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**THE ASSESSMENT OF SHORT-TERM GROWTH IN
CHILDREN ON CYTOTOXIC CHEMOTHERAPY**

A thesis submitted for the degree of
Doctor of Medicine
in the University of Edinburgh
by

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DECLARATION

No part of this thesis has been submitted in support of an application for another degree or qualification of this or any other university or other institute of learning.

All the studies performed as part of this thesis were approved by the local ethical committee and informed consent was given by all subjects and/or their parents.

DEDICATION

This thesis is dedicated to my wife, Shazia, and my parents whose support and encouragement provided the incentive to complete this work.

ABSTRACT

This thesis consists of two groups of prospective short-term studies; the first group evaluates the technique of knemometry and lower leg length (LLL) changes in a group of healthy children and adults; the second group of studies is based on short-term anthropometric changes in children on treatment for acute lymphoblastic leukaemia (ALL).

Studies in the healthy group were performed on 26 prepubertal children and 9 young adults. The first study refined the technique of knemometry by minimizing intra-observer bias; despite reducing bias knemometry remained a highly precise form of measuring changes in LLL. The next study in this group of subjects showed the presence of significant intradaily variation in the LLL of some fully grown adults and the influence of change in body weight and oedema on LLL. There was no distinct pattern of lower leg changes in these children when followed up for a period of 12 weeks by weekly measurements or when followed up daily for a period of 4 weeks. The growth pattern was non-linear in nature and the daily changes were not statistically related to nocturnal urinary growth hormone (uGH) excretion, a non-invasive marker of growth hormone secretion.

Studies of short-term growth in ALL were performed on 25 children 14 of whom had knemometry during the two year protocol for a median duration of 56 weeks. Conventional anthropometric studies as well as knemometry showed an adverse effect of intensive chemotherapy(CT) on growth. The effects on growth seemed to be transient with evidence of catch-up growth during less intensive periods of CT. Lower leg growth during continuation CT was comparable to that seen in healthy children and fluctuations in growth were associated with changes in neutrophil counts. The sitting height:height ratio reduced temporarily over the period of remission induction and although the initial deceleration in LLL improved immediately after induction, total axial growth took longer to improve suggesting a transient but disproportionately greater adverse effect of CT on the vertebral spine.

A study of biochemical markers of bone and collagen turnover in 16 subjects over the first six months of CT showed changes consistent with catch-up growth and similar to that found by knemometry. An uncoupling of bone turnover favouring bone degradation during the intensive phases of CT and favouring bone formation during the phase of catch-up growth was also evident.

Studies of uGH, insulin-like growth factor-I(IGF-I) and IGF Binding Protein-3(IGFBP-3) in the 8 subjects who had knemometry showed a possibility of a state of partial growth hormone resistance at presentation and during the first few weeks of CT where IGF-I and IGFBP-3 levels were not raised despite high uGH excretion. Catch-up growth, observed as a supraphysiologically high LLL velocity, occurred in the presence of high uGH levels and normal IGF-I and IGFBP-3 levels suggesting that catch-up growth might be partly independent of GH/IGF-I action.

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ABBREVIATIONS

ALL:	Acute Lymphoblastic Leukaemia
ALP:	Alkaline Phosphatase
Ara-C:	Cytosine Arabinoside
Asp:	Asparaginase
bALP:	Bone isoform of ALP
BBB:	Blood Brain Barrier
BMI:	Body Mass Index
cGY:	CentiGray
CNS:	Central Nervous System
CSF:	Cerebrospinal Fluid
CT:	Chemotherapy
CV:	Coefficient of Variation
Cyclo:	Cyclophosphamide
DNA:	Deoxyribonucleic Acid
Dnr:	Daunorubicin
DPD:	Deoxypyridinoline
Dxm;	Dexamethasone
Etop:	Etoposide
GH:	Growth Hormone
hd:	High Dose
Ht:	Height
ICTP:	Cross linked Telopeptide of Type I Collagen
IGF-I:	Insulin Like Growth Factor-I
IGFBP3;	IGF Binding Protein 3
it:	Intrathecal
iv:	Intravenous

LLL: Lower Leg Length
 LLLf: Lower Leg Length Fluctuation
 LLLV: Lower Leg Length Velocity
 LLLVd: Daily Lower Leg Length Velocity
 LLLVw: Weekly Lower Leg Length Velocity
 Mtx: Methotrexate
 OM: Original Method
 P95: 95th Percentiles
 PICP: Carboxyterminal Propeptide of Type I Procollagen
 PIIINP: Aminoterminal Propeptide of Type III Procollagen
 Pred: Prednisolone
 Pyd: Pyridinoline
 RM: Random Zero Method
 RT: Radiotherapy
 SDS: Standard Deviation Score
 SH: Sitting Height
 tALP: Total Alkaline Phosphatase
 TE: Technical Error
 uGH: Urinary Growth Hormone
 UKALL: United Kingdom Acute Lymphoblastic Leukaemia
 Vcr: Vincristine
 WSR: Wilcoxon Signed Rank Test
 Wt: Weight
 ΔSDS-BMI: Change in BMI-SDS from Baseline
 ΔSDS-Ht: Change in Ht-SDS from Baseline
 ΔSDS-Wt: Change in Wt-SDS from Baseline
 95%CI: 95% Confidence Interval
 6mp: 6-mercaptopurine

CHAPTER ONE

AIMS OF THE THESIS

The present studies were designed to investigate the short-term effects of chemotherapy on growth and bone metabolism in order to provide insights into the mechanism of any long-term changes. By employing recently developed indicators of growth hormone secretion and bone & collagen turnover, the study also evaluated the use of these markers in assessing the effects of chemotherapy on growth.

The aims of these studies were to:-

1. Evaluate the significance of short-term lower leg length fluctuations in healthy individuals.
2. Document the pattern of growth using knemometry and anthropometry in a group of children on treatment for ALL.
3. Evaluate the effects of chemotherapy on markers of GH secretion and bone & collagen turnover.
4. Investigate any relationships between the short-term anthropometric changes and those in the biochemical markers.

CHAPTER TWO

INTRODUCTION

Section 1. The assessment of short-term growth

Longitudinal growth studies allow assessment of individual growth patterns and differences in tempo of growth between individuals. The commonest method of accurately assessing growth in children has been by measuring total body stature with the stadiometer (Cameron, 1986). Because of the technical variability these measurements cannot reliably detect changes in growth velocities from less than six-month periods (Marshall, 1971); this is specially important when growth is assessed in populations where the growth rate might be particularly low such as prepubertal children as well as those suffering from growth-suppressing illness.

A. The Knemometer

Over the last two decades the knemometer (*knemo*: Greek for *lower leg*) has been developed to study the process of human growth in much finer detail (Valk, 1983). This instrument measures from the bottom of the heel to the top of the knee when the subject is sitting in a carefully controlled position with the knee flexed to an angle of just less than 90° (Fig.2.1). The instrument occupies an area of about 1.5m^2 and is 1.7m high, consists of a seat (A) that can be moved in horizontal and vertical planes so that the exact position of

the subject may be recorded and reproduced on subsequent occasions. In addition to the seating position the placement of the foot on the base is also standardised with a template(B) produced at the first measurement. The latter is made by lowering a plate(C) attached to a digital ruler(D) onto the knee. The vertical length is displayed on a digital meter(E) and the maximum length is obtained by moving the LL forward and backward and then medially and laterally until the digital meter displays the maximum value. Other controls present on the digiruler include a "reset" button which is used for zeroing the device and a "preset" button which is for calibrating the digiruler. Each measurement is derived from a set of 4 "estimations"; the most deviant of the 4 estimations is eliminated and the mean of the rest is calculated to provide a "measurement" (Wales & Milner, 1987). In between each estimation the subject walks a standard distance, such as around the knemometer, so that the conditions for each estimation are standardised.

Recently, portable hand-held knemometers have been devised and these are easier to use in younger children and infants who are too young to sit still for long enough (Bisgaard, 1993, Gibson *et al*, 1993). A portable knemometer for older subjects has also been recently described (Davies *et al*, 1996).

The knemometer can measure the LL non-invasively with an accuracy of 0.1mm and a reported technical error (TE, one standard deviation from the mean of repeated estimates) of 0.09mm-0.16mm (Hermanussen, 1988). It should be noted, however, that the operator is aware of the preceding estimates in the series and the measurement process is therefore subject to intra-observer bias as the operator inadvertently tries to equal the previous estimates and as a consequence reduces the technical error resulting in a measurement which might be artificially precise.

Besides studying the pattern of normal growth, the knemometer has also been used to predict the effect of growth promoting therapies such as Growth Hormone (GH) treatment (Wales & Milner, 1989), documenting the effect of corticosteroids in disease states such as asthma (Wolthers & Pedersen, 1992) and inflammatory bowel disease (Wales & Milner, 1989) and studying the effect of dialysis in children with chronic renal failure (Seidel *et al*, 1991).

B. Intradaily Variation of Lower Leg Length

Postural changes in height through the day have been quantified since the 18th century; Wasse demonstrated that height loss through the day in adults could be as much as '6/10s of an inch', occurred more in young adults and was due to changes in the back and not the legs (Wasse, 1724). The

knemometer has shown similar reductions in LL length (LLL) of children but no such studies have been performed in adults (Valk, 1983). The reduction is thought to be due to gravitational effects on the compressible components of the LL such as the soft tissue and the epiphyses. Physical activity prior to the knemometric measurement leads to a significant decrease in LLL in children and an increase in adults (Hermanussen et al, 1988(a)); this temporary effect tends to disappear 2 hours following activity.

C. Saltatory Growth

Variation in height velocity in relation to season (Butler et al, 1990) as well as so-called mid-childhood growth spurts (Berkey et al, 1983) have been well documented. Recent studies of infants looking at short-term changes in total recumbent length and head circumference have shown a saltatory process of 'incremental bursts' of growth on background stasis (Lampl et al, 1992). Moreover different parts of the body grow at different rates as suggested by differential longitudinal growth rate of the spine and subischial leg (Ashizawa & Kawabata, 1990).

