parameters

For the study participants, Ht was measured with a Harpenden stadiometer and used to calculate annual HV. Ht, Wt, and BMI at 12 months before baseline, at starting rhGH, at 12 months and at 24 months after starting rhGH. In the rhGH group, SH was measured and SILL was calculated from Ht and SH. SH and SILL were converted to SDS using the 1978 Tanner-White-house standards (275). The Ht, Wt and BMI were converted into SDS for chronological age using 1990 UK standards (273;274).

Pubertal status in rhGH group was systemically assessed clinically by a member of the research team and were available at baseline, 12months and 24 months, whereas in the control group (historical controls), pubertal data had been collected as part of clinical assessment by the clinical team and were available in 12 participants at baseline, 12months and 11 participants at 24 months. The participants were categorised as pre-pubertal (Tanner stage1), mid-pubertal (Tanner stages 2-3) and late pubertal (Tanner stages 4-5). Data for bone age (BA) were available for rhGH group at baseline, 12 months and 24 months. BA was determined using the Tanner (TW2) RUS method (277).

4.3.3 Biochemical assays

All laboratory parameters were taken at 9 am after overnight fasting. Systemic markers of disease activity including ESR, CRP, and serum albumins were evaluated at the study time points in both groups. Fasting glucose, glycosylated haemoglobin (HbA1c), insulin and C-peptide, triglycerides, cholesterol, were obtained at baseline, 12 and 24 months in the rhGH group. Glucose was measured in both centres using Abbott Architect c8000 (Abbott Laboratories Ltd, Maidenhead, UK) and between batch coefficient of variation (CV) was <3.9%. In 11 of the 14 children, an oral glucose tolerance test (OGTT) (n,11 children who were on treatment group during first 6 months on original trial) was also performed at baseline, 12 months and 24 months with plasma glucose levels before and 120 min after an oral glucose load (1·75g/kg up to maximum 75g). Impaired glucose tolerance and diabetes mellitus were defined according to the International Society for Paediatric and Adolescent Diabetes Clinical Consensus Guidelines 2014 (impaired glucose intolerance defined as fasting glucose level 5.6-6.9 mmol/l, 2 hour post load glucose level 7.8-<11.1 mmol/l) (306).

In Glasgow, insulin was measured using the Abbott Architect Analyser (Abbott Laboratories Ltd); with inter- and intra-assay CVs of <4.7% and <8%, respectively. In Liverpool, insulin was measured using Immunolite 2000 Analyzer (Siemens UK Ltd, Camberley, Surrey); with inter- and intra-assay CVs of <3.3% and <7.3%, respectively with standard. Fasting hyperinsulinemia was defined as fasting insulin (mU/l) ≥15 in tanner stage 1-2, ≥30 tanner stage 3-4 and ≥20 in tanner stage 5 (307) and this cut off point was used to categorised the participants at 24 months. As surrogate estimate of insulin resistance, the homeostatic model assessment (HOMA-IR) index was calculated as (fasting insulin (mU/I) × fasting glucose (mmol/l) / 22.5) (insulin resistance in children and adolescence defined as HOMA-IR, ≥4.5) (307).

C-peptide was measured in both centres using Immunolite 2000 Analyzer (Siemens UK Ltd); with inter- and intra-assay CVs of <3.3% and >6.3%, respectively (local reference range: 0.36-1.12 nmol/l). HbA1c measured using high performance liquid chromatography (local reference range: 4.8-6.6%).

Cholesterol and triglycerides were measured using the Abbott Architect c8000 (Abbott Laboratories Ltd); with inter- and intra-assay CVs of 0.8% and 0.4%, (local reference range: cholesterol, 0.36-1.12 mmol/l and triglyceride 0.4-1.5 mmol/l). IGF-1 was assayed in in both centres using a two-site chemiluminescent immunoassay (Nichols Advantage; Quest Nichols, Institute Diagnostic, Chantilly, VA, USA); with inter- and intra-assay CVs of <4% and <7.8%, respectively. To account for delayed puberty, IGF-1 was converted to SDS by adjusting for gender and BA (308).

4.3.4 Statistics

Analyses were performed using SPSS software version 22 (Newyork, USA). Non-parametric data presented with medians and ranges. Comparison between the groups was analysed using the Mann–Whitney test for continuous variables and Chi-square test for categorical variable. Changes between parameters were assessed at different time points analysed using repeated measure with Wilcoxon signed-rank tests and subsequently adjusted for multiple comparison using a Bonferroni correction. The association between variables was assessed using the Spearman's correlation coefficient. $p<0.05$ was considered as statistically significant. All graphs performed by GraphPad Prism software version 7(San Diego California, USA).

4.4 Results

4.4.1 Disease and laboratory characteristics

Disease location was L1 in 2/14 (14%) of the rhGH group and 1/14 (7%) of the control group (rhGH vs controls, p=0.54). L2 involvement was present in 5/14 (36%) of the rhGH group and 10/14 (71%) of the control group (rhGH vs controls, p=0.06). and L3 disease was present in 7/14 (50%) in the rhGH group and 3/14 (21%) in the control group (rhGH vs controls, p=0.11). L4 involvement was present in 6/14 (36%) of the rhGH group and 4/14 (42%) of the control group (rhGH vs controls, p=0.43). Perianal disease was present in 6/14 (42%) of rhGH and 3/14 (21%) in the control group (rhGH vs controls, p= 0.22). At baseline, indices of disease biomarkers were similar in both groups and did not show any significant change at 12 months and 24 months (Table 4-1).

4.4.2 Treatment characteristics

Table 4-1 summarises the treatment characteristics of both groups. At baseline, all children in both groups were on concomitant immunomodulator therapy. There were no significant differences in the use of 5-aminosalicylate, methotrexate, anti-TNF-α therapy, prednisolone or number of children undergoing surgical resection of bowel between groups' at all three time points. At baseline, the proportion of azathioprine use was significantly higher in rhGH group compared to the control (rhGH vs controls, p= 0.02).

Table 4-1: Disease and treatment factors

	rhGH			Control		
	Baseline	**12 Months**	**24 Months**	**Baseline**	**12 Months**	**24 Months**
ESR (mm/hr)	22.0 (3.0, 51.0)	15.0 (1.0, 30.0)	12.0 (1.0, 48.0)	22.0 (6.0, 62.0)	20.0 (5.0, 97.0)	7.0 (1.0, 44.0)
n (%) >20 mm/hr	7 (50)		4 (29)	6 (50)		3 (23)
CRP (mg/l)	8.0 (4.0, 42.0)	7.0 (4.0, 20.0)	10.0 (3.0, 35.0)	7.0 (4.0, 53)	13.0 (3.0, 69.0)	7.0 (7.0, 54.0)
n (%) >7 mg/l	6 (50)		8 (57)	5 (36)		6 (46)
Albumin (g/l)	38.0 (20.0, 50.0)	38.0 (19.0, 51.0)	38.0 (30.0, 43.0)	42.0 (31.0, 46.0)	37.0 (21.0, 44.0)	39.0 (30.0, 44.0)
n (%) <35 g/l	3 (21)		2 (14)	1 (7)		3 (21)
Medication n (%)						
5-aminosalicylate	6/14 (42)	4/14 (28)	4/14 (28)	10/14 (71)	10/14 (71)	9/14 (64)
Azathioprine	9/14 (64)[a]	8/14 (57)	8/14 (57)	3/14 (21)	3/14 (21)	4/14 (29)
Methotrexate	4/14 (28)	3/14 (21)	4/14 (28)	8/14 (57)	8/14 (57)	8/14 (57)
Anti-TNF-α	2/14 (14)	4/14 (28)	4/14 (28)	2/14 (14)	1/14 (7	5/14 (36)
Glucocorticoids	3/14 (21)	2/14 (14)	1/14 (7)	2/14 (7)	3/14 (21)	3/14 (21)
EEN	0/14 (0)	0/14 (0)	0/14 (0)	2/14 (14)	1/14 (7)	1/14 (7)
Surgery n (%)	2/14 (14)	2/14 (14)	0/14 (0)	2/14 (14)	1/14 (0)	1/14 (0)

Data presented as number, n; percentage, %, median (range). ESR, erythrocyte sedimentation rate; CRP, C - reactive protein; EEN, exclusive enteral nutrition. [a] p value<0.05 (rhGH group vs. control).

4.4.3 Anthropometric characteristics

Table 4-2 summarises median (ranges) changes of anthropometric and pubertal outcomes in rhGH compared to control group at each time point of study interval and figure 3-2 show individual data. At baseline, Ht SDS in rhGH group was significantly below zero (p=0.000), below their MPH SDS (p=0.00) and below control group (0.001). All children in the rhGH group grew at subnormal rate prior to starting rhGH therapy and showed significant improvement in HV; at 12 months representing a median percentage increase of 143.0% (32.0, 587.0). At baseline, Ht SDS in control was significantly below zero (p=0.000), below their MPH SDS (p=0.01) and there was a trend toward decrease HV at 24 months (p=0.13 vs. baseline). The median percentage of HV in control group at 12 months was 0.6% (-70.0, 85.0) (p=0.72).

In the rhGH group there was a signficant fall in median Ht SDS from -2.5 (-3.9, -1.0) at 12 months prior starting rhGH therapy to to -2.8 (-3.8, -1.4) at baseline (basline vs. 12 months prior to satring rhGH, p=0.03), median Ht SDS increased signficantly at 12months (12 months vs. baseline, p=0.002) and at 24 months (24 months vs. baseline, p=0.002). The increase in Ht SDS in the rhGH group was associated with an increase in SH SDS from -2.2 (-4.1, -1.2) at baseline to -1.8 (-3.8, -1.2) (12 months vs. baseline, p=0.012) at 12 months and to -1.8 (-2.8, -0.7) (24 months vs. baseline, p=0.012) at 24 months. Corresponding increase in SILL SDS which was -2.1 (-4.2, -0.8) at baseline, -1.4 (-3.7, -0.02) at 12 months (12 months vs. baseline, p=0.004) and -1.3 (-3.4, 1.2) at 24 month (24 months vs. baseline, p=0.004). In the control group, median Ht SDS remained unchanged at baseline, 12 and 24 months respectively. Although at baseline, Ht SDS of the rhGH group was significantly lower than the control group [vs. controls, p=0.66], by 24 months there was no significant difference between the two groups [vs. controls, p=0.66].

In rhGH group, median ΔHt SDS increased signficantly at 12 months (12 months vs. baseline, p=0.004) and at 24 months (24 months vs. baseline, p=0.004). In the control group, ΔHt SDS remained unchanged during study interval.

4.4.4 Body mass index, puberty & skeletal maturation

At baseline, median BMI SDS in the rhGH was significantly lower than the control group (rhGH vs. controls, p=0.04) and remained unchanged in both groups. Of the 14 children in the rhGH group, 8 (57%) showed pubertal progression at 24 months whereas 2 out of the 11 (18.1%) who had puberty assessed showed pubertal progression in the control group (Table 4-2). Median (range) BA at baseline, 12 and 24 months was 13.2 years (8.0, 14.4), 13.8 years (8.0, 15.2) and 14.8 years (10.1, 16.7) respectively. At baseline, median (range) of BA in rhGH group was not different from chronological age of control (rhGH vs. controls, p=0.51). Similarly, 24 months values was not different (rhGH vs. controls, p=0.41). Median BA delay in rhGH group at baseline, 12 and 24 months was 1.5 years (0.4, 3.9), 2.2 years (0.1, 4.2) (12 months vs. baseline, p=0.06) and 1.8 years (-0.3, 3.9) (24 months vs. baseline, p=0.14), respectively.

Table 4-2: Change in anthropometric measurements, body mass index and puberty over 24 months

	rhGH			Control		
	Baseline	**12 Months**	**24 Months**	**Baseline**	**12 Months**	**24 Months**
MPH SDS	-0.5 (-1.2, 0.1)			-0.6 (-2.1, 1.4)		
Ht SDS	-2.8 (-3.8, -1.4) [a,]	-1.9 (-3.2, -0.8)[b]	-1.6 (-2.4, -0.23) [a, c]	-1.5 (-2.6, -0.7)	-1.4 (-2.4, -0.5)	-1.3 (-2.2, -0.5)
HV (cm/year)	3.5 (1.0 , 7.0)[a]	7.8 (5.2, 13.5)[b]	7 (0.7, 11.3)[a, c]	5.5 (3.3, 12.6)	5.4 (1.1, 8.9)	3.8 (0.0, 7.2)
ΔHt SDS	-0.3 (-0.7, 0.3)[a]	0.6 (0.2, 1.0)	0.5 (-0.05, 0.9) [a, c]	0.01 (-0.2, 0.7)	0.05 (-0.5, 0.9)	-0.01 (-0.6, 0.5)
BMI SDS	-1.1 (-2.9, 0.3)[a]	-1.1 (-2.7, 0.7)	-1.1 (-2.3, 1.5)	-0.1 (-2.2, 4.5)	-0.4 (-2.3, 2.2)	-0.5 (-1.9, 1.8)
Pre-pubertal	3/14	3/14	2/14	4/12	4/12	4/11
Mid-pubertal	10/14	5/14	3/14	5/12	4/12	3/11
Late pubertal	1/14	6/14	9/14[d]	3/12	4/12	4/11

Data presented as number; median (range). MPH SDS, mid-parental height SD score; Ht SDS, height SD score; ΔHt SDS, change in height SD score; HV, height velocity; BMI SDS, body mass index SDS. [a] pvalue<0.05 (rhGH group vs. control), [b] p value<0.05 (baseline vs. 12 months), [c] p value <0.05 (baseline vs. 24months)

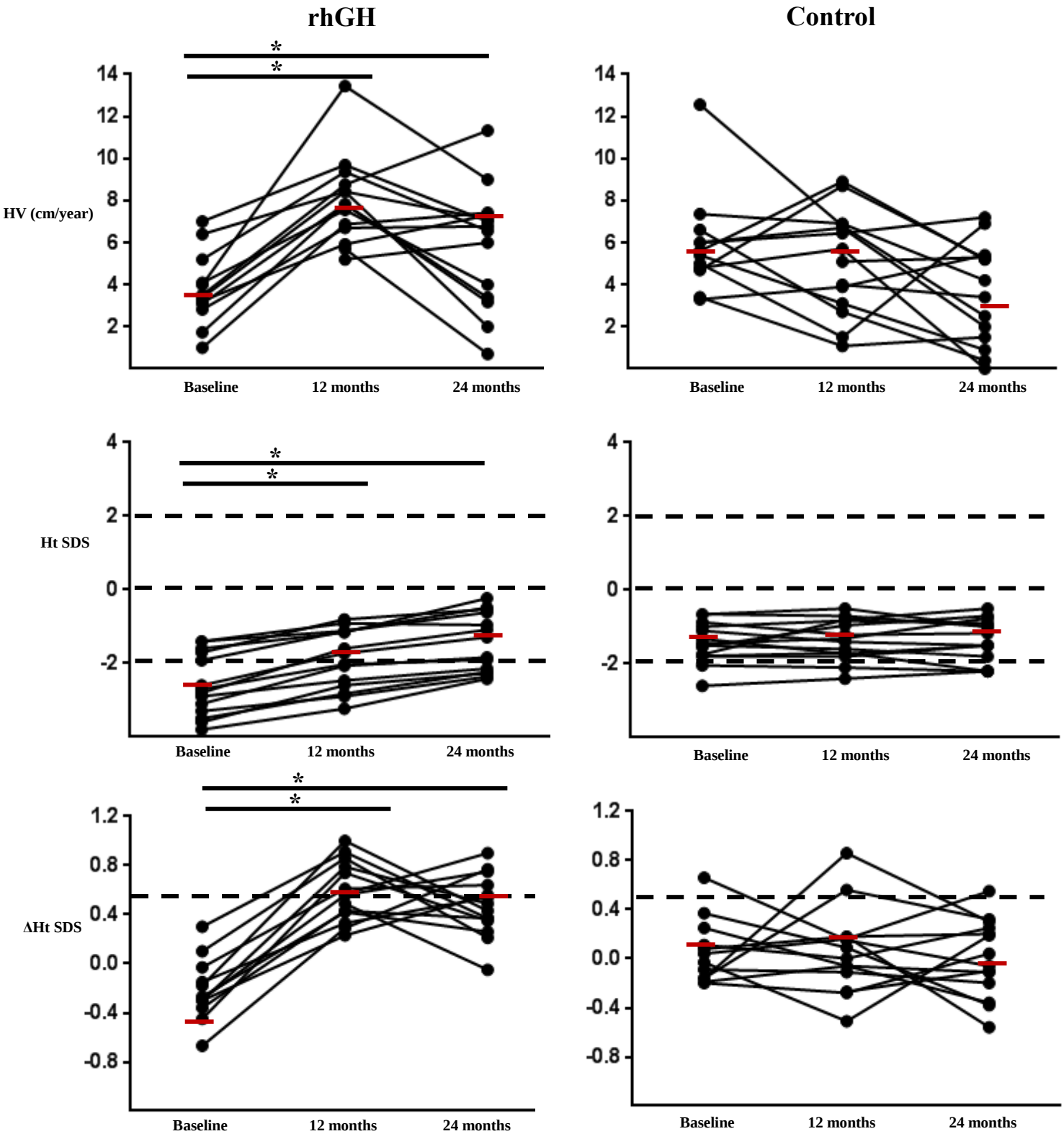

Figure 4-2: Individual change in anthropometric in rhGH (left) and the control (right) group. HV, height velocity (cm/years); Ht SDS, height SD score; ΔHtSDS, change in height SD score. Red solid lines represent the median values. Black dotted lines represent the 50th centile (0SDS), 3rd (-2SDS) and 97th (2SDS) centile. *indicate statistical significance ($p<0.05$).