Knemometry has shown that LL growth in healthy children is also seasonally variable (Gelander, 1994). LL growth can occur at a steady rate but also shows non-linear changes with intermittent leaps in growth as well as clear slowings or

shrinkage which might be associated with catabolic stress (Wales & Milner, 1987). It is not clear whether there is a definite periodicity as analytical techniques, such as moving averages, used in studies looking at periodicity are prone to aliasing when they incorporate possible outliers into the data and produce regular periodicity where none exists (Hermanussen, 1988(b)). More recently, highly accurate and precise radiogrammetric studies looking at growth in rabbits with metal pins inserted into the bone have documented a Gaussian distribution of velocities around an intermediate value, more indicative of continuous growth (Klein *et al*, 1994).

The episodic increase documented in previous studies could be partly due to intra-observer bias leading to an inaccurately low TE, as referred to earlier. However, the studies performed in healthy neonates which have been done by a process independent of observer bias have also revealed saltatory growth (Gibson *et al*, 1993a, Wales & Gibson, 1994).

D. Prediction of Long-term Growth

Early studies concentrated on studying the value of short-term knemometric studies in predicting the long-term efficacy of growth promoting therapy such as GH. The variability of LLL increments and the fact that these increments cannot reflect simultaneous growth in other parts of the skeleton,

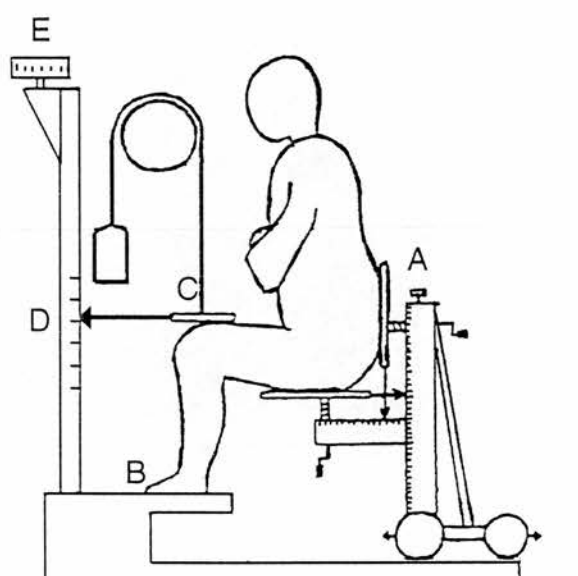
limit the role of knemometry in the prediction of long-term growth. For prediction of annual height velocity, the correlation coefficient using a 1-month increment is 0.069, for 3 months, 0.437 and for 6 months, 0.732 (Dean et al, 1990). To assess the effect of growth-promoting drugs an estimated difference of 0.37mm/wk over two 6-week periods would be necessary at a 5% level; this is equivalent to a statural growth response of at least 6.3cm/yr (Wit et al, 1987). A study of 12 girls with Turner Syndrome treated with GH revealed that a short-term growth response in LLL at one-month had only a 50% positive predictive value for a six month response in improved statural height, but had a 100% negative predictive value (Wales & Milner, 1989).

E. Therapeutic Intervention & Skeletal Growth

The study of short-term growth in relation to interventional strategies which might alter normal growth could provide an insight into the biology of the growth process as well as documenting any adverse effects which could be alleviated by modification of the treatment strategies. The effect of corticosteroids on growth and bone metabolism are well known although the pathophysiological basis is still not clearly understood (Tonshoff et al, 1996). Suppression of LL growth has also been documented in studies of short duration in children treated with steroids for Crohn's Disease, asthma and chronic neonatal lung disease (Wales & Milner, 1988,

Wolthers & Pedersen, 1992, Bisgaard, 1993, and Gibson et al, 1993b). A pilot study looking at LLL growth of 7 children receiving high-dose i.v. MTX and oral 6-MP following remission induction of ALL showed some evidence of growth retardation which was not statistically significant (Tammings et al, 1992). Assessment of growth factors and markers of bone turnover might provide a better understanding of the mechanism of the effect on growth (Wolthers et al, 1994).

Fig.2.1 The Knemometer



Section 2. The treatment of acute lymphoblastic leukaemia

Before cure could be achieved in children with acute lymphoblastic leukaemia (ALL), improvement of systemic treatment led to longer periods of remission which were accompanied by an increase in the rate of first relapse in the central nervous system (CNS) reported at around 50-80% (Evans *et al*, 1970, Price & Johnson, 1973) the ultimate outcome of which was death. Thus treatment protocols with the aim of preventing the development of overt CNS leukaemia were initiated and nowadays the combination of multiagent chemotherapy, better supportive care and, crucially, the addition of CNS prophylaxis, provide a child with ALL and no adverse risk factors, a greater than 70% chance of cure (5-yr survival) (Boring *et al*, 1994); this ever-increasing population is, however, at risk of long-term effects of therapy. Although specific approaches to therapy may differ from centre to centre, all modern treatment regimens comprise four major elements: remission induction, intensification/consolidation, CNS-directed therapy, and continuation of treatment.

A. Remission Induction

Complete remission is defined as the absence of clinical signs and symptoms of disease, the recovery of normal blood cell counts, and the recovery of normocellular bone marrow with fewer than 5% blast cells. Induction of remission typically involves administration of a glucocorticoid (dexamethasone or prednisolone), vincristine and L-asparaginase. The need for early aggressive therapy is supported by studies which have shown that more complete reduction of the leukaemic clone after the first 2 weeks of induction treatment predicts a favourable outcome (Miller *et al*, 1989, Gaynon *et al*, 1990). With present regimens, up to 98% of children achieve a complete remission.

B. Intensification Chemotherapy

"Intensification" refers to strategies of systemic chemotherapy that are more aggressive than those employed during remission or continuation treatment and their main aim is to retard drug resistance and control residual leukaemia (Rivera *et al*, 1991). Most protocols specify a period soon after remission induction when patients receive either increased doses of agents used previously or entirely new

agents with minimal cross-resistance. Although with this approach improved outcome has been reported (Clavell *et al*, 1986 and Gaynon *et al*, 1988), there is some doubt as to the benefit of this therapeutic strategy in low-risk groups (Camitta *et al*, 1980). The drugs used in this phase include daunorubicin, cytarabine, etoposide as well as the drugs used in "induction". A relatively new intensification regimen involves the use of parenteral methotrexate (Mtx) and 6-mercaptopurine (6mp) with the assumption that neither drug produces severe acute or long-term side effects (Camitta *et al*, 1989). There are often more than one period of intensification and in the current Medical Research Council trial in the United Kingdom (UKALLXI) there is an option for a prolonged third period of intensification which includes treatment with cyclophosphamide as well as the drugs listed above.

C. Prevention of CNS Relapse

Use of cranial irradiation (XRT) and intrathecal Mtx (itMtx), given after complete remission induction is essential as leukaemia in the CNS not only reduces chances of cure but also increases morbidity related to the need for intensive remission-retrieval therapy (Mulhern *et al*, 1989). Concern about the adverse

effects of XRT (Wallace & Shalet, 1992) have led to the replacement of XRT with age or size-adjusted doses of either high-dose iv or itMtx or triple agent i.t. chemotherapy consisting of hydrocortisone, cytarabine and Mtx (Tubergen et al, 1993 and Pullen et al, 1993). There is no long-term evidence that high-dose ivMtx is as effective as XRT in terms of CNS recurrence or chronic survival (Jenney et al, 1994). It was recently shown that amongst "intermediate" risk children survival, as well as event-free survival, was better in those treated with 1800cGy instead of itMtx alone (Tubergen et al, 1993).

D. Continuation Chemotherapy

As studies have shown that therapy lasting only for 15 months following remission was associated with a high rate of relapse (Chessels, 1982) and that therapy lasting for longer than 3 years did not provide any additional benefit (Eden et al, 1991), most treatment protocols discontinue all treatment for those in whom continuous complete remission has been achieved for two to three years. ALL is unique amongst cancers in that it requires such prolonged continuation treatment. This kind of treatment might be necessary to kill the more slowly dividing

leukaemic cells which might have been less susceptible to earlier chemotherapy (Pinkel, 1987) or it could simply suppress leukaemic cell growth to the extent that naturally programmed cell death, or apoptosis, can occur (Gale & Butturini, 1991). The mainstay of "continuation" treatment is a combination of oral 6-Mp and Mtx together with intermittent pulses of Vcr and Pred. Response to treatment with 6-Mp and Mtx appears more favourable when the combination is administered to the limits of tolerance, as determined by how low the neutrophil counts fall during treatment (Hale & Lilleyman, 1991 & Pearson *et al*, 1991). With no evidence of any long-term sequelae this mode of therapy is used for all children, including the low-risk individuals.

E. UKALL XI (92)

The children who were studied and received treatment for ALL followed the protocol designed for the UKALLXI(92) trial co-ordinated by the Medical Research Council of the United Kingdom which started in March 1992. The aim of this trial was to compare intensification of chemotherapy administered at different times and to test if CNS disease eradication can be successfully achieved without cranial radiotherapy. The treatment period ran for a

total duration of 2 years and included all the phases mentioned above (Fig.2.2). Details of the treatment regimen are presented on the schema on the following pages. Briefly, patients were stratified by white blood cell count on presentation for randomisation to CNS treatment which was given immediately after induction therapy(Weeks 1 to 4) and early intensification (Week 5) (Fig.2.3). Another block of (late) intensification therapy was administered at Week 20 and patients were randomised to receive or to not receive a further (Third) block of intensification therapy from Week 35 to Week42 (Fig.2.4). In between these blocks of intensive chemotherapy, and up to Week 100 following start of therapy, patients received continuation chemotherapy in 12 weekly cycles (Fig.2.5). XRT at 2400cGy was reserved for patients who had identifiable leukaemic blasts in the CSF; hyperleukocytosis, indicating a high risk of CNS relapse led to randomisation into either high dose ivMtx or XRT.