4.4.5 IGF-1

Figure 4-3 individual data of plasma IGF-1 SDS. Median plasma IGF-1 SDS adjusted for BA increased from -2.7 (-3.7, 2.9) at baseline to 0.3 (-2.1, 2.4) (12 months vs. baseline, p=0.014) at 12 months and to -0.6 (-3.1, 3.1) (24 months vs. baseline p=0.12) at 24 months. The proportion of IGF-1 SDS <-2 was 8 /11 at baseline decreased to 1/11 at 12 months (12 months vs. baseline, p=0.004) and 2/11 at 24 months (24 months vs. baseline p=0.02) respectively. At baseline, 12 and 24 months, no correlation observed between IGF-1 SDS with disease biomarkers (ESR, CRP, and albumin) or with Ht SDS. Similarly, no association observed between 12 months change in IGF-1 SDS with HV or Δ Ht SDS at 12 months. To be noted three participants had their IGF-1 value at baseline and 12 months and no 24 months data were available [(IGF-1 SDS: ID-1 baseline (-2.8), 12 months (1.5); ID-2 baseline (-3.1), 12 months (-2.5); ID-3 baseline (-0.4), 12months (1.4)].

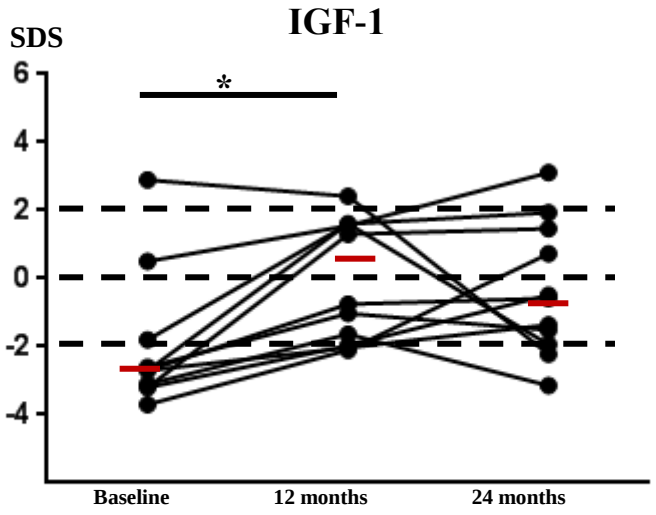

Figure 4-3: Individual changes in serum insulin like growth factor-1 SD score in rhGH following therapy with rhGH therapy.
Red solid lines represent the median values. Black dotted lines represent the 50th centile (0SDS), 3rd (-2SDS) and 97th (2SDS) centile. *indicate statistical significance ($p<0.05$).

4.4.6 Metabolic status

Table 4-3 summarises median (ranges) changes of glucose homeostasis and lipid status in rhGH at each time point of study interval and figure 4-4, shows individual data of fasting insulin, HOMA-IR and figure 4-5, shows individual data of OGTT. Median glucose, HbA1c, cholesterol and triglycerides remained unchanged over the 24 months. Despite an increase in median fasting insulin, C-peptide and HOMA-IR, median values remained within the normal reference range. No significant results observed in parameters of glucose homeostasis by sorting the participants according to ESR.

One participant, a 14.9-year-old boy who had a fasting insulin level (19 mU/l), within the reference range at baseline (Tanner stage-2; BMI SDS, 0.3; ESR, 26.0), who had not received GC throughout the study period developed hyperinsulinemia (24 mU/l) at 24 months (tanner stage-5; BMISDS, 1.5; ESR, 21.0) with normal OGTT (Fasting glucose 5.6 mmol/l, 2 hour 7.3 mmol/l and HbA1C (5.2%)). This participant was followed up 5 years after stopping rhGH therapy and he did not develop diabetes.

None of the 11 rhGH treated children who had an OGTT at 24 month was found to have asymptomatic diabetes mellitus. One participant, a 14.7-year-old girl at baseline (B3; BMI SDS, -1.1), who had not received GC throughout the study period and who had a normal OGTT at baseline (fasting glucose 5.1 mmol/l; 2-hr glucose 6.5 mmol/l; fasting insulin 11 mU/L) developed impaired glucose tolerance (fasting glucose 4.7 mmol/L; 2-hr glucose 9 mmol/L; fasting insulin 11.3 mU/L) at 12 months (B5; BMI SDS, -1.2; ESR, 9.0). By 24 months, her OGTT normalised (fasting glucose 4.6 mmol/l; 2-hr glucose 3.5 mmol/l; fasting insulin 9.1 mU/L) with no change in rhGH dose. At baseline, 12 and 24 months no correlation was observed between any of metabolic and disease parameters.

Table 4-3: Glucose homeostasis and lipid status following therapy with rhGH

				p-values	
	Baseline	**12 Months**	**24 Months**	**Baseline vs. 12 months**	**Baseline vs 24months**
Fasting Glucose (mmol/l)	4.7 (3.5, 5.5)	4.8 (4.0, 5.4)	4.9 (4.0, 6.2)	0.20	0.15
Insulin (mU/l)	4.3 (1.9, 19)	7.7 (3.5, 15)	8.4 (2.3, 24.2)	0.14	0.09
Tanner stage1-2(n, 4)	3.2 (1.9, 4.8)	7.4 (3.5, 11.5)	5.2 (3.5, 8.7)	0.14	0.47
Tanner stage 3-4(n, 4)	3.8 (2.9.5, 4.5)	7.8 (4.2, 15)	9.1 (6.2, 13.3)	0.14	0.14
Tanner stage 5 (n, 6)	8.5 (3.7, 19)	11 (4.0, 12.8)	9.2 (2.3, 24.2)	0.69	0.67
C-peptide (nmol/l)	0.34 (0.1, 1.15)	0.7 (0.3, 1.0)	0.6 (0.3, 2.5)	**0.01**	0.08
HbA1C %	5.3 (4.5, 6.3)	5.3 (4.7, 5.7)	5.2 (4.5, 5.9)	0.22	0.22
HOMA-IR	0.8 (0.4, 4.6)	1.5 (0.6, 3.5)	1.7 (0.5, 6.1)	0.08	**0.048**
HOMA-IR ≥4.5(n)	1	0	1		
Cholesterol (mmol/l)	3.5 (2.5, 5.5)	3.3 (1.9, 5.5)	3.3 (2.4, 4.5)	0.21	0.06
Triglyceride (mmol/l)	0.9 (0.68, 2.5)	0.8 (0.3, 2.2)	0.8 (0.3, 1.8)	0.56	0.53
Oral Glucose Tolerance Test (n, 11)					
Fasting Glucose (mmol/l) (n, 11)	4.7 (3.9, 5.1)	4.7 (4.1, 5.7)	5.0 (4.3, 5.8)	0.77	0.13
2 hours Glucose (mmol/l) (n, 11)	5.3 (3.5, 6.5)	4.9 (2.7, 9)	5.5 (3.2, 7.7)	0.44	0.96

Data presented as median (range); n, number. HbA1C (%), glycosylated haemoglobin; HOMA-IR, the homeostatic model assessment. Values in bold indicate statistical significance ($p < 0.05$).

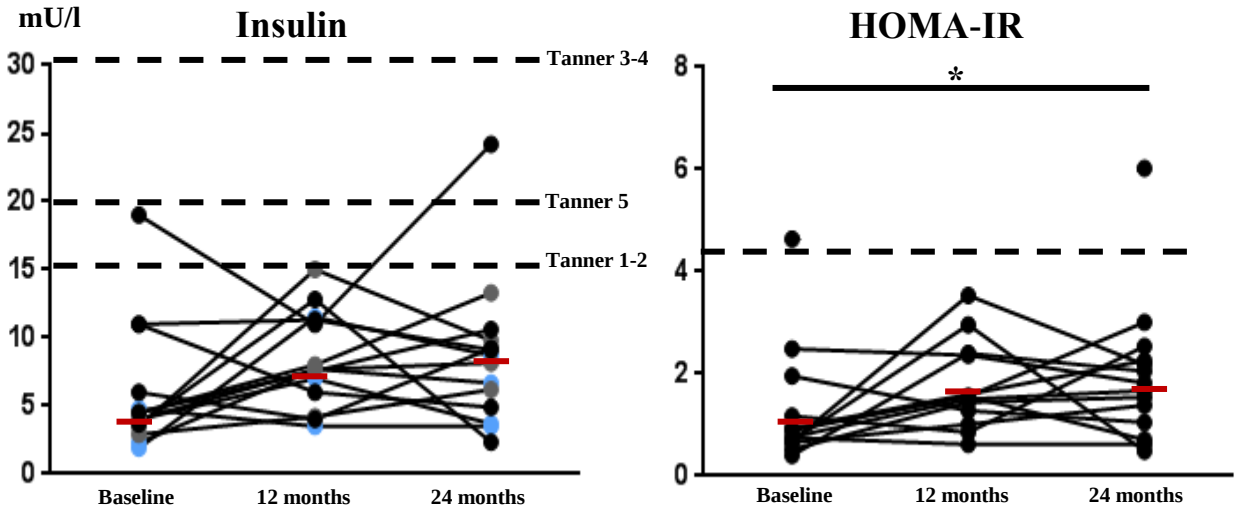

Figure 4-4: Individual change in fasting insulin (left), HOMA-IR (right).
HOMA-IR, the homeostatic model assessment following therapy with rhGH therapy. Red solid lines represent the median values. Blue filled circles represent fasting insulin data for participants who stayed at tanner stage 1-2 at 24 months, grey filled circles represent fasting insulin data for participants who were at tanner stage 3-4 at 24 months and black filled circles represent fasting insulin data for participants who were at tanner stage 5 at 24 months. Black dotted lines represent the cut-off point of fasting insulin and HOMA-IR according to Obesity Services for Children and Adolescents (307). *indicate statistical significance ($p<0.05$).

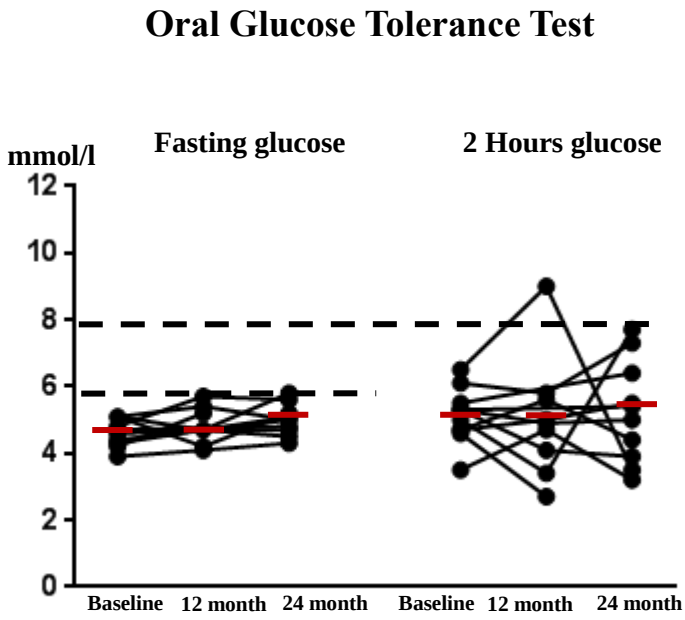

Figure 4-5: Individual change in fasting glucose (left), 2 hour glucose (right) after oral glucose tolerance test following therapy with rhGH therapy.
Red solid lines represent the median values. Black dotted lines represent the cut-off point of definition of impaired glucose tolerance to the International Society for Paediatric and Adolescent Diabetes Clinical Consensus Guidelines 2014(306).

4.5 Discussion

To date, several short-term studies of rhGH up to 12 months, suggest that rhGH therapy can promote short term growth in children with CD (258-263). The extended period of study in this current report clearly shows that in these children, rhGH therapy for 24 months at a dose of 67 mcg/kg/day leads to a sustained improvement in growth. Increasingly, an adequate growth response to growth promoting therapy is defined as ΔHt SDS of greater than +0.3 or +0.5 over the first 12 months of the intervention (283). In the current study, the median ΔHt SDS at 12 and 24 months was +0.6SDS and +0.5SDS, respectively. These data are comparable to a previous open label non-randomised study in 7 children with IBD using rhGH, 50mcg/kg/day, for 12 months (261). In another study that administered rhGH at 75 mcg/kg/day, ΔHt SDS was +0.7SDS for the subgroup of 18 children with CD who continued on rhGH for 12 months (259). The observed response was also comparable to other groups of children with chronic disease who has received rhGH for similar lengths of period (256;267;309). However, the key question does this translate into an increase in final height? Still remain unanswered.

Pubertal status is a potential confounder in this study. The treatment group had regular assessment of puberty and although puberty did progress in this group, over the two-year period of this study, the extent of bone age delay remained unchanged and, the increase in height was associated with an a proportionate increase in subischial leg length and sitting height suggesting that the improvement in height was not solely due to progression in puberty. In the historical control group, puberty was only examined in those where there were concerns about pubertal development thus explaining the lower percentage of pubertal progression in the control group. A greater proportion of controls were prepubertal at baseline and throughout the study. These factors could have contributed to a greater growth response in the rhGH-treated groups compared to controls. This heterogeneity in assessment makes the comparison between pubertal status weak and the complementary role of puberty on growth in this cohort cannot be overlooked.

Although there were some differences between the two groups with respect to the use of immunomodulator therapy with azathioprine use higher in the rhGH group compared to the control group, it is unlikely that these differences would have influenced the different growth response. Whilst the use of azathioprine may lead to maintenance of remission, it is unclear if its use or timing of introduction in CD has a positive impact on linear growth (127).

Although the two groups were matched for age, sex and duration of disease, the control group was not matched for Ht SDS as very short children around this period were all

recruited into the rhGH therapy trial. Whilst there is a possibility that the more marked short stature of the rhGH group provided them with a greater potential for catch up growth, we believe that this does not fully explain the improvement in growth that was observed in the treatment group especially as the disease status was similar throughout the two year period.

Besides a growth promoting effect, rhGH therapy has been reported to be associated with an improvement in disease status (268;269). In the current study, inflammatory markers were similar between both groups and remained unchanged over 24 months. The possibility that an improvement in disease secondary to rhGH therapy, itself, could be a contributing mechanism for improvement in linear growth is intriguing and would require a more detailed assessment of disease activity in future studies.

Patients with chronic inflammation are known to display insulin resistance (305) and this has also been observed previously in those with CD who received rhGH therapy (262). Therapy with even higher doses of rhGH at 75mcg/kg/day for one year in children with CD was associated with a rise in fasting insulin level without impairment in glucose tolerance (259). Whilst HOMA-IR rose with rhGH treatment in our cohort, this was not deemed clinically significant based on current consensus guidelines (307). The increase in HOM-IR was not associated with impaired glucose tolerance and was not as marked as observed in children with JIA at the same dose of rhGH (264). Nevertheless, we believe close monitoring of glucose homeostasis is required.

Finally, our results suggest that rhGH therapy might be associated with sustained improvement in linear growth in short pubertal children with mild or inactive CD. However, the current study was limited to a relatively small group of cases and historical controls hamper drawing firm conclusion. Thus, the results will be important to confirm in a larger group of subjects.

4.6 Conclusion

In conclusion, the results of our study indicate that the growth promoting effect of rhGH in children with CD that we previously observed over a period of 6 months is sustained over a longer period and this finding needs to be confirmed in a randomised clinical trial. In the meantime, the use of rhGH in children with CD and growth retardation needs to be undertaken very carefully with close monitoring of the IGF-1 status and glucose homeostasis.

Chapter Five

Recombinant Human Growth Hormone Therapy in Children with Crohn's Disease: Impacts on Bone Health

5.1 Abstract

Background: Data on bone mineral density (BMD) in children and adolescents with CD during rhGH treatment are not available.

Aims: To determine the effects of prolonged rhGH therapy on BMD by DXA of total body and lumbar spine.

Study design and subjects: Prospective study of eight children with CD (six male), aged 9 - 16.4 (median 14.8) years who were in puberty and received rhGH (67 mcg/kg/day) for 24 months.

Main outcomes: BMD by DXA (TB and LS) were measured at baseline, 6 months, 12 months and 24 months following rhGH therapy. DXA bone outcomes are expressed as sex, race, bone-age or height-age specific standard deviation scores (SDS) and compared with reference data. TB-lean mass and fat mass were measured by DXA and adjusted for sex and height. A biomarker of bone formation, P1NP, was measured in fasting blood and adjusted for sex and bone age. A biomarker of bone resorption, CTX, was measured in urine and reported as absolute values.

Results: At baseline, CD participants were short and had low TB-BMD, LS-BMD, LS-BMAD and TB-LM SDS. Based on wPCDAI: at baseline, 4/8 were in remission and 3/8 had mild disease; by 6 months, 5/8 were in remission and 3/8 had mild disease, and at 24 months, 4/8 were in remission and 4/8 had mild disease. Three out of eight participants were on GC at baseline, 2/8 were on GC at 6 months, and 1/8 at 24 months. At 6 months, P1NP levels increased a median of 24% (0.0, 1048.0) (p=0.02) and urinary CTX a median of 37% (9.0, 450.0) (p=0.04). Twenty-four months following rhGH therapy, participants showed significant improvement in height and 6/8 completed their pubertal growth. TB-BMD, LS BMD and LS BMAD remained unchanged at 24 months.

Conclusion: These data demonstrate persistent musculoskeletal deficits in children with inactive/mild CD after 24 months of rhGH therapy.

5.2 Introduction

Bone mass deficits are well described in children with CD (289). The intact GH-IGF1 pathway is a critical regulator of linear growth, osteoblast function, bone homeostasis and ultimately bone mass (73-75;284). In children with CD, systemic markers of the GH-IGF-1 axis are altered, suggesting that many may exhibit a range of functional GH insufficiency and resistance (101). In accordance with an anabolic effect of GH, previous studies on rhGH therapy in children with chronic inflammation such as JIA showed that, in addition to an increase in height, GH also has a positive effect on bone health and body composition (265;266;310). RhGH therapy may help to reduce detrimental effects of CD on bone health and muscle. However, the effect of rhGH on bone health in CD has scarcely been studied (260;261). Furthermore, there are no data on the long-term consequences of GH treatment on bone and body composition in paediatric CD. As an initial test of the hypothesis that GH treatment may increase BMD in children with CD, we carried out a small, preliminary pilot study to assess the changes in biomarkers of bone metabolism and bone mineral density in children with CD and growth retardation who received rhGH treatment for two years.

5.3 Methods

5.3.1 Study population

Eight out of thirteen children with CD (6male), who received rhGH at a dose of 67 mcg/kg/day as part of a six-month RCT at the Royal Hospital for Sick Children Glasgow (262) were studied for bone biomarkers and bone health by DXA. The participants at the Liverpool site were not included for the bone health assessment study as it was not feasible to conduct DXA scans at that location. Of the 13 children, rhGH was discontinued due to completion of linear growth (n=2), lost to follow-up (n=1), needle phobia (n=1) and post-colectomy in a child with UC (n=1). rhGH dose was adjusted for weight at each visit. Disease phenotype was classified using the Montreal criteria (5).
The original clinical trial was approved by the UK Multicentre Research Ethics Committee, and its conduct was approved by the Health Boards. Written informed consent was obtained from all parents and patients as appropriate for those patients on rhGH.

5.3.2 Study parameters and biochemical markers of disease and growth

Data on therapeutic interventions, anthropometry, puberty and disease biomarkers were collected and reported at baseline, 6 months, 12 months and 24 months as described previously in Chapter 4. Disease activity was assessed by wPCDAI as previously described in Chapter 2. Cut-off points for categorising the participants according to disease status, or GC exposure were defined as detailed in Chapter 2 and adapted in this chapter to categories DXA outcomes. To investigate the impact of pubertal progress on BMD and LM the participants were categorised based on pubertal status at 24 months as follows: stayed pre-pubertal (Tanner stage 1; n=1); progressed from pre-pubertal to early puberty (Tanner stage 2-3; n=1); completed their pubertal growth (Tanner stage 5; n=6).

5.3.3 DXA outcomes

DXA data were available for eight participants at baseline, 6 months, 12 months and 24 months. DXA scans acquisitions and analyses were described previously in Chapter 3. We adjusted for size using Ht-age and adjusted for skeletal maturation using BA for TB and LS using GE Lunar paediatric reference data of over 2000 US children (5-19 years). Body composition was adjusted for height using our local reference data of 201 (140 male)

children aged between 5-19 years. Bone mineral apparent density (BMAD) was calculated for LS and was adjusted to bone age (311).

5.3.4 Bone biomarkers

Biomarkers of bone turnover were measured at baseline and 6 months. A biomarker of bone formation, serum P1NP, was analysed in fasting blood with a radioimmunoassay kit (Intact P1NP; Orion Diagnostica, Finland) with inter- and intra-assay CVs of 7.8% and 8.3%, respectively. Gender and pubertal variation were adjusted for using published reference data (312). A biomarker of bone resorption, CTX-1, was measured in first morning voided urine with an enzyme immunesorbent assay (Immunodiagnostic Systems, UK) with inter- and intra-assay CVs of 4.3% and 6.9%, respectively. No reference data were available for urinary CTX-1.

5.3.5 Statistics

Analyses were performed using SPSS software version 22 (New york, USA). Non-parametric data presented with medians and ranges. Changes between parameters were assessed at different time points analysed using repeated measure with Wilcoxon signed-rank tests and subsequently adjusted for multiple comparison using a Bonferroni correction. Spearman's correlation coefficient was used to assess univariate relationship between continuous variables. SPSS mixed modelling was employed to explore the confounding effects of independent variables (IGF-1 SDS, wPCDAI, GC exposure, and TB-LM SDS) on TB-BMD and LS-BMD over the 24 months period. $p<0.05$ was considered as statistically significant. All graphs performed by GraphPad Prism software version 7(San Diego California, USA).

5.4 Results

5.4.1 Participant characteristics

Table 5-1 summarises the characteristics of the eight CD participants at the time of starting rhGH. The median (range) age at diagnosis of CD and age at rhGH initiation was 10.1 years (6.0, 13.8) and 14.8 years (9.0, 16.4) with median interval since diagnosis of 3.3 years (0.6, 10.1). Two participants had the disease for less than 18 months.

Table 5-1: Participant characteristics at time of starting rhGH therapy

	CD (n, 8)
Male, n (%)	6 (75%)
CA at Diagnosis (year)	10.1 (6.0, 13.8)
CA at starting rhGH (year)	14.8 (9.0, 16.4)
Duration of disease	3.3 (0.6, 10.1)
BA at starting rhGH (year)	13.3 (8.0, 14.4)
BA delay (year)	1.3 (-0.4, 2.2)
Tanner stage, n (%)	
Pre-puberty (tanner stage-1)	2 (25)
Mid-puberty (tanner stage 2-3)	5 (63)
Late puberty (tanner stage 4-5)	1 (12)
Ht SDS at starting rhGH	-2.4 (-3.3, 1.4)
BMI SDS at starting rhGH	-0.5 (-1.5, 0.3)
Disease location, n (%)	
Ileal (L1)	2 (25)
Colonic (L2)	4 (50)
Ileocolonic (L3)	2 (25)
Isolated upper disease(L4)	2 (25)
Behaviour (non-stricturing, non-penetrating)	8 (100)
Perianal	4 (50)

Data presented as number, n; percentage, %, median (range). CA, chronological age; BA, bone age; Ht SDS, height SDS score; BMI SDS, body mass index SDS score.

5.4.2 Disease, treatment characteristics and anthropometry

Table 5-2 summarises disease, treatment characteristics and anthropometry in eight CD participants at each visit. At baseline, based on wPCDAI, 4/8 (50%) participants were in remission, 3/8 (38%) had mild CD, 1/8 (12%) had moderate CD and none (0%) had severe CD. At 6 months and 24 months, all participants had quiescent disease (remission: n=4; mild disease: n=4). Three out of eight (38%) participants were on GC at baseline, 2/8 (25%) at 6 months and 1/8 (12%) at 24 months.

The percentage of participants with Ht SDS below the minimum reference range (Ht SDS ≤ -2) decreased from 6 (75%) at baseline to 3 (38%) at 12 months and 3 (38%) at 24 months. At baseline, 6/8 participants had entered puberty and 5/6 of participants were in mid-puberty. At 6 months, only one participant showed pubertal progress, from G2 to G4. However, 6/8 participants were in the late pubertal stage at 24 months. At baseline, the median (range) BA was 13.3 years (8.0, 14.4) (BA vs. CA, p=0.16), 14.1 years at 6 months, 14.5 years (8.0, 15.2) at 12 months and 15.3 years (10.1, 16.7) (BA vs. CA, p=0.23) at 24 months. Interestingly, we found that the bone age of those who were in late puberty at 24 months was ≥ 14.5 years for males and ≥ 13.5 years for one female. The BA of one male in early puberty was 10.1 and that of one female in pre-puberty was 11.2.

Table 5-2: Changes in disease activity, laboratory results, and treatment over the study interval

n, 8	Baseline	6 Months	12 Months	24 Months	p-values		
					Baseline vs. 6 months	Baseline vs. 12 months	Baseline vs. 24 months
wPCDAI	17.5 (0.0, 53.0)	7.5 (0.0, 35.0)	7.5 (0.0, 20.0)	13.7 (0.0, 25.0)	0.18	0.11	0.25
Remission	4/8	5/8	6/8	4/8			
Mild disease	3/8	3/8	2/8	4/8			
Moderate	1/8	0	0	0			
ESR (mm/hr)	24.0 (7.0, 53.0)	24.0 (4.0, 41.0)	21.5 (3.0, 30.0)	16.5 (5.0, 48.0)	0.49	0.14	0.78
CRP (mg/l)	8.0 (7.0, 42.0)	7.0 (7.0, 25.0)	7.0 (7.0, 20.0)	9.0 (3.0, 35.0)	0.50	0.56	0.23
Albumin (g/l)	35.0 (20.0, 44.0)	35.0 (25.0, 39.0)	37.0 (19.0, 41.0)	37.0 (30.0, 39.0)	0.83	0.91	0.23
Medication							
Glucocorticoids	3/8	2/8	1/8	1/8			
Aminosalicylates	2/8	2/8	2/8	2/8			
Mercaptopurine/Azathioprine	4/8	5/8	5/8	4/8			
Methotrexate	4/8	2/8	2/8	3/8			
Anti-TNF-α		2/8	3/8	1/8			
Exclusive enteral nutrition	0	1/8	0-	0			
Anthropometry							
Ht SDS	-2.4 (-3.3, -1.4)	-1.9 (-3.0, -1.2)	-1.4 (-2.9, -0.81)	-0.9 (-2.4, -0.2)	0.05	**0.035**	**0.035**
HV (cm/year)	3.4 (0.6,8.4)	9.1 (6.1, 11.6)	7.8 (5.9, 9.7)	6.9 (2.0, 11.3)	**0.036**	**0.036**	0.21
BMI SDS	-0.5 (-1.5, 0.3)	-0.6 (-1.45, 0.31)	-0.3 (-2.3, 0.73)	-0.8 (-1.7, 1.5)	0.89	0.48	0.87
Pre-puberty	2/8	2/8	1/8	1/8			
Early-puberty	5/8	4/8	2/8	1/8			
Late- puberty	1/8	2/8	5/8	6/8			

Data presented as number, n; median (range). wPCDAI, weighted paediatric disease activity index; ESR, erythrocyte sedimentation rate; CRP, C-reactive protein; Ht SDS, height SD score; BMI SDS, body mass index SD score. Values in bold indicate statistical significance ($p<0.05$).

5.4.3 DXA bone outcomes

Table 5-3 summarises the DXA bone outcomes at baseline, 6months, 12 months and 24 months among eight participants and Figure 5-2, shows individual data. At baseline, overall TB-BMD SDS $_{\text{Ht-age}}$ (p=1.00) was not different from zero while, TB-BMD SDS $_{\text{BA}}$ (p=0.010), LS-BMD SDS $_{\text{Ht-age}}$ (p=0.01), LS-BMD SDS $_{\text{BA}}$ (p=0.00) and LS-BMAD SDS BA (p=0.010) were below zero. No significant improvement was found in BMD at any of the measurement sites during follow-up. In a sub-analysis of disease status, GC exposure or pubertal progress, no significant results were found (Tables 5-4, 5-6 and 5-7).

Table 5-3: DXA bone outcomes at baseline, 6 months, 12 month and 24 month following rhGH therapy

	Baseline	6 Months	12 Months	24 Months	p-values		
					Baseline vs. 6 months	Baseline vs. 12 months	Baseline vs. 24 months
DXA	**(n, 8)**	**(n, 8)**	**(n, 8)**	**(n, 8)**			
TB-BMD SDS $_{Ht-age}$	0.1 (-2.3, 0.7)	-0.4 (-1.9, 0.3)	-0.7 (-2.2, 0.4)	-0.7 (-2.2, 0.1)	0.19	0.06	0.16
TB-BMD SDS $_{BA}$	-1.0 (-1.9, 0.1)	-1.1 (-2.0, -0.2)	-1.0 (-2.4, 0.0)	-1.2 (-1.7, -0.3)	0.12	0.21	0.57
LS-BMD SDS $_{Ht-age}$	-1.1 (-2.9, 0.6)	-1.4 (-2.0, 0.4)	-1.3 (-2.2, 0.7)	-0.8 (-2.9, 0.6)	0.94	0.73	1.00
LS-BMD SDS $_{BA}$	-1.7 (-3.0, -0.2)	-1.8 (-2.2, -0.2)	-1.6 (-2.8, 0.2)	-1.9 (-2.4, 0.1)	0.42	0.18	0.73
LS-BMAD SDS $_{BA}$	-1.1(-3.6, 0.8)	-0.9 (-2.1, 0.9)	-0.7 (-2.3, 1.5)	-0.9 (-1.6, 0.5)	0.89	0.15	0.49

Data presented as median (range). TB, total body; BMD, areal bone mineral density; BMAD, bone mineral apparent density; SDS $_{Ht-age,}$ adjusted for height age and sex; SDS $_{BA}$, adjusted for bone age and sex; LS, Lumbar spine.