Fig.2.2 UKALLXI(92)-Summary of Treatment Regimen and Options

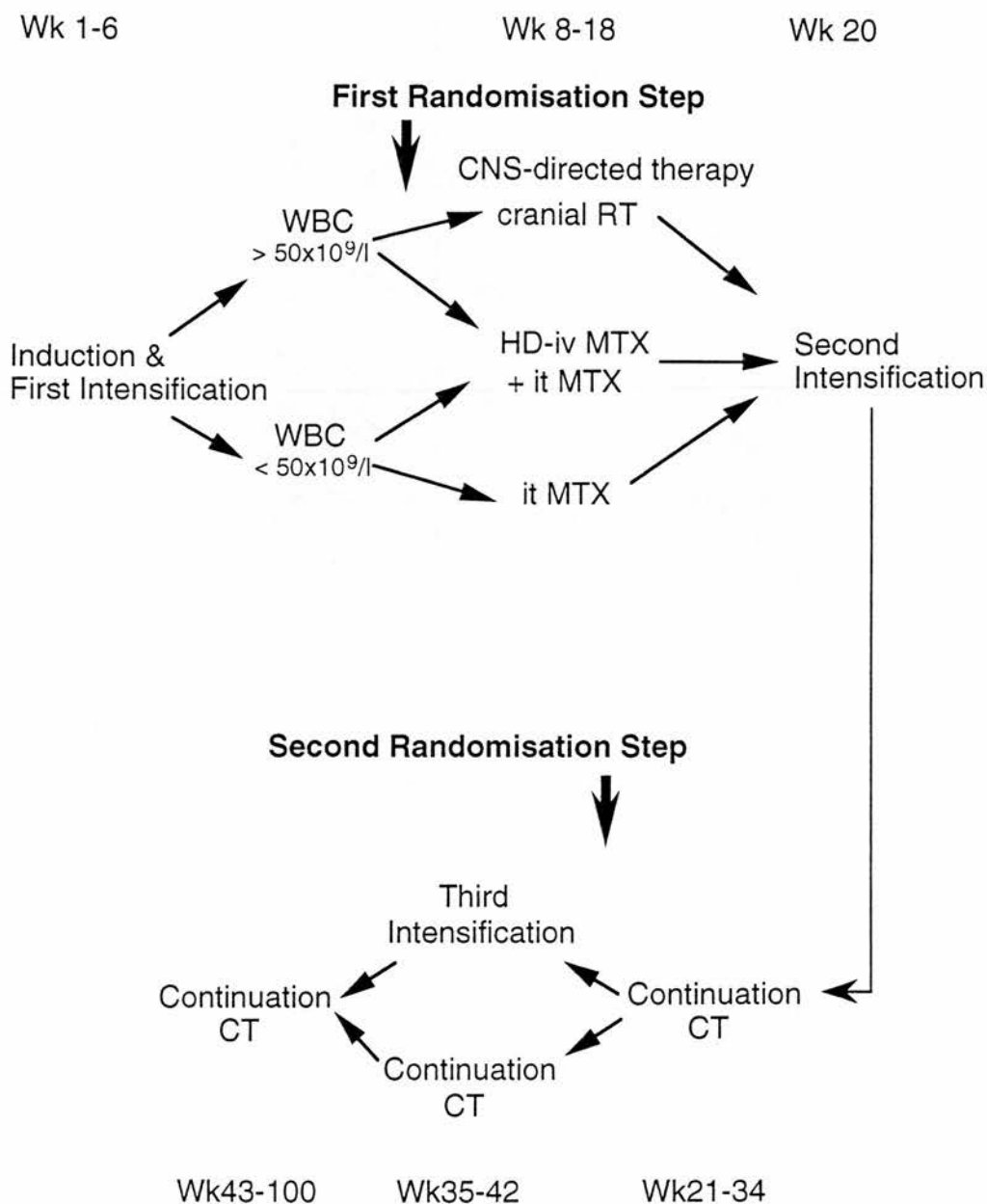
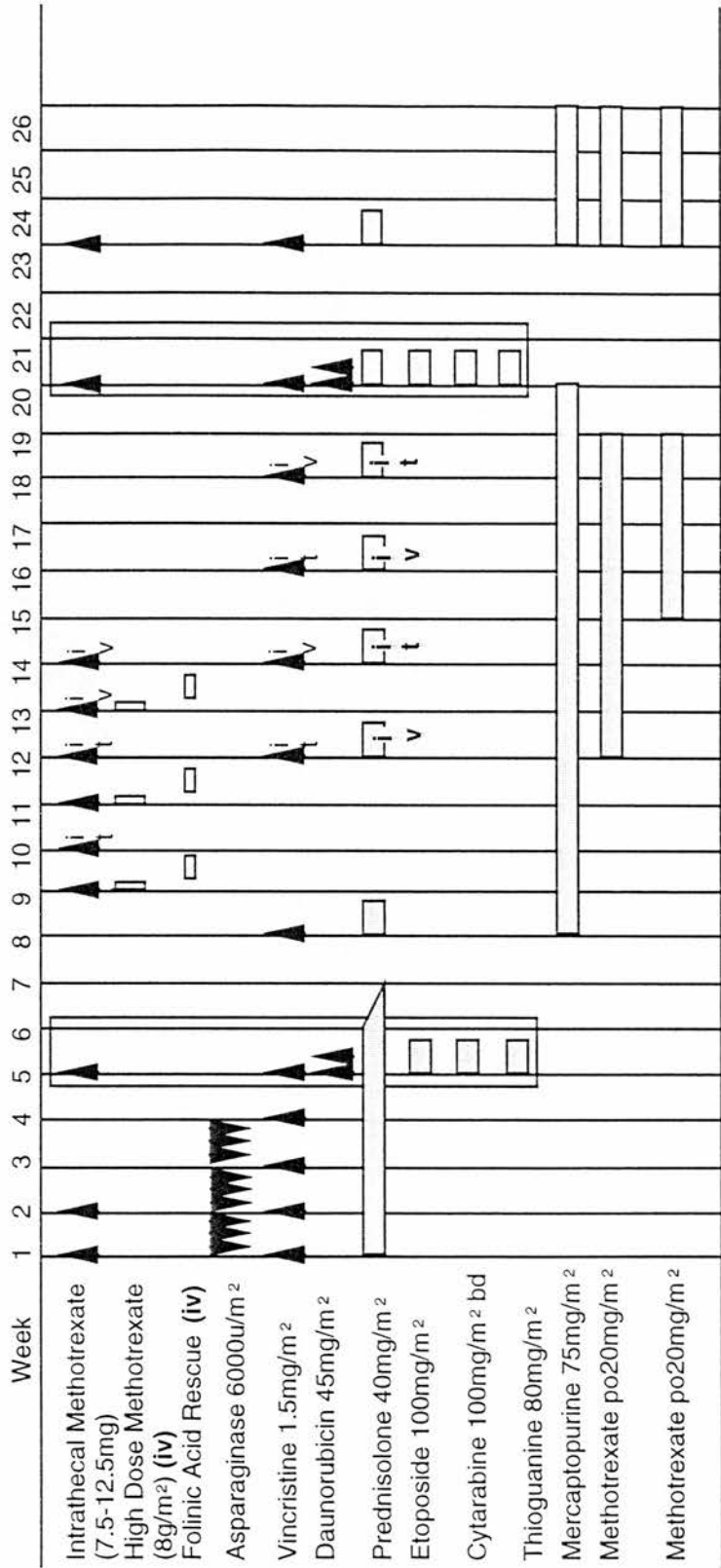


Fig. 2.3 Details of the first 6 months of UKALLXI(92) when CNS -directed therapy delivered as High Dose i.v.Methotrexate and/or i.t. Methotrexate



it - only administered in standard and continuing intrathecal methotrexate limb of CNS-directed therapy
 iv - only administered in High Dose Methotrexate iv and continuing intrathecal methotrexate limb of CNS-directed therapy

Fig.2.4 Details of Third Intensification Regimen (Wk35-42)

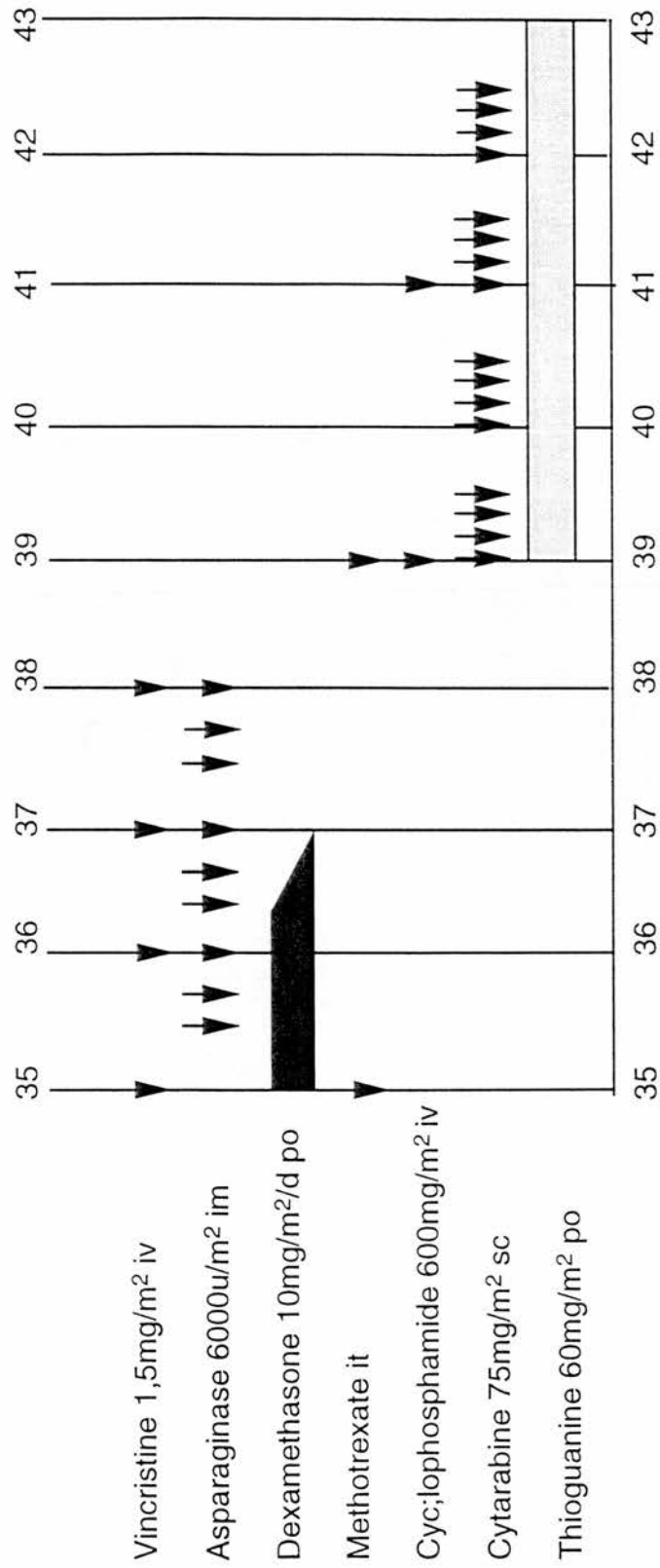
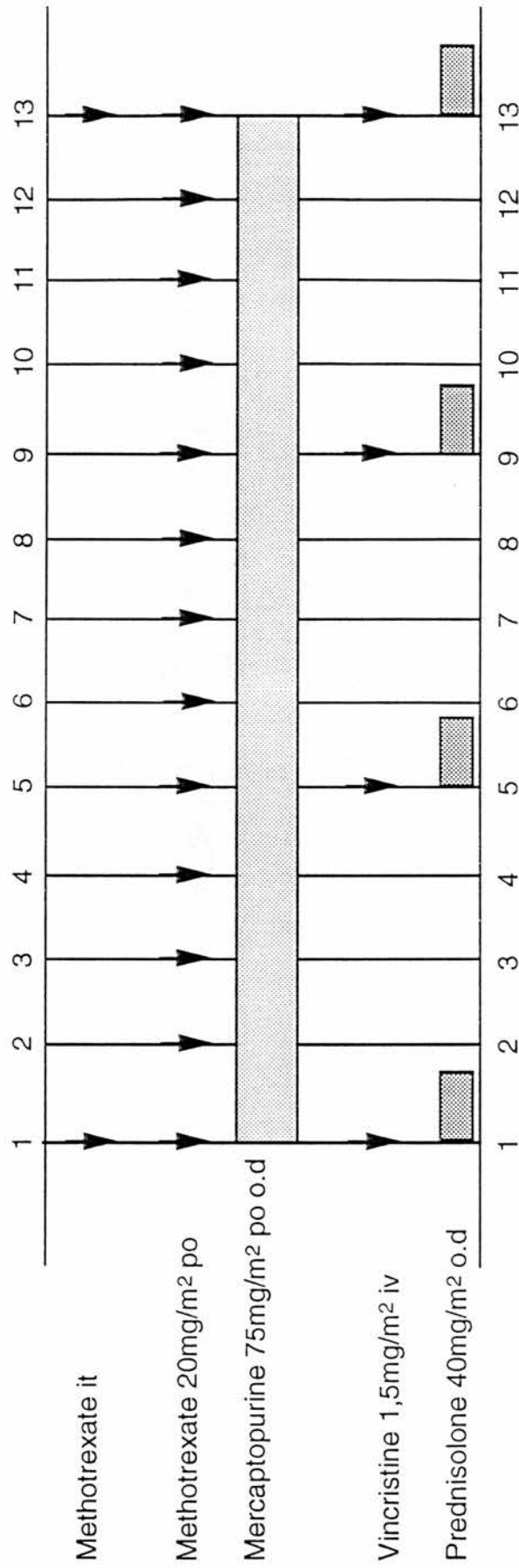


Fig2.5 Details of Continuation Chemotherapy Regimen



Section 3 The effect of individual drugs on the central nervous system and bone & collagen turnover

This chapter discusses the present evidence against individual treatment modalities for effects on the CNS, which may interfere with the central control of growth, and effects on bone and collagen metabolism. It should, however, be borne in mind that these drugs might have altered effects when given in combination (Mullenix *et al*, 1994 and Schunior *et al*, 1994). Section 6 will review studies of growth and bone & collagen metabolism in children treated with combined cytotoxic chemotherapy.

A. Vincristine (Vcr)

Vcr is a vinca alkaloid cytostatic drug which binds to mitotic spindle protein and thus arrests cell division. It also impairs axonoplasmic transport, prevents formation of neuro-filaments and disrupts those already formed; these effects lead to peripheral neuropathy which is dose-dependent. CSF concentrations of Vcr are less than 1% of peripheral levels (Johnson *et al*, 1973). However, the syndrome of inappropriate antidiuretic hormone secretion has been reported with its use, as well as other effects

such as confusion, hallucination and coma (Hildebrand, 1978). In vitro studies in isolated rat perfusion systems have shown significant inhibition of insulin-like growth factor-I (IGF-I) production in response to GH in the presence of Vcr (Morris, 1981). These studies also showed reduced uptake of (35S)-sulphate into porcine costal cartilage in response to IGF-I stimulation in the presence of Vcr. On the basis of these studies, IGF-I production as well as IGF-I action seem to be adversely affected.

B. Daunorubicin (Dnr)

Dnr is a glycosidic anthracycline antibiotic which intercalates between adjacent base pairs on a DNA strand. Dnr does not cross the blood brain barrier (BBB). The porcine costal cartilage bioassay in the presence of Dnr led to reduced uptake of (35S) and also (3H)-thymidine. No effects were seen on IGF-I production in the liver-bioassay (Morris, 1981).

C. Prednisolone (Pred) & Dexamethasone (Dxm)

These glucocorticoids cause a lysis of lymphatic tissue mediated by surface receptors of lymphocytes and lymphoblasts. Leukaemic lymphatic cells are specially susceptible to this lympholytic mechanism. The steroids have a diverse number of effects. They

can cross the BBB and have effects on mood, behaviour and brain excitability (Balis et al, 1987). Administration of daily Pred in adults leads to a reduction in integrated GH concentration as well as GH production rate and this inhibition is reduced on alternate day regimens (Thompson et al, 1983). The inhibitory action seems to be mediated by an enhancement of somatostatin tone resulting in poor GH response to growth-hormone releasing hormone stimulation (Giustina et al, 1990). This is supported by the observation that the steroid-induced decrease in growth of rats can be partially reversed by concomitant treatment with somatostatin antibodies (Wehrenberg et al, 1990).

Corticosteroid excess also has direct effects on the growth plate and bone. Local administration of DXM into one proximal tibial epiphysis significantly reduced linear growth in growing rabbits (Baron et al, 1992). Morphological studies have revealed disrupted angiogenesis in growth plate cartilage in rabbits (Brown et al, 1990) and inhibition of chondrocyte proliferation and cartilage matrix production (Annefeld, 1992) in response to systemic Dxm administration in rabbits. Studies in rats have shown that local IGF-I transcription is reduced on

inhibiting cell replication and synthesis of collagen and IGFs (Canalis & Avioli, 1992). Bone resorption is indirectly promoted by increased sensitivity of bone cells to parathyroid hormone (PTH) and secondary hyperparathyroidism due to steroid-induced renal and intestinal loss of calcium (Gennari *et al*, 1984). Direct promotion of resorption is supported by the finding that Dxm can enhance osteoclast generation in isolated mouse bone marrow cultures (Shuto *et al*, 1994).

D. Asparaginase (Asp)

Asp is an enzyme derived from *Escherichia coli* or *Erwinia carotovora* and hydrolyses L-asparagine, thereby depleting the supply of this amino acid as well as L-glutamine which cannot be synthesised by leukaemic cells. Although Asp does not cross the BBB, peripheral depletion of these amino acids results in a fall in CSF levels. Reported frequency of CNS toxicity varies from 25%-50% and range from lethargy, confusion to seizures and coma (Weiss *et al*, 1974).

E. Methotrexate (Mtx)

Mtx inhibits dihydrofolate-reductase by binding to it and inhibiting the production of tetrahydrofolate. This coenzyme is involved in the

production of purines and thymidylate, depletion of which results in the inhibition of DNA synthesis. When given i.v. in moderate or high doses, Mtx can reach CSF levels 1-3% of that in plasma (Bleyer & Poplack, 1978). Cranial irradiation as CNS-directed prophylaxis has been replaced by i.t. or high-dose i.v.Mtx administration in most protocols. White-matter lesions which could represent areas of demyelination or micro-infarction have been observed on magnetic resonance imaging (MRI) in children treated by this regimen (Bakke et al, 1993, Seidel et al, 1994). These changes could be transient (Lien et al, 1991, Wilson et al, 1991) but could persist as suggested by a correlation between the presence and type of cranial abnormalities and deterioration of neuropsychological function after Mtx treatment (Brouwers et al, 1985). An association between long-term Mtx therapy and osteoporosis and stress fractures has also been reported (Schwartz & Leonidas, 1984). In some cases these fractures do not heal until the withdrawal of Mtx (Stanisvalje & Babcock, 1977). The mechanism of this effect has not been elucidated but it could be related to the anti-folic effects of Mtx as the radiological features of affected bones are similar to those found in scurvy. The costal cartilage bioassay (Morris, 1981) studying abnormalities of substrate

uptake did not reveal any reduction in response to IGF-I in the presence of Mtx.