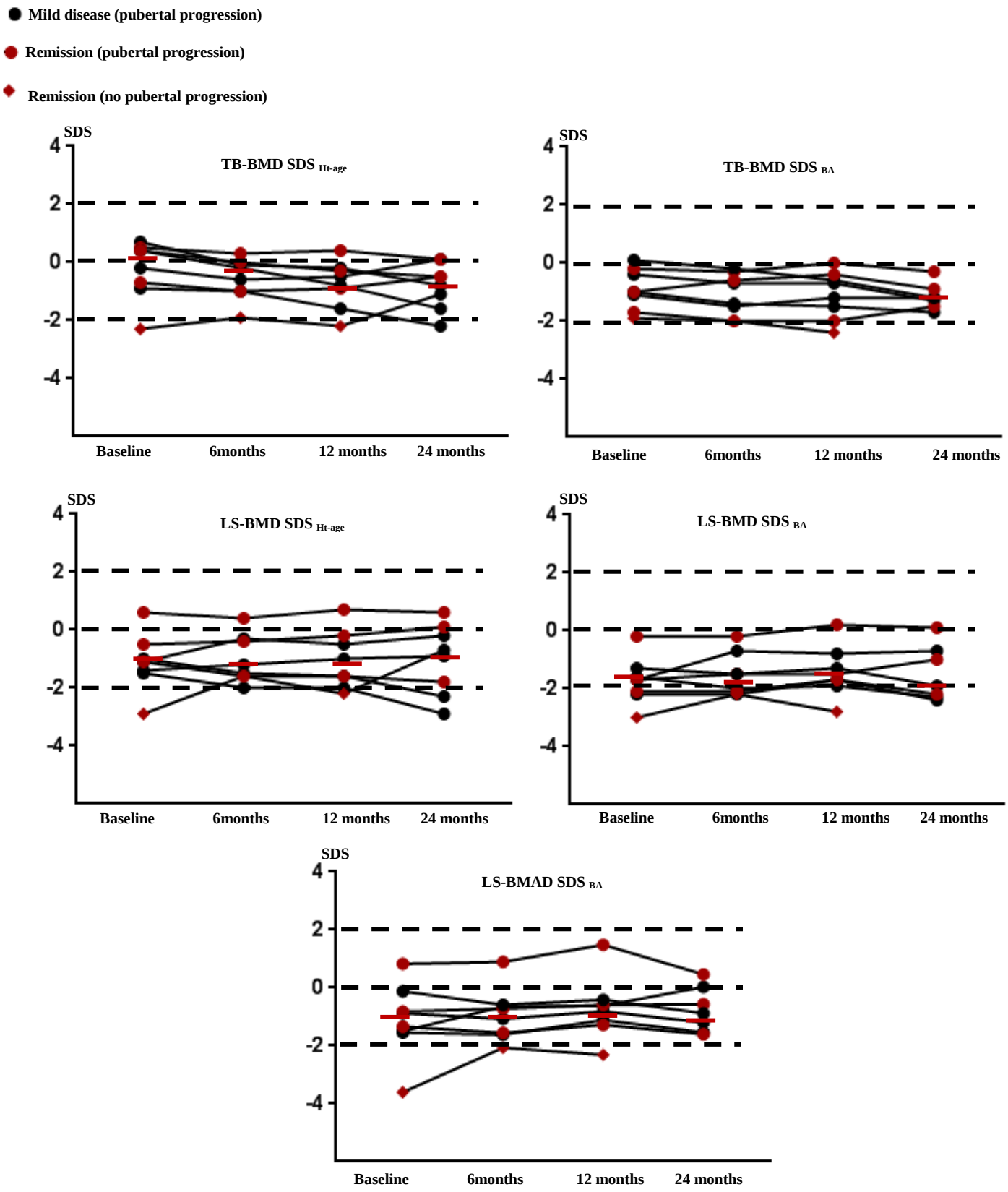

Figure 5-1: Individual changes in DXA bone outcomes at baseline, 6 months 12months and 24months following rhGH therapy

TB, total body; BMD, areal bone mineral density; BMAD, bone mineral apparent density; SDS $_{Ht\text{-}age,}$ adjusted for height age and sex; SDS $_{BA,}$ adjusted for bone age and sex; LS, lumbar spine. Black solid circle represents participants who had mild disease (based on wPCDAI) and progressed in puberty at 24 months, red solid circle represents participants who had remission and progressed in puberty whereas red sold triangle had remission and stayed at tanner stage 1 at 24 months. Red solid lines represent the median values. Black dotted lines represent the 50th centile (0SDS), 3rd (-2SDS) and 97th (2SDS) centile. *indicate statistical significance ($p<0.05$).

Table 5-4: DXA bone outcomes at baseline, 6 months, 12 months and 24 months following rhGH. Participants divided according to wPCDAI at 24 months.

n, 8	Baseline	6 Months	12 Months	24 Month	p-values		
					Baseline vs. 6 months	Baseline vs. 12 months	Baseline vs. 24 months
TB-BMD SDS $_{Ht\text{-}age}$							
Remission (n, 4))	-0.20 (-2.3, 0.5)	-0.5 (-1.9, 0.3)	-0.6 (-2.2, 0.4)	-0.5 (-1.1, 0.1)	0.58	0.20	1.0
Mild disease (n, 4)	0.2 (-0.9, 0.7)	-0.4 (-1.0, -0.1)	-0.7 (-1.6, -0.2)	-1.2 (-2.2, 0.1)	0.20	0.20	0.14
TB-BMD SDS $_{BA}$							
Remission (n, 4)	-1.4 (-1.9, -0.2)	-1.3 (-2.0, -0.3)	-1.2 (-2.4, 0.0)	-0.9 (-1.5, -0.3)	0.71	1.00	0.41
Mild disease (n, 4)	-0.7 (-1.0, 0.1)	-1.1 (-1.5, -0.2)	-1.0 (-1.5, -0.6)	-1.3 (-1.7, -1.2)	0.20	0.20	0.20
LS-BMD SDS $_{Ht\text{-}age}$							
Remission (n, 4)	-0.8 (-2.9, 0.6)	-1.0 (-1.6, 0.4)	-0.9 (-2.2, 0.7)	-0.3 (-1.8, 0.6)	1.00	0.47	0.59
Mild disease (n, 4)	-1.3 (-1.5, -1.0)	-1.4 (-2.0, -0.30)	-1.3 (-2.0, -0.5)	-1.6 (-2.9, -0.2)	1.00	0.85	0.47
LS-BMD SDS $_{BA}$							
Remission (n, 4)	-1.9 (-3.0, -0.2)	-1.8 (-2.2, -0.2)	-1.6 (-2.8, 0.2)	-1.0 (-2.2, 0.1)	0.18	0.18	0.29
Mild disease (n, 4)	-1.7 (-2.2, -1.3)	-1.8 (-2.2, -0.7)	-1.5 (-1.9, -0.8)	-2.1 (-2.4, -0.7)	1.0	0.28	0.71
LS-BMAD SDS $_{BA}$							
Remission (n, 4)	-1.1 (-3.6, 0.8)	-1.1 (-2.1, 0.9)	-0.9 (-2.3, 1.5)	-0.6 (-1.6, 0.5)	0.47	0.20	0.29
Mild disease (n, 4)	-1.2 (-1.5, -0.1)	-0.9 (-1.6, -0.6)	-0.7 (-1.1, -0.4)	-1.0 (-1.5, 0.03)	0.71	0.27	1.00

Data presented as median (range). TB, total body; BMD, areal bone mineral density; BMAD, bone mineral apparent density; SDS $_{Ht\text{-}age,}$ adjusted for height age and sex; SDS $_{BA}$, adjusted for bone age and sex; LS, lumbar spine.

Table 5-5: DXA bone outcomes at baseline, 6 months, 12 months and 24 months following rhGH. Participants divided according to glucocorticoid use.

n, 8	Baseline	6 Months	12 Months	24 Months	p-values		
					Baseline vs. 6 months	Baseline vs. 12 months	Baseline vs. 24 months
TB-BMD SDS $_{Ht\text{-}age}$							
GC naïve (n, 5)	0.4 (-2.3, 0.5)	-0.2 (-1.9, 0.3)	-0.8 (-2.2, 0.4)	-0.5 (-1.6, 0.1)	0.28	0.10	0.50
GC +/- (ID-1)	-0.2	-0.6	-0.5	0.1			
GC+/- (ID-2)	-0.9	-1.0	-1.6	-2.2			
GC+/+ (ID-3)	0.7	-0.1	-0.2	-0.8			
TB-BMD SDS $_{BA}$							
GC naïve (n, 5)	-1.0 (-1.9, 0.1)	-0.6 (-2.0, -0.2)	-0.6 (-2.4, 0. 0)	-1.1 (-1.5, -0.3)	0.50	0.50	0.85
GC +/- (ID-1)	-1.1	-1.5	-1.2	-1.2			
GC+/- (ID-2)	-1.0	-1.4	-1.5	-1.7			
GC+/+ (ID-3)	-0.4	-0.7	-0.7	-1.3			
LS-BMD SDS $_{Ht\text{-}age}$							
GC naïve (n, 5)	-1.0 (-2.9, 0.6)	-1.5 (-1.6, 0.4)	-1.6 (-2.2, 0.7)	-0.7 (-2.3, 0.6)	0.68	0.89	**1.0**
GC +/- (ID-1)	-1.4	-1.2	-1.0	-0.9			
GC+/- (ID-2)	-1.5	-2.0	-02.0	-2.9			
GC+/+ (ID-3)	-1.1	-0.3	-0.5	-0.2			
LS-BMD SDS $_{BA}$							
GC naïve (n, 5)	-1.7 (-3.0, -0.2)	-1.5 (-2.2, -0.2)	-1.5 (-2.8, 0.2)	-1.5 (-2.2, 0.1)	0.41	0.19	0.72
GC +/- (ID-1)	-2.2	-2.2	-1.7	-2.4			
GC+/- (ID-2)	-1.6	-2.0	-1.9	-2.3			
GC+/+ (ID-3	-1.7	-0.7	-0.8	-0.7			
LS-BMAD SDS $_{BA}$							
GC naïve (n, 5)	-0.8 (-3.6, 0.8)	-0.7 (-2.1, 0.9)	-0.6 (-2.3, 1.5)	-0.7 (-1.6, 0.5)	0.89	0.23	0.14
GC +/- (ID-1)	-1.5	-1.6	-1.1	-1.5			
GC+/- (ID-2)	-0.9	-1.1	-0.8	-1.2			
GC+/+ (ID-3)	-1.5	-0.6	-0.6	0.03			

Data presented as median (range).TB, total body; BMD, areal bone mineral density; BMAD, bone mineral apparent density; SDS $_{Ht\text{-}age}$, adjusted for height age and sex; SDS $_{BA}$, adjusted for bone age and sex; LS, lumbar spine; GC, glucocorticoid; +/- ID-1, glucocorticoid at baseline and stopped before 6 months; +/- ID-2, glucocorticoid at baseline and stopped after 6 months; GC+/+, glucocorticoid throughout the study period.

Table 5-6: DXA bone outcomes at baseline, 6 months, 12 months and 24 months following rhGH. Participants divided into two groups based on pubertal status at 24 months

n, 8	Baseline	6 Months	12 Months	24 Months	p-values		
					Baseline vs. 6 months	Baseline vs. 12 months	Baseline vs. 24 months
TB-BMD SDS $_{Ht\text{-}age}$							
Late-puberty (n, 6)	0.1 (-0.9, 0.5)	-0.4 (-1.0, 0.3)	-0.7 (-1.6, 0.4)	-0.5 (-2.2, 0.1)	0.08	0.08	0.12
Early-puberty (ID-3)	0.7	-0.1	-0.2	-0.8			
Pre-pubertal (ID-7)	-2.3	-1.9	-2.2	-1.1			
TB-BMD SDS $_{BA}$							
Late-puberty (n, 6)	-1.0 (-1.7, 0.1)	-1.0 (-2.0, -0.2)	-0.9 (-2.0, 0. 0)	-1.2 (-1.7, -0.3)	0.24	0.46	0.34
Early-puberty (ID-3)	-0.4	-0.7	-0.7	-1.3			
Pre-pubertal (ID-7)	-2.9	-2.0	-2.4	-			
LS-BMD SDS $_{Ht\text{-}age}$							
Late-puberty (n, 6)	-1.1 (-1.5, 0.6)	-1.4 (-2.0, 0.4)	-1.3 (-2.0, 0.7)	-1.4 (-2.9, 0.6)	0.14	0.34	0.23
Early-puberty (ID-3)	-1.1	-0.3	-0.5	-0.2			
Pre-pubertal (ID-7)	-2.9	-1.6	-2.2	-0.7			
LS-BMD SDS $_{BA}$							
Late-puberty (n, 6)	-1.7 (-2.2, -0.2)	-1.8 (-2.2, -0.2)	-1.6 (-1.9, 0.2)	-2.1 (-2.4, 0.1)	0.41	0.14	0.53
Early-puberty (ID-3)	-1.7	-0.7	-0.8	-0.7			
Pre-pubertal (ID-7)	-3.0	-2.0	-2.8				
LS-BMAD SDS $_{BA}$							
Late-puberty (n, 6)	-0.9 (-1.5, 0.8)	-0.9 (-1.6, 0.9)	-0.7 (-1.3, 1.5)	-1.0 (-1.6, 0.5)	0.17	0.17	0.12
Early-puberty (ID-3)	-1.5	-0.6	-0.6	0.03			
Pre-pubertal (ID-7)	-3.6	-2.1	-2.3	-			

Data presented as median (range). TB, total body; BMD, areal bone mineral density; BMAD, bone mineral apparent density; SDS $_{Ht\text{-}age}$, adjusted for height age and sex; SDS $_{BA}$, adjusted for bone age and sex; LS, lumbar spine.

5.4.3.1 Correlation of DXA bone outcomes with disease and anthropometry

At baseline, 6 months, 12 months and 24 months no significant correlation was observed between BMD at different sites by using different methods of adjustment and disease biomarkers (ESR, CRP) or disease duration. At 6 months, significant negative correlation was observed between wPCDAI with LS-BMD SDS $_{\text{Ht-age}}$ (r=-0.77, p=0.044) and LS-BMD SDS $_{\text{BA}}$ (r=-0.91, p=0.006). At 24 months, significant negative correlation was observed between wPCDAI and TB-BMD SDS $_{\text{BA}}$ (r=-0.84, p=0.018).

At baseline, 6 months, 12 months and 24 months no significant correlation was observed between BMD at different sites by using different way of adjustment and Ht SDS. Similarly, no significant correlation was observed 24 months change in BMD at different site by using different way of adjustment and 24 months change in Ht SDS.

5.4.4 Whole body composition outcomes by DXA

Table 5-6 summarises the DXA body composition outcomes at baseline, 6 months, 12 months and 24 months among of eight participants with result of each visit limited to those with a 24 months visit to facilitate comparisons across the visits. Figure 5-8 shows individual data. At baseline, participants had low TB-LM SDS but were not significantly different from zero (p=0.11) and remained unchanged during the study. The median TB-FM SDS was also not significantly different from zero (p=1.0) and remained unchanged. At baseline, 6 months, 12 months and 24 months no significant correlation was observed between TB-LM SDS and disease biomarkers (wPCDAI, ESR, CRP, albumin) or duration of disease. At baseline, significant positive correlation was observed between TB-FM SDS and CRP (r=-0.87, p=0.012) but no significant correlation was observed between TB-FM SDS and other disease biomarkers or disease duration. At 6 months, 12 months and 24 months no significant correlation was observed between TB-FM SDS and disease biomarkers (wPCDAI, ESR, CRP, albumin).

At baseline and 6 months no significant correlation was observed between TB-LM SDS and bone parameters measured by DXA. At 12 months, significant positive correlation was observed between TB-LM SDS with TB-BMD SDS $_{\text{Ht-age}}$ (r=0.83, p=0.010) and LS-BMD SDS $_{\text{Ht-age}}$ (r=0.74, p=0.035). At 24 months, significant positive correlation was observed between TB-LM SDS with TB-BMD SDS $_{\text{Ht-age}}$ (r=0.76, p=0.029).

Table 5-7: Change in body composition by DXA, at baseline, 6 months, 12 months and 24 months following rhGH therapy

	Baseline	6 Months	12 Months	24 Months	p-values		
					Baseline vs. 6 months	Baseline vs. 12 months	Baseline vs. 24 months
DXA	**(n, 8)**	**(n, 8)**	**(n, 8)**	**(n, 8)**			
TB-LM SDS	-0.8 (-2.4, 3.0)	-0.4 (-1.2, 0.1)	-0.6 (-2.3, 0.5)	-1.0 (-2.0, 0.1)	0.41	0.89	0.21
TB-FM SDS	0.04 (-0.6, 1.8)	-0.5 (-0.7, 0.8)	-0.6 (-0.9, 0.3)	-0.2 (-0.8, 1.4)	0.13	0.16	0.78

Data presented as median (range). TB, total body; LM SDS, lean mass adjusted for height and sex. FM, fat mass adjusted for height and adjusted for height and sex.

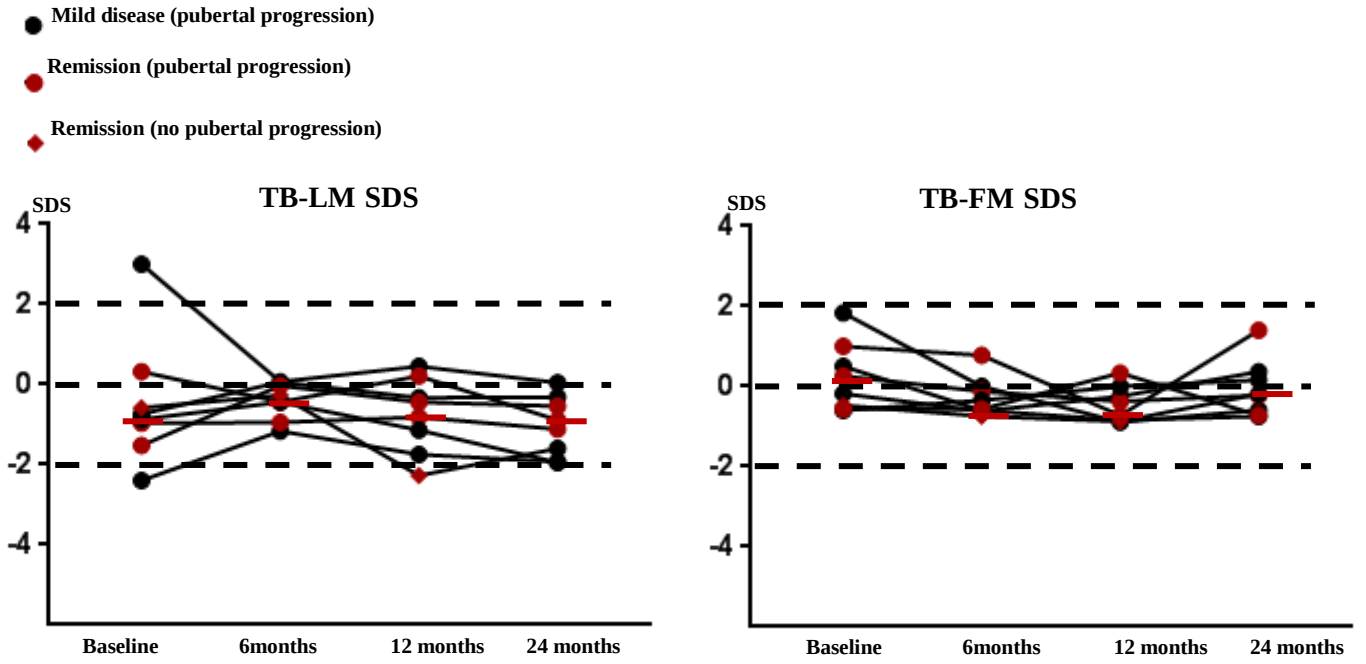

Figure 5-2: Individual changes in body composition by DXA at baseline, 6 months, 12 months and 24 months following rhGH therapy
TB, total body; LM SDS, lean mass adjusted for height and sex. FM, fat mass adjusted for height and sex. Black solid circle represents participants who had mild disease (based on wPCDAI) and progress in puberty at 24 months, red solid circle represents participants who had remission progress in puberty whereas red sold triangle had remission and stayed at tanner stage 1 at 24 months. Red solid lines represent the median values. Black dotted lines represent the 50th centile (0SDS), 3rd (-2SDS) and 97th (2SDS) centile.

5.4.5 Bone biomarkers

There was a significant median percentage change of P1NP from baseline to 6 months of 24% (0, 1048) (p=0.02), and of CTX from baseline to 6 months of 37% (9, 450) (p=0.04). At baseline, P1NP SDS was -3.6 (-7.9, -0.9) and it increased significantly to -2.4 (-3.7, 0.4) at 6 months (p=0.012). At 6 months, P1NP SDS improved in six participants, while participants 4 and 6 remained unchanged (Figure 5-1). The median (range) of urinary CTX significantly increased from 6625 μg/l (1800, 8780) at baseline to 9800 μg/l (6620, 9900) at 6 months (p=0.028). The median IGF-1 SDS was -2.7 (-3.7, 2.9) at baseline and to -2.8 (-3.7, 4.1) at 6 months (p=0.78), and to 1.5 (-2.5, 2.4) at 12 months (12 months vs. baseline, p=0.10) and -1.4 (-2.2, 3.1) at 24 months (24 months vs. baseline, p=0.40). Apart from participants 3 and 5, IGF-1 SDS remained unchnaged (Figure 5-1)

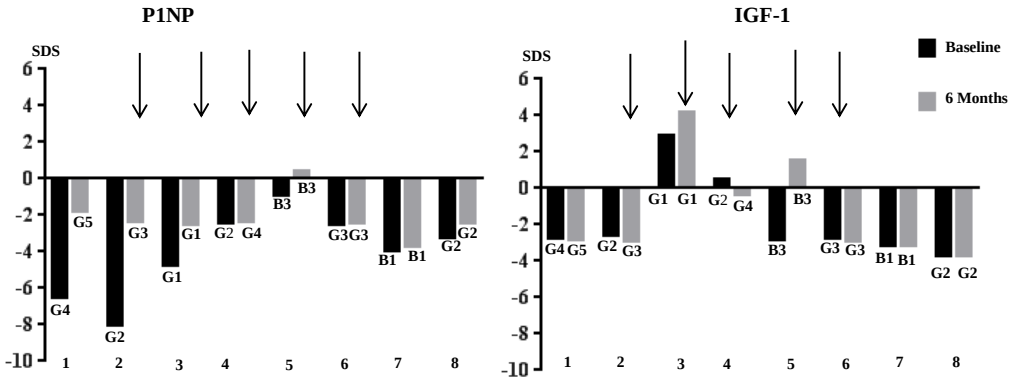

Figure 5-3: Individual changes in P1NP SDS (right) and IGF-1 SDS (left)
Baseline (black columns) and 6 months (grey columns) following therapy with rhGH therapy. P1NP, procollagen type 1 N-terminal propeptide; IGF-1 SDS, insulin like growth factor-1; SDS, SD score adjusted for sex and bone age; G1or B1, tanner stage 1; G2, tanner stage 2; G3or B3, tanner stage 3; G4, tanner stage 4; G5, tanner stage 5. Participants who had remission at 6 months (based on wPCDAI) are indicated by arrows and the rest had mild disease at 6 months.

5.4.5.1 Correlation of bone biomarkers with disease, anthropometry and DXA bone outcomes

At baseline and at 6 months, no significant correlation was observed between bone biomarkers and disease biomarkers. Similarly, no significant correlation was observed between bone biomarkers and height at baseline and at 6 months. No correaltion obseved bewtween 6 months changes in P1NP ,CTX with HV at 6 months.

5.4.5.2 Correlation of bone biomarkers with DXA bone outcomes

At baseline, significant positive correlation was observed between P1NP SDS and LS-BMD SDS $_{\text{Ht-age}}$ (r=0.81, p=0.014). At 6 months no significant association was observed between P1NP and BMD measured by DXA. The 6 month change in P1NP SDS was not correlated with BMD measured by DXA at 24 months. At 6 months, a significant positive correlation was observed between IGF-1 SDS and LS-BMD SDS $_{\text{Ht-age}}$ (r=0.85, p=0.007) and at other time points, no significant correlation was observed between IGF-1 SDS and BMD by DXA.

5.4.6 Multivariate mixed-model regression analysis for DXA bone outcomes

Table 5-8 displays the results of multivariate mixed-model regression analysis of the relationship of DXA bone outcomes with wPCDAI, GC (Yes/No), IGF-1 SDS and TB-LM SDS during the 24-months study interval. TB-BMD $SDS_{Ht\text{-}age}$ was significantly positively associated with TB-LM SDS independent of wPCDAI, GC use and IGF-1 SDS. LS BMD $SDS_{Ht\text{-}age}$ was positively and negatively associated with IGF-1 SDS and GC exposure respectively. LS BMD SDS BA was positively associated with IGF-1 SDS independent of other confounders.

Table 5-8: Mixed model assessment of the effect of inflammation, glucocorticoid use, growth biomarkers adjusted for bone age and lean mass on DXA bone outcomes at each time points during 24 months study

Independent variables	**Dependent Variables**									
	TB-BMD SDS $_{Ht-age}$		**TB-BMD SDS $_{BA}$**		**LS-BMD SDS $_{Ht-age}$**		**LS-BMD SDS $_{BA}$**		**LS-BMAD SDS $_{BA}$**	
	Estimate (SE)	**p-value (95%CI)**	**Estimate (SE)**	**p-value (95%CI)**	**Estimate (SE)**	**p-value (95%CI)**	**Estimate (SE)**	**p-value (95%CI)**	**Estimate (SE)**	**p-value (95%CI)**
wPCDAI	-0.009 (0.013)	0.456 (-0.04, 0.02)	-0.006 (0.009)	0.508 (-0.02, 0.01)	-0.010 (0.012)	0.468 (-0.03, 0.02)	-0.008 (0.010)	0.472 (-0.03, 0.01)	-0.0199 (0.0146)	0.178 (-0.05,01)
GC	-0.226 (0.316)	0.475 (-0.88, 0.42)	-0.076 (0.230)	0.328 (-0.55, 0.16)	**-0.698 (0.313)**	**0.035 (-1.3, -0.05)**	-0.289 (0.266)	0.286 (-0.83, 0.25)	-0.199 (0.366)	0.588 (-0.94,0.54)
IGF-1 SDS	0.093 (0.074)	0.220 (-0.06, 0.24)	0.053 (0.054)	0.328 (-0.06, 0.16)	**0.201 (0.072)**	**0.011 (0.05, 0.35)**	**0.180 (0.062)**	**0.006 (0.05, 0.31)**	0.113 (0.085)	0.194 (-0.61,0.29)
TB-LM SDS	**0.302 (0.134)**	**0.032 (0.03, 0.58)**	0.158 (0.098)	0.113 (-0.04, 0.35)	0.223 (0.133)	0.105 (-0.05, 0.49)	0.077 (0.113)	0.499 (-0.15, 0.30)	0.047 (0.155)	0.765 (-0.27,0.36)

TB, total body; BMD, areal bone mineral density; BMAD, bone mineral apparent density; BMAD, bone mineral apparent density;SDS $_{Ht-age,}$ adjusted for height age and sex; SDS $_{BA}$, adjusted for bone age and sex; AP, anteroposterior; LS, lumbar spine; LM, lean mass; IGF-1 SDS, serum insulin like growth factor-1SD score; GC, glucocorticoid. Values in bold indicate statistical significance ($p<0.05$).

5.5 Discussion

This chapter reports the results of a two-year longitudinal study of rhGH therapy on bone health and lean mass measured by DXA in CD children with short stature. Our data for first time showed that rhGH increased height, biomarkers of bone formation and resorption at 6 months, and persistent bone mineral density deficiency of total body and lumbar spine, adjusted for size and skeletal maturation.

It is evident that inflammation has a detrimental effect on bone health in CD, and controlling inflammation may lead to improvement in trabecular bone, cortical structure and muscle mass (156;237). The disease was clinically inactive in most of our participants at 24 months. Participants were further categorized according to their disease status to examine whether rhGH therapy has a beneficial effect on those who entered remission compared with those who had a mild case of the disease and we found that patients with inactive disease still had impaired bone health. This finding confirms our initial observation (Chapter 3) and further emphasizes that patients with inactive CD still manifest impaired bone mass and muscle. It is also crucial to highlight that the disease duration in this small cohort was long. However, there is currently no consensus on the relative importance of disease duration and bone recovery and further research is needed to shed light on this issue.

Apart from disease, other confounder that may influence the bone health in our cohort was GC exposure. The inverse association that was observed between LS BMD and GC exposure would be consistent with GC resulting in sustained reductions in bone formation due to decreased osteoblast differentiation, function, and life span and increases in bone resorption by osteoclasts (191;192). Although 5/8 of our small cohort of CD patients were not on GC during the study interval, they did not demonstrate improvement in BMD scores, contrary to previous reports on recovery of density with less GC exposure (153). However, it is challenging to identify the independent effect of GC therapy in CD. Muscle is another potential factor that influences bone health (299). We observed a significant positive association between lean muscle mass and bone in this cohort. The persistent low BMD observed in our participants may be related, in part, at least, to the fact that they have low lean mass. Thus, it seems that increased LM (for example through nutrition and physical activity) in combination with GH treatment would be worth further exploration.

Linear growth is a major driver of periosteal apposition by which bones can increase in length and width leading to greater bone strength (31). Bone mass attainment, on the other hand, is also closely related to pubertal growth (50). Evidence from JIA data speculated on

the beneficial effect of rhGH therapy during puberty (266;310). In our study, there was significant improvement in height and most of the participants (6/8) completed their pubertal growth during the 24-months study follow up; however, when we examined the sub-group who completed their puberty, no parallel improvement was observed in bone or muscle mass. Concurring with evidence of the change in BMD lagging behind the pubertal growth spurt by 0.6-0.7 years (50), we may not assume that the duration of follow-up in our study was insufficient to see favorable effects of rhGH on bone.

Some available data from GH-deficient mice demonstrated that rhGH therapy commencing during puberty rescued the quantity of trabecular bone but not the structure (313;314). In addition, data from JIA children showed that rhGH therapy is associated with improvement in bone geometry (266). Although we have addressed the confounding effect of growth and skeletal maturation on BMD, our study was limited by using DXA which does not take into account parameters of bone strength other than bone mass. Thus, our final conclusion on the benefit of GH therapy on bone health in CD children cannot be definitively drawn in the absence of detailed study of bone microarchitecture and geometry.

Serum IGF-1 levels is a biochemical response to rhGH and usually rises during treatment with rhGH (283). However, CD patients can be GH resistant (101). In fact, the observed persistent low systemic IGF-1 SDS despite supra-physiological doses of rhGH in those children suggests the possibility of partial rescue of GH resistance following rhGH therapy. Normalisation of IGF-1 through rhIGF-1 therapy may perhaps result in improvement of bone deficits and future research is needed to evaluate this.

The current study is limited by a small sample size, lack of control, and the fact that other determinants of bone strength were not investigated in our cohort. Therefore, our results may be insufficient to make a final conclusion. Nevertheless, the current pilot study is important as first the study that extensively explored the long term effect of rhGH on biomarkers of bone turnover and BMD and body composition in this particular population. Thus we can only postulate that future larger studies are needed using high resolution bone imaging to evaluate the effect of rhGH alone and in combination with rhIGF-1 or in combination with exercise on bone and body composition in CD.

5.6 Conclusion

This long term study depicts that bone and muscle deficiencies persist in chronic CD children over 24 months of rhGH therapy. Although the biomarkers of bone turnover increased and most children completed pubertal growth, bone mineral density did not increase and this may be due to partial recovery of the GH-IGF-1 axis and/or decreased

muscle mass. Further studies are needed to explore the effect of rhGH, targeting those with short disease duration and exploring the benefit of contemporaneous rhIGF-1 or exercise.

Chapter Six

Assessing the Feasibility of Injectable Growth-Promoting Therapy in Crohn's Disease

6.1 Abstract

Background: Despite optimal therapy, many children with CD experience growth retardation.

Objectives: To assess the feasibility of performing RCT of injectable forms of growth promoting therapy; and to survey the attitudes of children with CD and their parents to it.

Methods: A face to face questionnaire surveyed willingness to consent to future participation in the RCT. Eligibility to the survey was any young person (with their parent/guardian) with CD whose Ht SDS was ≤1.0. Of 118 questionnaires, 94 (80%) were returned (48 by young people and 46 by parents).

Results: The median age of the patients in the survey was 14.3 years (range 7.0 to 17.7) and 35 (73%) were male. Median Ht SDS was -1.2 (-3.0, 0.2) and significantly lower than the median mid-parental Ht SDS was -0.6 (-3.1, 1.4) (p=0.003). Overall, 21 (44%) young people and 22 (48%) parents were willing to take part in the proposed RCT. Common reasons for not taking part in the RCT were either fear of injections (44%) or not concerned about their height (44%).