F. 6-Mercaptopurine (6mp)

6mp is a hypoxanthine analogue, which after several metabolic conversions is incorporated in DNA as a false nucleotide, resulting in cell death. CSF levels of 6mp are about 45% of those of plasma during maintenance (Schouten *et al*, 1984). However, no specific neurotoxic effects have been reported. The porcine costal cartilage bioassay in the presence of 6mp did not lead to altered uptake of (35S)-sulphate or (3H)-thymidine. However, IGF-I production in response to GH in the isolated liver-bioassay was reduced (Morris, 1981).

G. Cytosine-Arabinoside (Ara-C)

Ara-C is a pyrimidine analogue, which selectively inhibits DNA synthesis by the incorporation of the active metabolite Ara-C triphosphate into DNA. Levels up to 40% of those in plasma can be reached in the CSF if Ara-C is administered by continuous i.v. infusion which are, however, less neurologically toxic than intermittent high doses of the drug (Lockhart *et al*, 1994). The porcine costal cartilage bioassay in the presence of 6-MP lead to altered uptake of (3H)-thymidine only. IGF-I

production in response to GH in the isolated liver-bioassay was minimally reduced (Morris, 1981).

H. Cyclophosphamide (Cyclo)

Cyclo belongs to the group of nitrogen mustards which act as alkylating agents. Only minute amounts of Cyclo penetrate into the CSF. Nausea and vomiting may be the result of a direct effect on the CNS; with high doses, SIADH has been observed. The porcine costal cartilage bioassay did not show any major alterations in the presence of Cyclo (Morris, 1981).

I. Etoposide (Etop)

Etop is a cytotoxic semi-synthetic epipodophyllotoxin which acts by producing protein-linked DNA strand breaks by inhibition of DNA topoisomerase used in condensing or decondensing supercoiled DNA. Neurotoxicity is rarely reported with Etop with somnolence occurring in about 3% of cases.

Section 4 Markers of Growth Hormone Secretion

Optimal evaluation of GH secretion has been an important problem in paediatric endocrine practice. Tests for GH secretory ability need to be sensitive, specific, practical, reproducible and safe. The traditional serum GH provocation tests do not fulfil any of these criteria and this has generated an interest in simple, harmless and practical methods of measuring markers which might reflect physiological GH secretion.

A. Urinary Growth Hormone (uGH)

The major form of GH in urine is a 22kd peptide. Most of the secreted GH is degraded by the liver, kidneys and the peripheral tissues and less than 0.001% of the GH secreted by the pituitary is excreted in the urine (Baumann & Abramson, 1983); renal function can, therefore, have a profound effect on uGH levels (Hattori *et al*, 1988). uGH can be measured by prior dialysis and concentration of the urine followed by a double-antibody radioimmunoassay with a sensitivity of 0.3ng/ml (Hansenn, 1974). More recently described methods employing enzymelinked immunosorbent assays and immunoradiometric assays are sensitive down to 0.4-4pg/ml (Porquet *et al*, 1992).

The appropriate method of expression of uGH output is controversial. Results can be expressed by assessing timed urine collections (ng/12hrs) based on chronological age, weight, surface area or creatinine excretion (MacGillivray, 1993).

Since the amount of GH present in timed urine collections from healthy subjects is extremely small, standardisation on the basis of age, weight, or surface area results in considerable overlap of values between groups. Normalising data on the basis of creatinine excretion can also lead to significant error because creatinine is dependent on lean body mass which changes with age, puberty and clinical state. Due to these uncertainties, some investigators have reported the absolute outputs of uGH in nanograms per time interval without any standardisation steps (Albini et al, 1991, Granada et al, 1992).

In most studies uGH excretion correlates positively with plasma GH concentration as assessed by physiological or provocation tests in children who are not in advanced puberty. However, the overlap of uGH values between normal and short (GH-deficient and Idiopathic Short-Stature) children precludes the test as an adequate screening test for GH-deficiency (Albini et al, 1991). Another disadvantage of the uGH test is its range of variability in the same child.



This results from a combination of assay variation and variability in renal handling of GH (MacGillivray, 1993). As minor changes in renal function can influence the variability in consecutive uGH levels in the same subject it would be useful to compare the renal handling of GH with other freely filtered proteins (Skinner *et al*, 1993). A further reason for the observed variability could be that there is real biological variation in GH secretion (Thalange *et al*, 1996) but this remains to be proven. The variation error can be reduced by collecting repeat samples (Skinner *et al*, 1993).

uGH assays have not been used to gather information about relative outputs of GH over time, nor to assess changes in uGH in relation to therapeutic interventions aimed at increasing or decreasing GH production. Such investigations could provide a non-invasive method of studying abnormal growth provided that uGH levels reflect short to medium-term growth.

B. Insulin-Like Growth Factor I (IGF-I)

IGF-I is a 7.6kD single chain polypeptide which is regulated by a combination of autocrine, endocrine and paracrine factors. The major regulator of circulating IGF-I is GH and the main site of production is the liver (Hynes *et al*, 1987). The synthesis of IGF-I by osteoblastic cells is enhanced mostly by parathyroid hormone (PTH) and to a lesser extent by

GH. Skeletal IGF-I also enters the circulation but whether plasma IGF-I reflects local tissue levels is undetermined; it is possible that the endocrine and paracrine control as well as effects of IGF-I are distinct from each other. The actions of IGF-I can be classified into two groups; short-term metabolic and long-term mitogenic effects. The metabolic effects are similar to those of insulin with negative feedback inhibition of GH secretion. In combination with GH, IGF-I has proliferative effects on epiphyseal chondrocytes (Lindahl *et al*, 1987). Following GH-induced differentiation of target cells, IGF-I acts to promote tissue growth by clonal expansion. Besides its direct proliferative action, in-vitro studies have shown that IGF-I also promotes bone and collagen growth by promoting Type I collagen synthesis, increased proteoglycan synthesis in chondrocytes, maturation of the osteoblast phenotype and inhibition of skeletal-specific collagenases (Rosen *et al*, 1994). There is some early evidence that IGF-I might be involved in osteoclast activation, too (Slootweg *et al*, 1992). In paediatric practice, IGF-I levels have been used primarily for evaluation of GH-secretion (Juul *et al*, 1994). IGF-I levels are, however, subject to the same "overlap" problem as uGH as discussed above. In addition, its levels do show some circadian variation (Minuto *et al*, 1981) and depend on the state of nutrition (Monaco *et al*, 1992).

C. IGF Binding Proteins-3 (IGFBP-3).

The systemic and local effects of IGFs are modulated by a group of proteins, the insulin-like growth factor binding proteins (IGFBPs) which have a high affinity and specificity for the IGFs. Of the six IGFBPs so far described, IGFBP3 is the most abundant protein in serum. It is produced by most cell types including osteoblasts and although the core protein has a molar weight of 28.7kD, it exists in the circulation as a 150kD complex with an acid labile 85kD glycoprotein and one of the two IGFs (Rosen *et al*, 1994). Factors known to influence IGFBP3 levels include GH levels and age and puberty (Blum & Ranke, 1990); unlike IGF-I, IGFBP3 reduces only with prolonged restriction (Young *et al*, 1992). Release of IGF from the complex is thought to occur following a lowering in pH and is mediated by IGFBP proteases (Clemmons *et al*, 1986). IGFBP3, therefore, seems to inhibit unnecessary insulin-like activity, prolong the half-life of IGFs and enhance the biological response of target tissues to IGFs by directing them to particular cell types.

The value of IGFBP3 as a marker for GH secretion has only recently been studied and whether it is a more reliable indicator than IGF-I is still debated. As IGFBP3 levels alter IGF-I bioavailability they might reflect GH secretion better than IGFI; the methodology involved in IGF radioimmunoassay needs preliminary IGFBP extraction which if not performed

optimally can lead to inaccurate results (Brier *et al*, 1991); the IGFBP3 assay has a higher reference range than the IGF-I assay, making the assay more sensitive to the detection of decreases in GH secretion (Blum *et al*, 1990). It is possible that changes in IGF-I levels are more sensitive to GH secretion, explaining the circadian rhythm, and IGFBP provides a more integrated measure of GH secretion. Finally, there is some evidence that IGFBP3 levels are more reproducible when performed repeatedly as compared to IGFI (Blum *et al*, 1993). However, it has recently been shown that IGFBP3 measurement might be as reliable as IGFI in assessing GH secretion (Hasegawa *et al*, 1994).

Section 5. Biochemical markers of bone & collagen turnover

Somatic growth involves synthesis and deposition of collagen and the study of biochemical markers of bone and collagen turnover provides the means of further understanding the physiology of growth as well as the mechanism by which growth could be disrupted by specific pathological processes. This section will consist of a brief review of some of the markers which have been used in these studies.

A. The Basic Multicellular Unit

Sequential replacement of old bone with new occurs within a defined quantum of bone, the basic multicellular unit (BMU). The cycle begins with multinucleated osteoclasts which originate from haemopoietic stem cells. These cells cause bone dissolution by release of protons and proteases. 5 to 7 days of resorption by the osteoclasts produce a resorption lacuna. After a short quiescent phase, bone formation in this area proceeds over a span of approximately 100 days. Mesenchymal-derived osteoblasts synthesize the new bone by forming an organic matrix which is later mineralized. The frequency of BMU activation is dependent on the nature of the activating signal (eg. hormones, gonadal steroids, cytokines and growth factors) and possibly, skeletal architecture (Parfitt *et al*, 1996). The time span mentioned above is that

for post-pubertal bone and it is possible that it differs in growing children and is variable from site to site. The markers of bone and collagen turnover are illustrated in Figure 2.6.

B. Markers of Bone Formation

The developmental sequence of an osteoblastic cell phenotype has been divided into three consecutive phases: proliferation, extracellular maturation and mineralization (Stein *et al*, 1990). Mesenchymal cells committed to the osteoblast lineage only produce Type I Collagen which occurs early during the proliferation of the precursor cells. The expression of alkaline phosphatase (ALP) starts immediately after cessation of cell proliferation, reaches a maximum during the phase of matrix maturation and declines as matrix mineralization commences. Among the genes expressed at this point are those for the calcium binding proteins, osteocalcin and osteopontin.

i. Procollagen Type I Carboxy-Terminal Propeptide (PICP).