Conclusion: Around 40% of young people and parents surveyed would take part in an RCT of growth promoting therapy. Allaying fears about injections may result in higher recruitment rates.

6.2 Introduction

Around one quarter of cases of CD are diagnosed in children under 18, with the incidence in childhood increasing (315). Growth failure is a common manifestation and may be the first presentation of disease (20). Despite advances in CD treatments, around 20% of affected children may continue to grow slowly (84) and remain short on reaching their final adult height (90;91).

The cause of growth failure is multi-factorial, and includes poor nutrition, inflammation and corticosteroid treatment. The mechanisms involve a disturbance of GH-IGF-1 at peripheral and central levels (99). Although the exact mechanisms of disturbance of the GH-IGF-1 axis in CD are still not fully elucidated, the abnormalities may range from functional GH deficiency to GH resistance with low circulating IGF-1 (81;99-101). Current treatment is to improve growth using steroid-sparing anti-inflammatory medication (212).

Preliminary evidence from the use of rhGH therapy in CD children indicates potential efficacy despite the expected insensitivity to GH (259;260;262). However, there are no RCT that investigate rhIGF-1 alone or in combination with rhGH on growth of children with CD.

There are concerns with regards to the acceptability of conducting such a trial to both patients with CD and their families. It is important to establish whether it is feasible to conduct a trial of this nature. We therefore undertook a feasibility study to survey attitudes of patients and parents towards the proposed RCT.

6.3 Methods

A British study group collaborating with the Medicines for Children Clinical Trials Unit (MC- CTU) examined the feasibility of a trial of injectable growth promoting drugs with a view to establish an RCT.

Two questionnaire surveys were developed for two groups; potential participants for the trial and their parents. These questionnaires were developed by the trial management group and their format was based on a successful earlier questionnaire survey by the MC-CTU (316). As the questionnaire was a survey on patients' willingness to consent to a future trial, it did not require research ethics committee approval, which was confirmed by the Barts Health NHS Trust R&D office. Information sheets were written for the clinical teams, which included a description of the four proposed arms of the RCT: (1) treatment to combat inflammation; (2) added injections of rhGH; (3) added injections of rhIGF; and (4) added rhGH and rhIGF-1.

Content validity was assessed through questionnaires piloted on five patients and their parents. They were amended based on feedback and then sent to a wider group. The questions (Appendix-1 and Appendix-2) included demographic data: age, height on the day of attending clinic and parental height. Specific questions included their degree of concern regarding their height, attitudes to injections to improve growth, willingness to join an RCT, and any previous growth promoting drugs. All questionnaires were anonymous and no patient identifiable data were collected. Parents and patients in the same family were given a single study number to enable comparison of responses.

6.3.1 Subjects

Questionnaires were distributed in two paediatric IBD clinics (Barts Health (London) and Royal Hospital for Sick Children (Glasgow)) to consecutive participants between 1st March and 31st July 2014. The target recruitment at each of the centres was at least 30 patient questionnaires and 30 parent questionnaires. The child questionnaire was completed by a patient with CD who fulfilled the eligibility criteria (Ht SDS was ≤ 1.0). One of the child's parents completed the parental questionnaire. A healthcare worker was available to explain the meaning of any questions that a child did not understand, but was not involved in recording the answers.

Completed questionnaires were sent back to the MC CTU for initial result collation. For the study participants, Ht was measured with a Harpenden stadiometer and converted into

standard deviation scores (SDS) for chronological age using 1990 UK standards (274). MPH and MPH SDS were calculated from reported parent heights.

6.3.2 Statistics

Data were analysed using Minitab software version 17. Descriptive analyses were used for all variables. Comparison between the patients and their parents and between subgroups was analysed using the Mann–Whitney test. Non-parametric data are presented with medians and ranges. For categorical variables percentages were calculated. The degree of agreement between responses of children and their parents was represented using the kappa statistic with 95% confidence interval and I would like to thank Professor Thomas Jaki for performing this analysis. Missing responses were not included in the descriptive analyses. $p<0.05$ was considered as statistically significant. All graphs were prepared by GraphPad Prism software version 7 (San Diego California, USA).

6.4 Results

6.4.1 Demographic characteristics

The overall response rate was 48 (80%) out of 60 questionnaires were completed by patients and 46 (77%) out of 60 were completed by parents. Patient's demographics, anthropometric and clinical information are presented in Table 6-1. Median Ht SDS at time of approach was -1.2 (-3.0, 0.9) and median MPH SDS was -0.6 (-3.2, 2.4) [Ht SDS vs. MPH SDS, p=0.004). 4 (9 %) of young people had been specifically treated with a growth promoting therapy before (n =2 received rhGH and n=2 received testosterone).

Table 6-1: Demographic, anthropometry and clinical characteristics

	Total 48 (80%)
Age/year (range)	14.3 (7.0, 17.7)
Sex (M) n (%)	35 (73)
Ht SDS (range)	-1.2 (-3.0, 0.9)*
MPH SDS (range)	-0.6 (-3.1, 1.4)
Treated for Growth Problem, n (%)	4 (8)
Family history of CD, n (%)	8 (17)

MPHSDS, mid-parental height SDS; HtSDS, height SDS score**;** CD, Crohn's Disease. * $p<0.05$ (Ht sds vs MPH SDS).

6.4.2 Participants response

The responses from young people and parents to survey's questions are summarised in Table 6-2. In total 3 (6%) young people and 5 (11%) parents were very concerned about height, 19 (40%) young people and 11 (24%) parents were slightly concerned about height and 26 (54%) young people and 29 (63%) parents were not concerned about height(Figure 6-1). In 31 cases there was agreement with regards to how concerned a child and their parent were with the child's height (κ=0.42 95% CI (0.23 to 0.71)), representing moderate agreement.

The majority of respondents, 42 (88%) young people and 40 (87%) parent, agreed that doctors should try to find a better treatment for growth in CD; 20 (42%) young people and 25 (54) parents believed that opportunity of gaining extra height was worth a year of daily injection. 36 (75%) young children and 34 (74%) parents agreed to attend an extra visit to have child's growth and other things checked (e.g. quality of life) if it sometimes means an extra visit (1 or 2 extra in a year). With respect of being comfortable of entering the study without being able to choose the treatment; 20 (44%) of parents said yes and 24 young people (50%) answered yes. Regardless of concern about height, 21 (44%) young people and 22 (48%) parents were willing for RCT participation.

Table 6-2: The responses from young people and parents to survey's questions

			Total	
	Question	**Response**	**Parents (n=46)**	**Child(n=48)**
1	**How concerned are you about your child's height?**	Not concerned Slightly concerned Very concerned Missing	29 (63%) 11 (24%) 5 (11%) 1 (2%)	26 (54%) 19 (40) 3 (6%)
2	**Do you think it is worth doctors trying to find a better treatment for growth in crohn's disease?**	Yes No Missing	40 (87%) 4 (9%) 2 (4%)	42 (88%) 5 (10%) 1 (2%)
3	**Do you think that the opportunity of gaining extra height is worth a year of daily injections?**	Yes No Missing	25 (54%) 19 (41%) 2 (4%)	20 (41%) 28 (58%) 0
4	**We have explained that in an RCT you are not able to choose which treatment your child would receive. Would you be comfortable with this?**	Yes No Missing	20 (44%) 25 (54%) 1 (2%)	24 (50%) 23 (47%) 1 (2%)
5	**Would you and your child be willing to attend to have your child's growth and other things checked (e.g. quality of life) if it sometimes means an extra visit (1 or 2 extra in a year)?**	Yes No Missing	34 (74%) 10 (22%) 2 (4%)	36 (75%) 12 (25%) 0
6	**If the RCT we had in mind was happening now, would you be willing for your child to join?**	Yes No Missing	22 (48%) 23 (50%) 1 (2%)	21 (44%) 27 (56%) 0

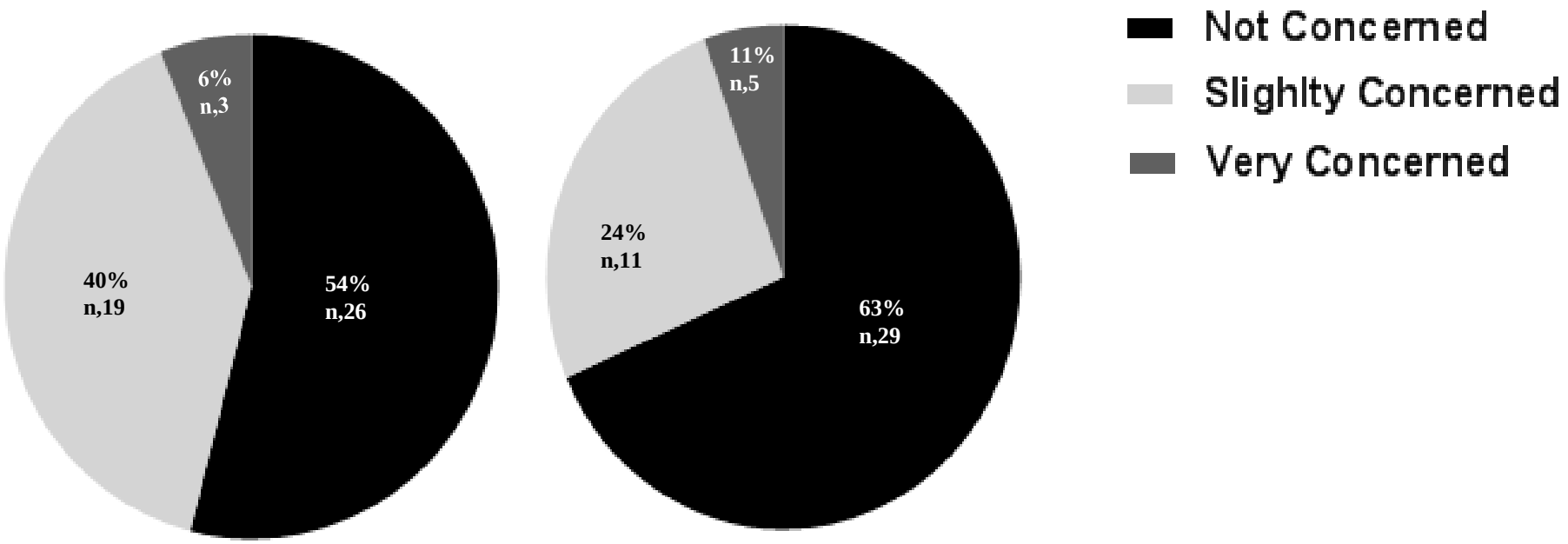

Figure 6-1: Young people's (on the left) and parents' (on the right) attitude about height

6.4.3 Children and parents attitude about height and participation in RCT

Cross-tabulation of questions was examined to understand if a child's attitude to their height influenced their willingness to have injectable treatment. The results of cross-tabulation of Question 1 (How concerned are you about your/your child height?) and Question 6 (If the RCT we had in mind was happening now, would you be willing for your child to join?) are shown in Tables 6-3& 6-4.

Although they were not concerned about their height, 6/26 (23%) patients were willing to participate in an RCT [median Ht SDS (range) for children who were willing, -0.6 (-2.2, 0.2), and not willing, -0.7 (-1.5, 0.8), to participate in the RCT, p=0.9]. Moreover, 12/19 (63%) young people who were slightly concerned about their height responded that they would be happy to participate in the RCT [median (range) Ht SDS was -1.6 (-3.0, -0.07) compared to 6/19 (31%) who were slightly concerned and not willing to participate, -1.3 (-2.0, -0.6), p=0.5]. All very concerned young people were willing to take part if the RCT went forward, and they all had heights more than 1 SD below the mean [median (range) Ht SDS -2.1 (-1.2, -2.5)] (Figure 6-2).

Although they were not concerned about their children's growth, 11/29 (38%) parents were willing to have their children participate in the RCT. The median (range) Ht SDS of these children was -1.2 (-3.0, 0.2) compared to -0.7 (-2.2, 0.9) for children of the 18/29 parents who were not concerned and not willing to participate in the RCT, p=0.61]. In contrast, 7/11(64%) of parents who were slightly concerned were willing for their children to join the RCT. There was no difference in median (range) Ht SDS [-1.4 (-2.3, -0.6) vs -1.1 (-1.6, -0.6) respectively, p=0.63] between these two groups. In addition, 4/5 (80%) of very concerned parents were willing for their children to participate in the RCT [median (range) Ht SDS -1.8 (-2.5, -1.1)] (Figure 6-2).

Table 6-3: **Cross-tabulation of questions was examined to understand if a child's attitude to their height influenced their willingness to have injectable treatment**

		Question 6 If the RCT we had in mind was happening now, would you be willing to join?	
		Total	
Question 1 **How concerned are you about your height?**		No	Yes
	Not concerned n (%)	20 (77%)	6 (23%)
	Slightly Concerned n (%)	7 (37%)	12 (63)
	Very concerned n (%)	0 (0%)	3 (100%)

Table 6-4: **Cross-tabulation of questions was examined to understand if parent's attitude to their children height influenced their willingness to have injectable treatment**

		Question 6 **If the RCT we had in mind was happening now, would you be willing for your child to join?**	
		Total	
Question 1		No	Yes
How concerned are you about your child's height?	Not concerned n (%)	18 (62%)	11 (38%)
	Slightly Concerned n (%)	4 (36%)	7 (64%)
	Very concerned n (%)	1 (20%)	4 (80%)

Missing data for question 1 =1, Missing data for question 6 =1

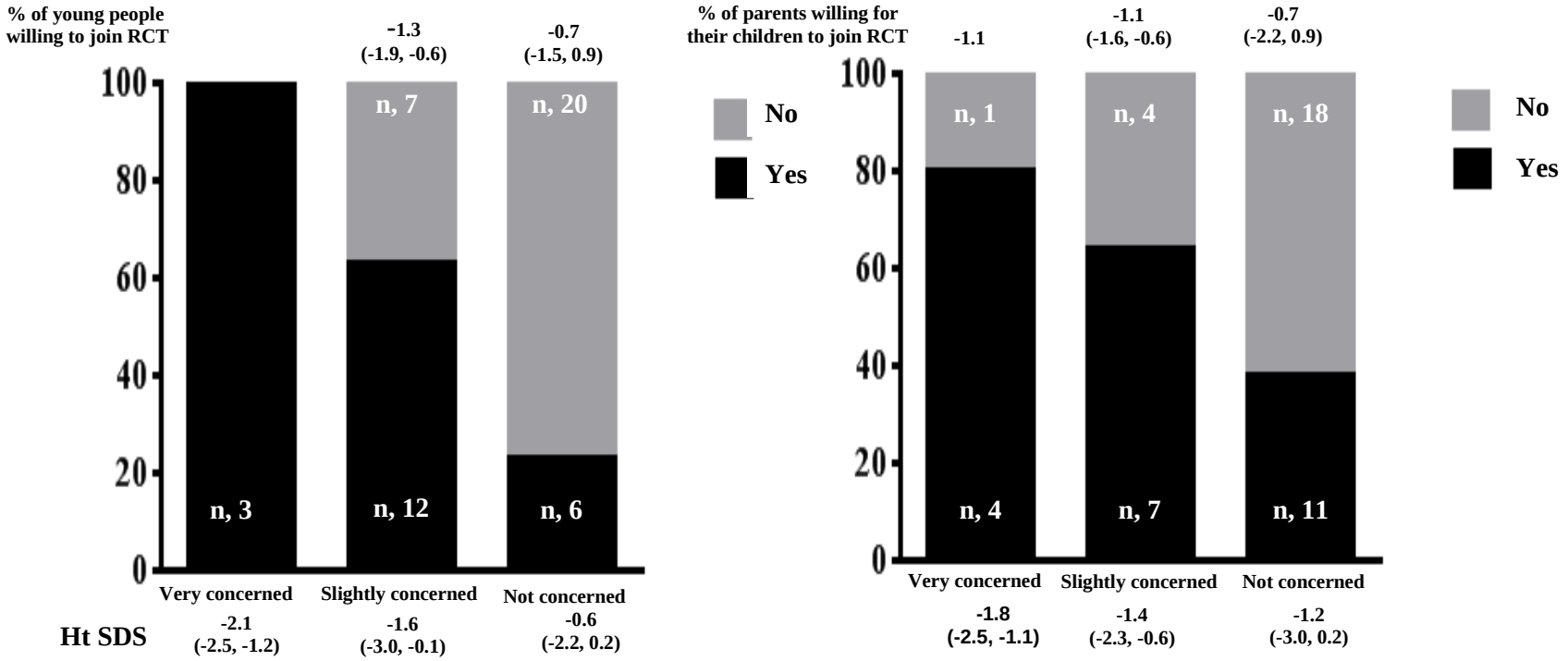

Figure 6-2: Yong people (left) and parents (right) attitude on height and willingness to participate in a randomised controlled trial
RCT, randomised controlled trial; Ht SDS, height SD score.

6.4.4 Anthropometric characteristic of children based on their attitude and willingness to participate in RCT

The median Ht SDS [-1.5 (range, -3.0, -0.7)] in concerned young people was lower than in the non-concerned group [median (range) Ht SDS -0.7 (-2.2, 0.9); p=0.0009] (Table 6-5; Figure. 6-3). However, no differences were found in gender distribution (16M/6F vs. 9M/7F, p=0.98) or MPH SDS [-0.6 (-3.1, 0.5) vs. -0.6 (-2.3, 1.4), p=0.82] among concerned and non-concerned children. The 21 (44%) children who were willing to participate in the RCT were shorter [median (range) with a Ht SDS -1.5 (-3.0 to 0.2] than the 27 (56%) who were not willing to participate in RCT [median (range) Ht SDS -0.9 (-1.9 to 0.9), p=0.01] (Table 6-5; Figure. 6-3). However, there were no differences among the two groups with respect to their age and gender, as the median age (range) was 14.7 years (7.0 to 17.3; 14M/7F) in the former group and the median age (range) was 14.1 years (10.0 to 17.7, p=0.5; 21M/6F, p=0.39) in the latter.

Table 6-5: Different in age, gender and anthropometry between young people according to concern about height and willing to participate in RCT.

	Concerned about height(n, 22) Slightly concerned (n, 19)+ Very concerned(n, 3)	**Not concerned about height (n, 26)**
Age(year)	15.1 (10.0, 17.7)*	13.9 (7.0, 17.2)
Sex(M/F) (n)	16/6	19/7
HtSDS	-1.5 (-3.0, -0.7)*	-0.7 (-2.2, 0.9)
MPHSDS	-0.6 (-3.1, 0.5)	-0.6 (-2.23, 1.4)
	Willing to participate in RCT (n, 21)	**Not willing to participate in RCT (n, 27)**
Age(year)	14.7 (7.0, 17.3)	14.1 (10.0, 17.7)
Sex(M/F)(n)	14/7	21/6
Ht SDS	-1.5 (-3.0, 0.2)*	-0.9 (-1.9, 0.9)

Median(range). M, male; F, female; Ht SDS, height SD score. * $p<0.05$.

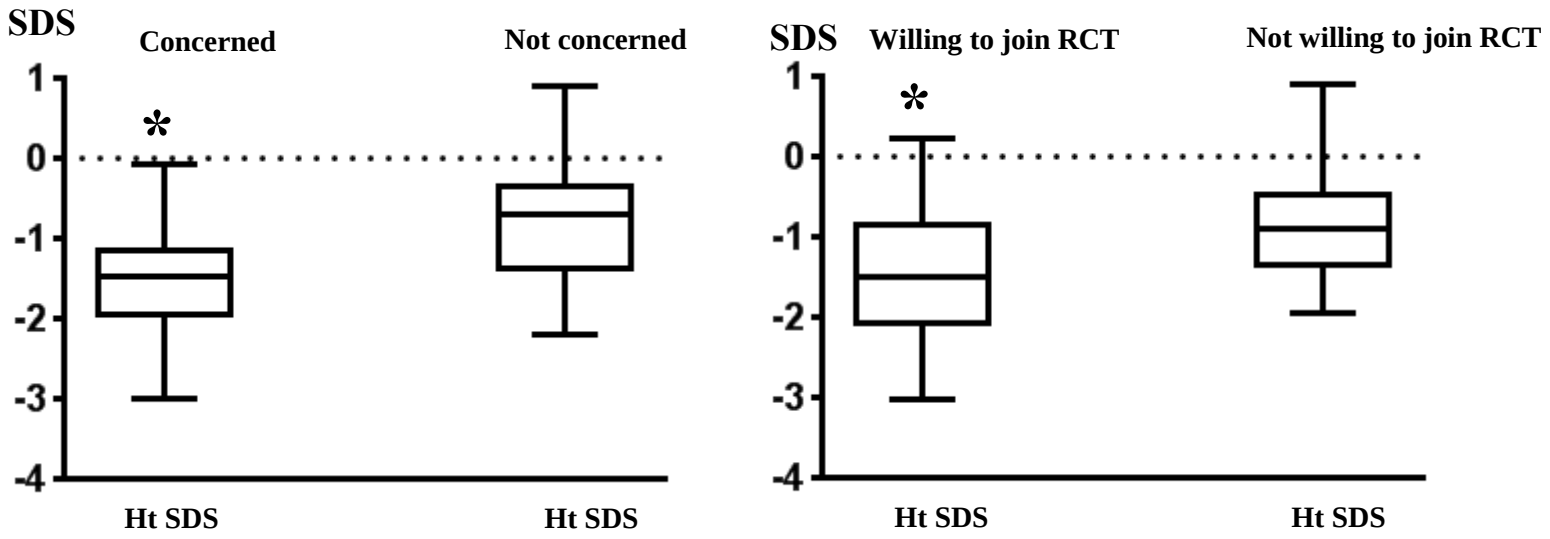

Figure 6-3: Differences in height (SDS) between young people who were concerned and those who not concerned about their height(Left)and different heights (SDS) between young people who willing to participate in randomised controlled trial and those who not willing(Right).
Ht SDS, height SD score. *p<0.05.

6.4.5 Major factors influencing decision making in willing to join RCT

The major reasons for not wishing to participate in the RCT were identified by 18 out of 27 of young people (Figure 6-4): Eight (44%) of them stated the fear of injections; 8 (44%) stated that they were not concerned about their height; 1 (6%) participant was already on many drugs; and 1 (6%) difficulty in taking time off from college.

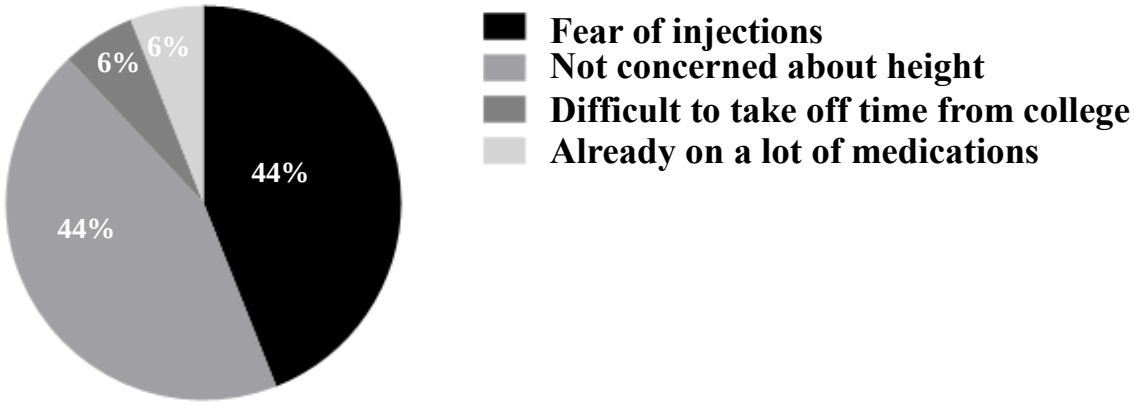

Figure 6-4: Major reason for why young people not willing to participate in randomised controlled trial

6.5 Discussion

This is the first feasibility study of an RCT in growth promoting therapy in children with CD. It is also the first quantitative study to survey the attitude of young people with CD and their parents towards endocrine therapy for growth promotion in an RCT. The possible treatments, in addition to optimal anti-inflammatory therapy (standard treatment), includes daily injections of rhGH; rhIGF-1; or rhGH and rhIGF-1. Many young people with CD and their parents would take part in an RCT of growth promoting therapy despite only a minority being very concerned about their height. However, answers may differ when confronted with consent to an ongoing trial, rather than a hypothetical one. Concerns about height were more likely in those who were shorter; and shorter children were more likely to consider this additional therapy to promote their growth. The results of our survey are in agreement with a preliminary RCT on rhGH in children with IBD (262).

Reports show that boys are more vulnerable to the psychological burden of being short than are girls (317), however, we did not find any gender difference in their concerns over height. The majority of our participants were boys (73%), and this may have influenced our results. Major reasons for not taking part in the proposed RCT reported by young people in the survey were fear of injections and not being concerned about their height. Alleviating such concerns in eligible participants may result in higher recruitment rates. Regardless of concern about height, majority of young people and parents were willing for RCT participation. However, the degree of concern about height correlated with an interest in taking part in the proposed RCT, with 100% of very concerned patients willing to participate. Similarly, in a survey examining patients' perceptions of faecal microbiota transplantation for UC, all patients with severe UC were willing to take part (318); thus the patients most affected by the condition it seems are the most likely to agree to any proposed study.

Although currently there is a lack of conclusive data on rhGH in children with IBD, the initial results from the published studies as well as the study described in this thesis suggest that rhGH may have a positive effect on growth in the short term (258-262). The available evidence has shown the growth-promoting effect of rhGH on children with mild disease activity and growth retardation. Thus, the effect of rhGH on CD children with intractable inflammation and growth retardation remains unanswered. There is a need to perform larger, more conclusive studies of rhGH therapy which explore this issue.

Given that a substantial proportion of children may remain short despite use of optimal therapy (84) and considering that the abnormality may occur at multiple levels of the GH-IGF-1 axis, the possible use of other forms of growth-promoting agents such as rhIGF-1, either alone or in combination with rhGH for promoting growth, also warrants further investigation. The studies of effects of IGF-1 on growth on CD have not been described yet, partly because of the theoretical risk of colon cancer in patients with high levels of circulating IGF-1. However, by using mathematical modelling to determine the dose of rhIGF-1 that could be prescribed to maintain serum IGF-1 level within the physiological range study may inform the design of future clinical trials (319).

One of the limitations of the survey was that exploring the reasons for patients' concern at being short was beyond the scope of a questionnaire on willingness to consent. Mason et al (98) published the first study which showed that short stature is associated with adverse quality of life measured by IMPACT-III in the subdomain of body image. It would, therefore, be beneficial to assess the impact on quality of life in any future trial involving the use of growth promoting therapies.

6.6 Conclusions

In summary, this study indicated that it was feasible to consider the initiation of a randomised controlled trial of growth therapy in children with CD with the majority of those surveyed were interested despite a minority being very concerned about their height. By targeting the shorter young people as well as alleviating fears about injections to them, a future trial would be likely to achieve higher recruitment rates.

Chapter-7

General Discussion and Future Direction

7.1 General discussion

The studies presented in this thesis investigated the effects of anti-TNF-α and rhGH therapy on linear growth and bone health in CD children.

The impact of anti-TNF-α on linear growth, IGF-1 axis and biomarkers of bone turnover were investigated in Chapter-2. My data showed that receiving anti-TNF-α maintenance therapy for 12 months was associated with a significant improvement in growth velocity and ΔHt SDS, in those who had potential to grow at time of anti-TNF-α induction, although no benefit was seen in more mature patients. These observations are consistent with previous data showing that anti-TNF-α therapy is associated with linear growth improvement in children with chronically active CD in early puberty (88;240). In accordance with these previous reports (88;240), anti-TNF-α therapy was not associated with a significant improvement in Ht SDS in my study, may suggest that control of inflammation improves growth velocity sufficiently to prevent further deterioration in height but is unable to improve overall height. This finding suggests that if anti-TNF-α therapy is given to CD patients with growth potential, attained height may still be sub-optimal of what would be expected in a healthy population. Thus, if growth is of concern, the role of adjuvant therapy to boost height in these children is worth further exploration.

The GH-IGF-1 axis is a crucial signalling pathway that impacts on linear growth, bone, and muscle development (73;74). It is assumed that disturbances in the GH–IGF-1 axis are one of many factors contributing to growth retardation in CD children (112;114;115). However, in the current study no obvious disturbances in this axis were noted.

Consistent with previous studies (149;150;178), I found a state of low bone turnover at the time of starting anti-TNF-α, as indicated by markers of bone formation (BSAP) and bone resorption (β-CTX). Marked increases in bone formation biomarkers and moderate increases in bone resorption markers were also observed following anti-TNF-α therapy, in line with previous evidence (237;243). Whether this improvement in disease status and biomarkers of bone metabolism translated to improvement in bone mass was explored further in Chapter-3.

The Chapter-3 prospective study explored the impact of anti-TNF-α on bone mineral density and muscle mass and function. BMD was assessed at different body sites including TB, LS by DXA, and weight and non-weight bearing limbs by pQCT. Muscle mass was assessed at TB by DXA and muscle cross-sectional area was assessed at radius by pQCT. Muscle function in the non-dominant hand was assessed by MIGF. My results revealed improvement in growth and bone formation following anti-TNF-α and there was

insufficient evidence to show a change in bone health or muscle mass and function as assessed by imaging.

A recent study of anti-TNF-α therapy in children and adolescents with CD did find an improvement, but not normalization, of trabecular bone density and cortical structure using pQCT (237). It is of note however, that my study cohort consisted of a group of children with a longer duration of disease (3.1 vs. 2.1 years), and a greater degree of lean mass deficits. The degree of recovery from cytokine-induced bone growth retardation has been demonstrated to be dependent on the duration of exposure (120;248). However, two additional studies found no improvement on bone health after anti-TNF-α with disease duration similar/longer than our cohort (241;254). Using exclusive enteral nutrition in ten newly diagnosed children with CD normalized trabecular density and muscle area using pQCT at 12 weeks (223). Pooling my findings with the available evidence raises a question about the proper time for intervention to see recovery of bone health in CD children. Further studies are needed to determine if there is window for intervention and recovery of bone health in CD children.

Catch-up in bone mass is greater in younger children with CD following controlling the inflammation (153;237), and my study consisted of a large proportion of adolescents in later stages of puberty, with a limited window for improvement of skeletal growth. Additionally, the possibility of a lag between catch-up linear growth and bone growth may also explain our results (302). Despite significant improvement in disease activity index in our cohort, there is the possibility of persistent mild, chronic inflammation (149). Unfortunately, we did not have information of endoscopic re-evaluation or faecal calprotectin to inform on this. The findings of low muscle mass and strength in my cohort is consistent with previous studies of adolescents with CD (115;153-155;199;200). This study also revealed a strong independent positive association between muscle and bone density and geometry following anti-TNF-α therapy in paediatric CD. In accordance with the mechanostat theory (299), it is possible that sustained muscle deficit may hinder skeletal acquisition in paediatric CD following anti-TNF-α therapy. Thus, the role of adjuvant interventions to improve muscle strength such as exercise/nutrition may be beneficial in CD after controlling inflammation, and warrants further studies.

Another possible explanation to be considered from our analysis is the positive association between ALS SDS and trabecular BMD SDS, and negative association between IGFBP-3 and tibia cortical thickness SDS. This raises the possibility that the IGF axis may contribute to trabecular and cortical geometry impairment seen in CD children. A reduction in trabecular BMD and cortical bone volume is present in ALS-Knockout mice,

which is explained by the inability to form ternary complex, resulting in the marked reduction in circulating IGF-1 (77;300). Recently, data from our group, investigating women with childhood onset type 1 diabetes mellitus showed a significant inverse correlation between ALS and trabecular bone separation (301). This raises the possibility that ALS could play a role in the regulation of trabecular bone, and this might be exerted via IGF-1 independent mechanisms. However, preclinical studies are needed to verify this assumption. Regardless of proposed possible mechanisms behind the lack of improvement in bone in this cohort, it seems that inflammation insult to the skeleton is not completely removed even with anti-TNF-α therapy. The role of adjuvant therapy to improve musculoskeletal development in children with CD such as nutritional, exercise or manipulation of the GH/IGF axis requires further exploration.