Type I collagen, the most abundant collagen in the body and is present in most tissues but especially bone, is synthesized as a larger protein, type I procollagen. The extra material is present at the carboxy as well as the amino terminal of the protein molecule and is necessary for

preventing premature association of the molecules into collagen fibrils. These extension domains are cleaved on extracellular secretion of the procollagen. PICP is not metabolised but rapidly cleared intact by the liver reticuloendothelial cells. Serum PICP can be measured by a commercial radioimmunoassay (Melko *et al*, 1990) and its concentration is closely related to the rate of the histomorphometrically assessed bone matrix formation (Eriksen *et al*, 1993). PICP maybe a good indicator for catch-up growth after severe disease in children (Hyams *et al*, 1989). Circadian variation has been noticed in PICP levels and a positive relationship between 24hr PICP levels and GH secretion and growth velocity (Saggese *et al*, 1994).

ii. Bone Alkaline Phosphatase (bALP)

The function of this enzyme is not known with certainty but it is associated with the plasma membrane of the osteoblast, which buds out to form the matrix vesicles seen in developing bone. It may be involved in extracellular breakdown of pyrophosphate, a potent inhibitor of calcium phosphate deposition. Total ALP (tALP) activity levels measured by simple colorimetry correlates with bone formation rate but a drawback of this method relates to the presence of isoenzymes originating from tissues other than bone. This is less of a problem in healthy children in whom most of the tALP is derived from bone. The activity of the liver isoform might be

affected by drugs affecting hepatic function and at times of impaired growth it might be appropriate to measure bALP specifically by methods such as wheat germ affinity electrophoresis (Crofton, 1992) or immunoradiometric assay (Gomez *et al*, 1995). It is not yet clear, however, whether the bALP measurements give significant new information over the total activity.

C. Markers of Bone Resorption

Osteoclasts express a number of lysosomal enzymes and proteins which produce an acidic environment and promote bone resorption. Breakdown of collagen fibres results in a mixture of peptides and amino acids. The regions of the collagen molecule that participate in covalent cross-linking between molecules seem to be less susceptible to the action of these enzymes.

i. Pyridinoline (PYD) & Deoxypyridinoline (DPD)

Collagen molecules in fibrils are cross-linked by pyridinium structures which are heterogeneous with respect to their degree of hydroxylation and glycosylation. PYD is found in fibrillar (mature) collagen from several tissues, including soft tissues, but it is particularly characteristic of Type II collagen of cartilage and the Type I of bone and dentin. Due to the large amount of bone collagen in humans, most of the PYD detected in the urine is derived from bone. DPD is

another variant which almost exclusively originates from bone collagen. The above pyridinium crosslinks can be simultaneously measured by high pressure liquid chromatography. There is generally good correlation between accelerated bone turnover and the urinary excretion of the cross-links (Seibel *et al*, 1992).

ii. Cross-linked Carboxyterminal Telopeptide of Type I Collagen (ICTP)

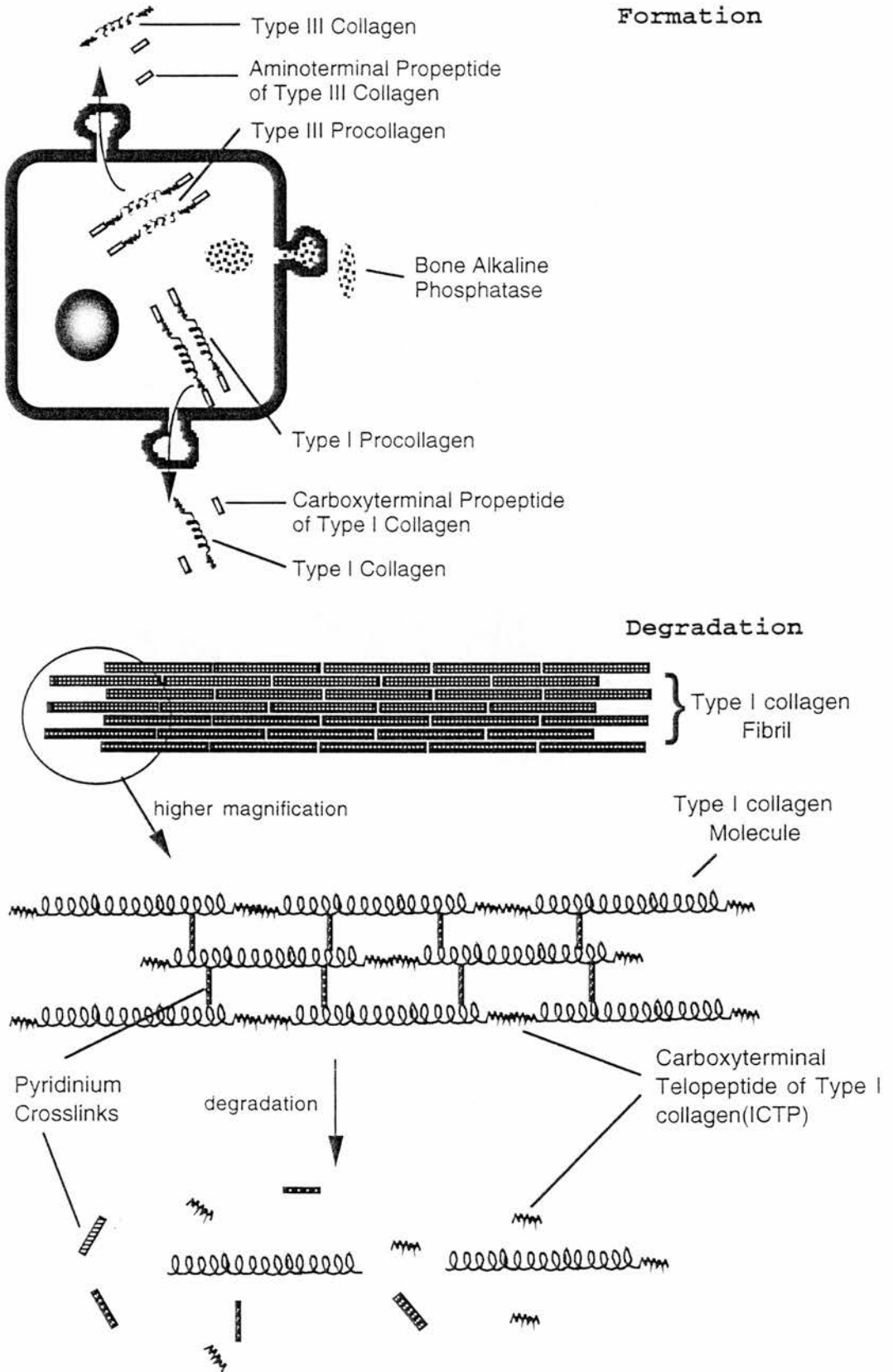
Inter and intra-crosslinking between collagen molecules occurs at the short non-helical ends of the collagen molecules known as telopeptides and ICTP is liberated from the carboxyterminal end of Type I collagen when the latter is degraded. ICTP can be assayed from plasma using a commercially available radioimmunoassay and preliminary data suggests that it is a marker of bone resorption (Eriksen *et al*, 1993).

D. Bone-Exclusive Markers of Collagen Synthesis

The above markers are all related to bone collagen synthesis or breakdown. **Procollagen Type III Amino-Terminal Propeptide (PIIINP)** is cleaved off during the synthesis of Type III collagen in a fashion similar to PICP. Type III collagen is present in almost all tissues except bone and cartilage. The serum concentration measurable by a radioimmunoassay has been shown to correlate with the somatic

growth rate in healthy children whereas PICP levels reflect bone and linear growth (Graham 1989, Trivedi *et al*, 1991). Circadian variation, when studied, was not evident in relation to PIIINP levels (Sagesse *et al*, 1994). Simultaneous measurement of both PICP and PIIINP should give an indication of the relative amount of both bone and soft-tissue collagen formation.

Fig.2.6 Markers of Bone and Collagen Turnover



Section 6 Growth and bone metabolism during cytotoxic chemotherapy

The effects of XRT on growth have now been extensively studied and well documented (Wallace & Shalet, 1992) and this mode of treatment is now only used in a small number with CNS disease or at a high risk of CNS-relapse. The effects of cytotoxic chemotherapy (CT) on growth are less well understood. This section will review the present literature on the effect of CT on growth and bone metabolism.

A. Cytotoxic Chemotherapy & Growth

The first indication that CT by itself might have a harmful effect on growth in children with ALL, was the report that a more intensive chemotherapeutic regimen, but a similar radiotherapy regimen, showed worse growth retardation (Kirk *et al*, 1987, Clayton *et al*, 1988). The second study also showed that catch-up growth following the end of CT did not compensate completely for the earlier fall in height (Ht) standard deviation scores (SDS). Thun-Hohenstein *et al* (1992) compared children who received treatment with one of two protocols of different intensity (Berlin-Frankfurt-Munster (BFM) or non-BFM), and reported that those receiving the less intensive CT showed less growth impairment. The intensive treatment group received significantly more cyclophosphamide, anthracyclines, nitrosureas and vincristine.

The initial decline in HT SDS during the period of treatment is felt to be due to CT rather than XRT (Hokken-Koelega et al, 1993). This study also showed that catch-up growth which only started after CT ended restored CT-only children to their original HtSDS 5 years after diagnosis and CT, by itself, only had a temporary effect on growth.