In children with CD, systemic abnormalities of the GH-IGF-1 axis are reported, suggesting that many may exhibit a range of abnormalities in secretion and sensitivity of the GH-IGF axis (101). From the results presented in Chapter-2, and consistent with the existing evidence (203;235;239;240;245;249), it seems that targeting cytokines using anti-TNF-α therapy leads to statistically significant, but clinically modest, height gain. Therefore, if maximising growth is the concern, adjuvant therapy combined with other forms of growth-promoting therapy may also need to be considered. Given gaps in knowledge on the effect of rhGH, especially at higher dose and for long duration, on linear growth and glucose homeostasis in children with CD, the third study (Chapter-4) was conducted to investigate this question in the cohort of CD children. In the first long term case-controlled study of rhGH at 0.067 mg/kg/day, which is almost double the replacement dose, we demonstrated a sustained effect on linear growth over a 24-month period, in short pubertal CD children compared with untreated control patients. However, findings from this study should be interpreted with caution as the sample size was small and puberty is a potential confounder. Data may therefore not be applicable to prepubertal CD children. Furthermore, it is noteworthy to highlight that the participants in our cohort had mild or inactive disease so our finding cannot be applied to those with growth failure and intractable disease. A further large RCT will be required to determine whether rhGH therapy can potentially improve growth in this population regardless of pubertal progress and disease status.

It is known that rhGH treatment is associated with a decrease of insulin sensitivity in some studies (320). However, my analysis in this study revealed that rhGH therapy for 24 months was not associated with deleterious effect on glucose homeostasis in this cohort. Given that children with inflammatory conditions may also be at risk of developing insulin resistance as a result of the inflammatory process (305) as well as the use of concurrent GC

therapy (259;264), I believe close monitoring of glucose homeostasis is required when rhGH therapy is used in this cohort in both a clinical setting and for research purpose.
Following the findings in Chapter-3, work performed in Chapter-5 aimed to examine the role of adjuvant therapy with rhGH on bone health in children with inactive/mild disease status. This study is the first report of the results of a 24-months prospective study of rhGH therapy on bone health and lean mass, measured by DXA, in a small cohort of children with mild/inactive CD.
Though the sample size was small, my data indicated 24 months of rhGH in paediatric CD was not associated with improvement in bone density and body composition, despite an increase in biomarkers of bone formation at six months, after adjusted for skeletal maturation and improvement linear growth, and the consideration that most of children (6/8) had completed their pubertal growth. This may be due to partial recovery of the GH-IGF-1 axis and/or persistent decreased muscle mass. A state of functional GH insensitivity can exist in children with chronic disease (101), and whilst early evidence show that use of relatively high dose rhGH may overcome this insensitivity and lead to improvement in linear growth (259;260;262), the possibility of differential end organ insensitivity should be considered. Whilst serum IGF-1 levels have been shown to increase with rhGH therapy in children with CD (283), the observed persistent low systemic IGF-1 SDS, despite supra-physiological doses of rhGH in this small cohort of children, suggests the possibility of partial rescue of GH resistance following rhGH therapy. A study in female rats showed that combined rhGH and rhIGF-1 therapy leads to significantly higher systemic IGF-1 paralleled with improvement in cortical bone mass (321). Thus, normalisation of IGF-1 with rhIGF-1 therapy (on its own or in combination with rhGH) may result in greater improvement of bone deficits, and future research is needed to evaluate this.
My analysis also confirms potential imperative influence of muscle on bone health (299). Thus, the challenge remains for further research to identify management strategies for low muscle mass and function in CD and to examine whether addressing lean mass deficit can improve bone health. Therefore, future clinical studies assessing mechanical-loading interventions are required. It is also possible that DXA bone assessment may not be sufficiently detailed to give information on changes of bone structure following rhGH therapy in these children. Newer methods of assessment using high-resolution pQCT or microMRI may allow non-invasive evaluation of bone microenvironment and should be considered in future studies in CD. Finally, as participants in this study had disease of long duration, the question of a possible optimal window of recovery is also raised. However, so

far, there is no general agreement on the relative importance of disease duration on bone health recovery.

Currently there is a lack of conclusive data on rhGH in children with CD, the initial results suggest that rhGH may have a positive effect on growth in the short term (258-262). The available data are limited by small sample size and are restricted to children with CD who only have mild disease activity and growth retardation. Thus, to date, the effect of rhGH on CD children with intractable inflammation and growth retardation has not been examined and larger, more conclusive studies of rhGH therapy that explore this issue are still needed. Work presented in Chapter-6 was the first piece of research that surveyed the attitude of young people with CD and their parents towards endocrine therapy for growth promotion in an RCT. The participants were questioned about their attitude to take part in one of the four proposed arms of the RCT including (1) standard treatment to combat inflammation; (2) additional injections of rhGH; (3) additional injections of rhIGF; and (4) a combination of tow injections of rhGH and rhIGF-1. Many CD children and their parents would participate in an RCT despite only a minority being very concerned about their height. Concerns about height were more likely in those who were shorter; and shorter children were more likely to consider this additional therapy to promote their growth. Not being concerned about their height and fear of injections were foremost explanations stated by CD children in the survey for not participating in the proposed RCT. Thus, higher recruitment rates may be achieved in eligible participants by alleviating such concerns. However, responses could vary when challenged with consent to an on-going trial, rather than a hypothetical one. Exploring the explanations for patients' concern at being short was beyond the scope of a survey on willingness to consent. Recently our group (98) published the first study which showed that short stature is associated with adverse quality of life measured by IMPACT-III in the subdomain of body image. Thus, it would certainly be of interest for future research to consider the impact of poor growth, short stature and delayed puberty on the quality of life of these children.

7.2 Conclusion

The data reported in this thesis found that combatting inflammation with anti-TNF-α was associated with modest clinical improvement in height, but with no observed beneficial effect on musculoskeletal health. Overall, no definitive conclusions can be made regarding the effect of anti-TNF-α therapy on bone health, as conclusive data are still lacking. If growth is of concern, the data suggest that targeted therapy with rhGH, or in combination with rhIGF-1, during critical periods of growth is worthy of further exploration. The use of

high dose rhGH for 24 months was associated with a growth-promoting effect but no influence was observed on bone and body composition. Further larger randomised studies are needed to definitively evaluate the effects of rhGH on growth and musculoskeletal development in paediatric CD. The careful monitoring of glucose homeostasis while using rhGH in children with CD and growth retardation is also recommended. The sustained muscle deficit in children with CD hinders skeletal acquisition following anti-TNF and rhGH therapy, thus muscle mass and strength are imperative contributors for bone health in CD. Interventions to preserve or increase muscle mass can be one of the important strategies that may improve bone health. From surveying CD children it seems to be feasible to consider the initiation of a randomised controlled trial of growth therapy in those children for whom growth is a valid concern.

7.3 Strength and limitations

Specific strengths and limitations of the thesis results have been comprehensively presented in each respective chapter. In summary, the major strengths are the evaluation of two treatment strategies in two models of CD, looking at both growth and bone health in children with CD in the context of active inflammation by using anti-TNF-α, and the role of adjuvant therapy alongside rhGH therapy in children with mild/inactive CD. Chapter-3, although preliminary due to sample size constraints, is the first study to have examined the effect of anti-TNF-α on IGF-1 and binding protein and related them to bone health in the context of paediatric chronic inflammation. Secondly, this study provides a broad view of skeletal health at different body sites through the use of DXA and pQCT. Thirdly, this is the first study that charts the effect of anti-TNF-α therapy on changes in upper limb muscle function in paediatric patients with CD. Chapter-4 and Chapter-5 are important as the first study that extensively explored the long-term effect of rhGH on growth, IGF-1, glucose homeostasis, biomarkers of bone turnover, bone density and body composition in this particular population.

The main limitation of the studies presented in this thesis related to sample size constraints, which restricted the analyses of potential confounding factors on growth and bone health. However, we tried to overcome this issue through the use of mixed model regression analysis to obtain association between variables. In the anti-TNF-α studies, lack of knowledge of drug trough levels may have impacted on our ability to ensure anti-TNF-α optimisation, and without a comparable control group, we were unable to examine the protective role of anti-TNF-α. In relation to evaluation of bone parameters, although the DXA and pQCT methods provided information about BMD and bone geometry at different

body sites, both methods were insufficient to characterise the microarchitecture of trabecular and cortical bone, which are crucial components of bone strength. Furthermore, in the absence of data on sex steroid levels, their relationship with changes in bone density could not be taken into account. Although, it was more feasible to use validated survey of child self-assessment of puberty (Chapter-2 & Chapter-3) instead of investigator examination, it potentially leads to misclassification of pubertal stage. However, we overcome this limitation by measuring BA and use it for further analysis.
Measurements of pQCT geometry were adjusted only for height, without taking age into consideration, so may yield error when comparing individuals of the same height with different age and pubertal maturation, ignoring the influence of sexual hormones on bone and muscle development. Moreover, the studies are limited by a reliance on validated disease activity index to assess disease status, which is not a valid assessment of mucosal healing in comparison with endoscopic re-evaluation and assessment with faecal calprotectin.

7.4 Implication for clinical practice and further research

Given that the majority of CD children present in early or mid-adolescence, there is a limited window of opportunity for improvement in growth, and a combined approach may be needed that targets both disease and growth. However, in the absence of conclusive data, rhGH therapy should be considered only in those with suboptimal growth despite controlling inflammation. Because the results of this thesis suggest that inflammation-the insult to the skeleton is not completely removed, even with anti-TNF-α therapy, the role of adjuvant therapy to improve musculoskeletal development in children with CD such as nutrition and exercise may be useful.
Based on work relating to growth and bone promoting therapy in children with CD, the following future research should be considered:

1. Firstly, an in-depth understanding of the mechanism behind the insult to bone cells and the potential for spontaneous recovery following proinflammatory cytokines exposure should be addressed through preclinical study. Such research may aid clinicians in a more focused approach to delivery of management for bone health.

2. Large prospective studies are still needed to verify the effect of anti-TNF-α on bone health.

3. RCT in children with active CD and growth failure to compare the four treatment strategies (combat inflammation, combat inflammation and rhGH therapy, combat inflammation and rhIGF-1 or combat inflammation plus rhGH and rhIGF-1) on growth and bone health.

Given the abnormality may occur at multiple levels of the GH-IGF-1 axis (101), the possible use of other forms of growth-promoting agents such as rhIGF-1, either alone or in combination with rhGH for promoting growth should be considered. There are a number of reasons why combination treatment of rhGH with rhIGF-1 may be more beneficial for growth and metabolism. Human data has shown that combined therapy resulted in a higher serum concentration of IGF-1 compared with IGF-1 alone, probably linked to the negative feedback effect of IGF-1 on GH secreted by the pituitary (322). The addition of rhGH to rhIGF-1 may reverse the insulin suppressive effects of the latter and may have anti-catabolic effects on protein synthesis and muscle mass (322-324). Furthermore, administration of rhIGF-1 alone results in hypoglycaemia, thus concomitant treatment with rhGH may attenuate the glucose-lowering effect of rhIGF-1. An experimental rat model of uraemia showed that concomitant treatment with rhIGF-1 and rhGH was more effective in improving growth than rhIGF-1 or rhGH therapy alone, and inhibited the hypoglycaemia that may occur with use of rhIGF-1 alone (325).
Finally, the use of IGF-1 may itself counter the insulin-resistant state that can be induced by the use of high-dose rhGH therapy in a group of children who may already have a degree of insulin resistance, due to their state of chronic inflammation and glucocorticoid excess (259). A double-blind placebo-controlled crossover trial of seven pre-pubertal cystic fibrosis children showed that rhIGF-1 therapy for 6 months was associated with an improvement in glucose homeostasis (326). More recently, Rao et al. (319) reported the use of mathematical modelling to determine the dose of rhIGF-1 necessary to maintain circulating IGF-1 in the physiological range in children with IBD and growth failure, which may aid in selecting therapy dosage for children with CD to maintain IGF-1 level within an acceptable range.

4. RCT to assess the effect of exercise alone or in combination with rhGH-IGF-1 therapy on the bone health of children and adolescents with inactive CD.

In keeping with muscle-bone unit theory (299), the results of studies in this thesis consistently showed that muscle mass and strength are important contributors to bone

health of subjects with CD. Thus interventions to encourage physical activity may be beneficial for bone health.

Appendices

Appendix-1: Young People Survey

1. About you:

a. How old are you? _____years, _____months *(example: 12 years, 8 months)*

b. Are you: Male ☐ Female ☐

c. Please tell us your height as measured at today's clinic *(the clinic staff will be happy to tell you this if they haven't already done so)*
Height _____centimetres Date measured: dd / mm / yyyyy

d. Please can you tell us your parents heights (if they haven't completed their own form):
Mother:_____feet _____inches (_____centimetres)
Father: _____feet _____inches (_____centimetres)

e. Have you ever been treated for a growth problem? Yes ☐ No ☐ Don't know ☐

If yes, what treatment did you have:
_____
_____
_____

2. About your views:

a. How concerned are you about your height? Not concerned ☐ Slightly concerned ☐ Very Concerned ☐

b. Do you think it is worth doctors trying to find a better treatment for growth in Crohn's disease? Yes ☐ No ☐

c. Do you think that the opportunity of gaining extra height is worth a year of daily injections? Yes ☐ No ☐

d. We have explained that in an RCT you are not able to choose which treatment you would receive. Would you be comfortable with this? Yes ☐ No ☐

e. Would you be willing to attend to have your growth and other things checked (e.g. quality of life) if it sometimes means an extra clinic visit (1 or 2 extra in a year)? Yes ☐ No ☐

f. If the RCT we had in mind was happening now, would you be willing to join? Yes ☐ No ☐

You don't have to give us a reason, but it would help us if you could provide us with more information about your response to (f) in the space below:

_____

_____

g. Has your mum or dad also completed a survey questionnaire? Yes ☐ No ☐

Please add here any information that you think would be helpful to the doctors thinking about developing this study:

_____

_____

_____

Thank you for taking the time to complete this survey. Please place the questionnaire in the envelope provided, seal the envelope and hand it in to clinic staff.

Appendix-2: Parent Survey

3. About you:

a. Are you the parent of a child with Crohn's disease? Yes ☐ No ☐

(If no, please do not complete the rest of the survey)

b. Do you also have inflammatory bowel disease? Yes ☐ No ☐

c. Please can you tell us your height and that of the other parent:
Mother: _____feet _____inches (_____centimetres)
Father: _____feet _____inches (_____centimetres)

4. About your child:

a. Age: _____years, _____months *(example: 12 years, 8 months)*

b. Is your child: Male ☐ Female ☐

c. Please tell us your child's height as measured at today's clinic *(the clinic staff will be happy to tell you this if they haven't already done so)*
Height _____centimetres Date measured: dd / mm /yyyy

d. Has your child ever been treated for a growth problem? Yes ☐ No ☐ Don't know ☐

If yes, what treatment was given:

_____

_____

_____

5. About your views:

a. How concerned are you about your child's height? Not concerned ☐ Slightly concerned ☐ Very Concerned ☐

b. Do you think it is worth doctors trying to find a better treatment for growth in Crohn's disease? Yes ☐ No ☐

c. Do you think that the opportunity of gaining extra height is worth a year of daily injections? Yes ☐ No ☐

d. We have explained that in an RCT you are not able to choose which treatment your child would receive. Would you be comfortable with this? Yes ☐ No ☐

e. Would you and your child be willing to attend to have your childs growth and other things checked (e.g. quality of life) if it sometimes means an extra clinic visit (1 or 2 extra in a year)? Yes ☐ No ☐

f. If the RCT we had in mind was happening now, would you be willing for your child to join? Yes ☐ No ☐

You don't have to give us a reason, but it would help us if you could provide us with more information about your response to (f) in the space below:

_____

_____

g. Has your child also completed a survey questionnaire? Yes ☐ No ☐

Please add here any information that you think would be helpful to the doctors thinking about developing this study:

_____

_____

_____

Thank you for taking the time to complete this survey. Please place the questionnaire in the envelope provided, seal the envelope and hand it in to clinic staff.

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