In theory, CT can affect the mechanisms controlling growth at different points as discussed before. Minor white-matter lesions have been seen on MRI in children who have received CT only regimens for ALL (Bakke et al, 1993); there is a possibility that these lesions could affect GH secretion if they occurred around the hypothalamo-pituitary area, albeit temporarily. Physiological GH secretion profiles during investigation have been found to be satisfactory, except in one case where it was profoundly depressed and was related to high-dose DXM treatment; this was the only child investigated on Dxm treatment (Marky et al, 1991). Another study looking at serial changes in the GH-IGF axis in children receiving craniospinal irradiation for brain tumours showed a transient decrease in IGF-1 and IGFBP-3 levels in the presence of normal GH levels in the first 6 months of therapy when treatment was particularly intensive (Nivot et al, 1994). The changes coincided with a fall in BMI and plasma concentration of total proteins indicating a defective nutritional status

and the possibility of a transient GH resistant state. Hepatic dysfunction which might be due to the CT used could also contribute to reduced levels of IGF-1 production.

B. Bone Metabolism and Chemotherapy

Although children with ALL might present with osteoporosis at diagnosis it has become evident that osteoporosis of variable severity can also occur during treatment (Schwartz, 1984, D'Angelo, 1989) and could be related to the effects of CT. Following completion of CT for ALL, Gilsanz et al (1990) found significant reduction of bone mineral density in only those children who received XRT. Nussey et al (1994) related reduced bone mineral density in long-term survivors of XRT for ALL to GH deficiency. Another study raised the question whether altered calcium and magnesium homeostasis in children on treatment is a possible cause of abnormal bone turnover (Atkinson et al, 1989).

Further evidence of the involvement of CT came from a study looking at final Ht and sitting height (SH) in survivors of childhood solid tumours. The males who had received both CT and radiotherapy to the spine had a greater decrement in SH, but no association was found between radiation therapy to the spine without CT and subsequent growth of the spine (Makiperna, 1990).

CHAPTER THREE

SHORT-TERM CHANGES IN LOWER LEG LENGTH IN HEALTHY
INDIVIDUALS

Short-term growth studies using knemometry have revealed a saltatory pattern of growth in children while total body length or stature measurement reveals a more continuous pattern. The precision of the technique is likely to dictate the pattern of growth and any measurement bias will affect that precision. The first section of this chapter investigates the presence of observer bias as well as the presence of any learning curve for the technique.

The pattern of growth in healthy "normally-growing" children remains controversial and before any studies of interventional therapy, it is important to document the nature of this growth in healthy children as performed by the same group of measurers and analysed uniformly. This group of children would provide some reference data against which groups of other children on treatment can be compared but they would not act as strict controls, primarily as they do not suffer from any illness. In order to study changes in LLL which might be artefactual a group of fully grown adults was also studied.

The relationship between changes in LLL and weight is described in Section 2; Section 3 studies relationships between fluctuations in LLL and concurrent excretion of urinary Growth Hormone. Utilising this information the pattern of growth in children is discussed in Section 4.

Section 1. Comparison of two different techniques of knemometry

A. Introduction

The method of knemometry in children as described by the original investigators is now universally accepted (Valk et al, 1983). Knemometry is easy to learn and although some investigators have suggested that there should be a learning period (Hermanussen et al, 1988), there are no data available evaluating how long it takes to become proficient. Using the Valk knemometer, which has an accuracy of 0.1mm, the technical error (the mean standard deviation in a series of n independent measurements) (TE) has ranged between 0.09mm to 0.16mm in previous studies (Hermanussen, 1988). As no modifications of the original method have been described it is likely that the measurer in previously reported studies is not blind to previous estimations in the set from which each measurement is derived.

Methods which employ "blind" lower-leg length estimations have been developed for the neonatal knemometer where the estimations are printed at some distance from the measurer (Gibson et al, 1993a). The TE has been reported to be higher at 0.3-0.5mm and a learning curve was also found using this method.

In this study a simple modification of the original technique, designed to reduce the intra-observer bias, was investigated. This technique was also used to evaluate the presence of a learning period for the technique, information which is important for prospective users.

B. Patients & Methods

Over the period between May 1993 and August 1994, 50 children aged 4.2-12.8 years and 8 healthy adults had knemometry performed. The children consisted of 26 healthy volunteers from a neighbouring primary school, 18 children on cytotoxic chemotherapy and 6 children with cystic fibrosis. Individual children were measured at the same time of the day (within an hour's time interval from the first measurement), on a weekly basis, for periods ranging from one to ten months, depending on the group. The adults were measured in the last two months of the study. Measurements were performed at either one-weekly or two-weekly intervals.

Each measurement was derived from a set of 4 "estimations"; the most deviant of the 4 estimations was eliminated and the mean of the rest was calculated to provide a "measurement" (Wales & Milner, 1987).

Measurements were performed using two methods; the Original Method (OM) was performed as described by Hermanussen (1988)

in Chapter One; Section 1(A). The Random Zero Method (RM) differed from OM in that the baseline reading on the preset meter was altered by the measurer before each estimation when the measuring platform was resting at zero. The preset meter was covered with a piece of paper when the alteration was made so that the measurer was not aware of the baseline reading until the end of the estimation. Each estimation was recorded as a set of two readings and deduction of the baseline from the actual digital read-out provided the estimation.

C. Statistical Analysis

The technical error (TE) for each measurement was derived from the standard deviation of the set of 3 estimations which make up each measurement as described in "Methods". The TE and coefficient of variation (CV) for each measurement were calculated and grouped with that of other measurements performed in the same month. The overall average TE and CV of the two groups (OM & RM) were presented as median values with their respective 5th and 95th percentile values (P5 & P95). The Wilcoxon Signed Rank test was used as a non-parametric test to compare differences between groups.

D. Results

413 measurements were performed over the 10-month period; the RM group consisted of 314 measurements and the OM group

consisted of 99. The monthly breakdown of these measurements is detailed in Table 3.1.

Mean lower leg length velocity in the children was 0.39mm/wk (95%CI 0.26-0.53). The mean *fluctuation* in leg length which was calculated by transforming all the velocities into positive values before obtaining the mean was 0.86mm/wk (95%CI 0.76-0.95). This value provides an idea of the amount of change per week that can be detected irrespective of whether it represents an acceleration or deceleration.

Median TE in the RM and OM group was 0.15mm (P5-0, P95-0.65) and 0.11mm (P5-0, P95-0.37), respectively. Median CV in the RM group was 0.06% (P5-0, P95-0.21), not significantly different than 0.06% (P5-0, P95-0.16) which was the CV in the OM group. Adults were measured in the last 2 months of the study period by the Random Zero method only. Median TE was 0.36mm (P5-0, P95-0.7) in April and 0.12mm (P5-0, P95-0.58) in May.

TE and CV reduced in both groups over the period of study (Table 3.2). To analyse any trend the values for the September and October subgroups in the RM group were excluded from evaluation as they consisted of relatively small samples, as was the November sub-group of the OM group. The TE reduced over the first 4 months when RM was used on a substantial number of occasions (Table 3.2).

Differences of TE and CV between consecutive period of measurements did not reach significance because of the wide distribution of the values (Fig.3.1). However, grouping together the TE values for the first 4 months (Nov,Dec,Jan, Feb) revealed a median value of 0.2mm (P5-0.05, P95-0.87) which was significantly higher than the TE for the following 3 months at 0.15mm (P5-0,P95-0.55) ($p=0.04$, WSR).

E. Discussion

The physiology of short-term changes in human growth is still poorly understood. Although instruments such as the knemometer might shed light on changes not very far from the cellular level, their limitations as well as their advantages must be thoroughly assessed. Knemometry, as performed traditionally, is subject to intra-observer bias which arises as the measurer is aware of the previous estimation in the series. A reported TE of 0.09mm (Hermanussen, 1988) is comparable to more precise techniques such as kyniklometry (Hermanussen et al, 1992) in which reproducible estimations are more likely due to better localisation of reference points of measurement.

The RM technique described in this study eliminates the observer bias very simply. It can be performed without any structural modification to the original knemometer. However, there is one extra step: deducting the baseline reading from the initial estimation to provide the real estimation.

Another advantage of RM is that it provides a better guide to the length of the training period alluded to in previous studies (Hermanussen *et al*, 1988). We saw a trend towards lower TE in the RM as well as the OM groups but this was only significant for the TE values in the former group. This trend might have become statistically significant by the high outlying values at the beginning of the study but the fact that there were more TE values near zero by the end of the study support the suggestion of a real learning curve (Fig.3.1). The lower TE in the last 2 months were not due to the introduction of adults into the study as the TE for that specific sub-group was slightly higher than the overall value for that month. The low TE values for the first two months in the RM group were unrepresentative as the sample size was small and the RM measurements were also being performed with extra care as it was a newly introduced method. Subjectively, RM took much longer initially than in the latter months.

On the basis of our results we would recommend a period of 3 to 4 months for learning if the knemometer is used as frequently as described above. By using RM, each operator can also evaluate their own technique looking for an improvement and subsequent plateauing of the TE. To improve the measurement process further, it would be helpful for the operator if the knemometer could be electronically adapted so

that the series of estimates could be stored, the most deviant estimate omitted and a TE calculated instantly. If the latter is above an arbitrary limit, then there is scope to repeat the measurements immediately.

To summarise, the Random Zero Method reduces observer bias on the part of the measurer but does have a higher TE than reported before. This will clearly have implications on interpreting patterns of short-term growth with multiple fluctuations which might well be an artefact of measurement rather than a real phenomenon. The method also allows independent evaluation of operator proficiency. The knowledge of the length of the training period would help new investigators who are designing studies involving knemometry

Table 3.1. Distribution of measurements according to method (Random Zero vs. Original) and month of study. Figures in parantheses denote the number of measusurements performed in adults.

	Random Zero Method	Original Method
Aug	0	35
Sep	8	35
Oct	11	23
Nov	36	6
Dec	29	0
Jan	22	0
Feb	35	0
Mar	24	0
Apr	39 (10)	0
May	110 (60)	0

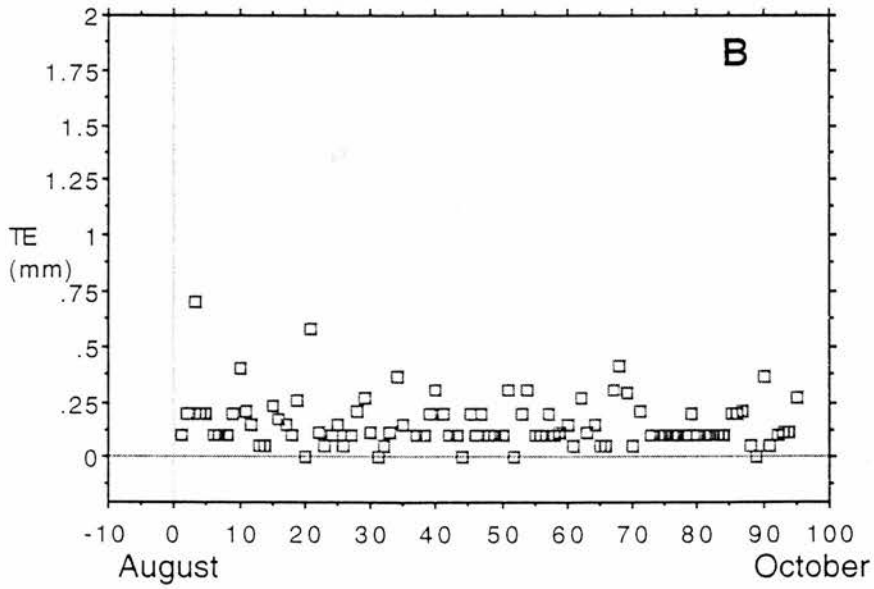
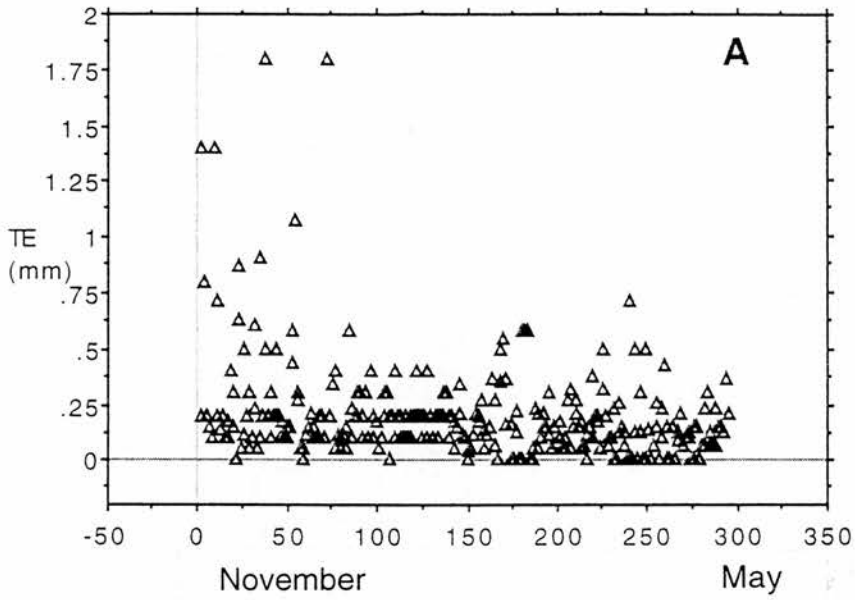
Total 314

99

Table 3.2 Median monthly Technical Error(TE) and Coefficient of Variation(CV) of measurements performed by the Original Method(OM) and the Random Zero Method (RM).5th and 95th Percentile values in parantheses.

	TE-RM	TE-OM	CV- RM	CV-OM
Aug		0.15 (0.03-0.55)		0.04 (0-0.27)
Sep	0.13 (0.06-0.2)	0.12 (0.03-0.31)	0.06 (0.02-0.09)	0.07 (0-0.15)
Oct	0.11 (0.01-0.47)	0.1 (0.04-0.28)	0.04 (0-0.06)	0.04 (0.01-0.14)
Nov	0.17 (0.08-1.05)	0.17 (0-0.30)	0.10 (0.02-0.4)	0.10 (0-0.10)
Dec	0.20 (0.08-0.36)		0.10 (0.02-0.22)	
Jan	0.20 (0.05-0.4)		0.10 (0.02-0.2)	
Feb	0.16 (0.05-1.15)		0.08 (0.02-0.40)	
Mar	0.13 (0.05-1.0)		0.06 (0.02-0.31)	
Apr	0.14 (0-0.58)		0.06 (0-0.18)	
May	0.11 (0-0.59)		0.04 (0-0.13)	

Fig.3.1 Scattergram of individual TE values of the random zero method(A) and the original method(B) plotted in chronological fashion.



Section 2. The Relationship of Short-term changes in Lower Leg Length and Body Weight.

A. Introduction

As the knemometer measures the complete lower leg, it is possible that these changes may represent alterations in the composition of subcutaneous tissue, as well as changes in bone and cartilage turnover and growth at the cellular level. Previous studies have shown anecdotal evidence of an inverse as well as a direct relationship between LLL and weight (Hermanussen *et al*, 1988, Seidel *et al*, 1991).

As the knemometer is increasingly being used to study short-term changes in LLL in conditions associated with substantial weight changes (Wales & Milner, 1988) it is important to delineate clearly the relationship of weight to LLL. This study investigates this association by documenting LLL and weight changes in a group of healthy children and adults.

B. Subjects & Methods

Between May 1993 and August 1994, 26 pre-pubertal children (Grp A) aged 4.2-10yrs (M:F, 12:14) and 9 healthy adults aged 29-39yrs (M:F, 5:4) had lower leg and weight measurements. Individual children were measured at the same time of the day, on a weekly basis for 12 weeks. In 7 adults (Grp B) knemometry was performed daily, twice a day, about 8 hours

apart for 6 weeks; in the remaining 2 (Grp C) who were both women, measurements were performed once, three days a week, for a period of 8 months. During the study, one of the Group C subjects became pregnant. All the other women had regular menstrual periods.

Knemometry was performed as previously described (Chapter 3; Section 2(B)). Subjects in Group A and B were measured by myself whereas those in Group C were measured by 2 other observers as well as myself. Weight was recorded with standardised clothing on the same Avery Scales with an accuracy of 0.05kg and a standard deviation of 0.05kg from the mean for 3 repeated measurements. All measurements were performed at approximately the same time of day and to facilitate the enlistment of subjects standardisation for ingestion, micturition and defecation was not instituted.

C. Statistical Analysis

LLL changes were expressed as LLL velocity (LLL_v) as well as *fluctuations* (LLL_f) which represent the relative and the absolute amount of change that can be detected between consecutive measurements, respectively. Body weight was also similarly calculated. In Grp A, the changes were expressed over weekly intervals and in Grp B & C, the changes were expressed over daily intervals. Some Group A measurements were performed at 2-weekly intervals. In Grp B & C, data

analysed for assessing change consisted only of measurements performed on consecutive days. Precision of knemometric measurements was expressed as TE. Normally distributed data was described by mean value and 95% Confidence Interval (95%CI) whereas skewed data was described by median value and 5th and 95th Percentile values (P5, P95). Spearman's correlation coefficient was used to assess any association between variables. Inter-group differences were assessed by the Wilcoxon Signed Rank (WSR) test.

D. Results

Measurements & Technical Error

241 measurements were performed in Grp A, 110 pairs in Grp B and 123 in Grp C. The Median TE in children (Grp A) was 0.15mm (P5-0, P95-0.58) and in adults (Grp B & C) was 0.12mm (P5-0, P95-0.58).

LLL and BW Changes in Group A - Children

Mean derived LLLv was 0.39mm/wk (95%CI, 0.26-0.52) and mean derived BWv was 0.08kg/wk (95%CI, 0.01-0.13). Mean LLLf was 0.83mm/wk (95%CI, 0.74-0.91) and mean BWf was 0.26kg/wk (95%CI, 0.23-0.29). The median overall change in LLL and BW from the start to the end of the study was 4.8mm (range, 2.4-10.8) and -0.6kg (range, -1.4-0.5). There were no significant differences between the two sexes in the group. There was some evidence for a positive (Fig.3.2) and none for an

inverse relationship between BWv and LLLv in individual subjects (Fig.3.3). The positive relationship was also evident on cumulative analysis of the data (Fig.3.4).

LLL and BW Changes in Group B - Adults

Twice a day measurements in these adults revealed a significant amount of intra-daily variation in 2 individuals; in one, there was an increment (Table 3.3). For longitudinal velocity and fluctuation analysis, the mean of each day's measurement in every individual was used. Mean derived LLLv was 0.07mm/day (95%CI, -0.03-0.16) and mean derived BWv was -0.02kg/day (95%CI, -0.06-0.03). There was no inter-sex difference. However, median LLLf was higher in women at 0.16mm (P5-0, P95-0.7) as compared to 0.1mm (P5-0, P95-0.48) in men (p=0.02). This was also true for median BWf which was 0.15kg (P5-0, P95-0.54) as compared to 0.1kg (P5-0, P95-0.5) in men (p=0.04). The median overall change in LLL and BW from the start to the end of the study was, -0.05mm (range, -0.9-2.6) and -0.23kg (range, -1.7-0.9). There was no evidence of a temporal relationship between BW and LLL changes in the adults.

LLL and BW Changes in Group C - Pregnancy Data

Fig.3.5 displays the changes in LLL and BW seen in the 2 subjects in this group. As evident in Grp B individuals, there was no significant relationship between day-to-day variation in BW and LLL in either individual. However, over

the duration of the pregnancy LLL reduced while BW increased until a point was reached (beginning of 3rd trimester, in this case) when LLL started to increase; this increase coincided with the appearance of leg oedema associated with the pregnancy.

E. Discussion

Changes in lower leg length as assessed by the knemometer are likely to be multifactorial. While actual growth and remodelling of the cellular components of the leg must contribute, it is likely that some of these changes are due to measurement error (previous section) as well as other physiological factors such as gravity and body weight (Hermanussen *et al*, 1988).

The effect of body weight changes on LLL was briefly described by Hermanussen as a weak direct relationship when data from different children were analysed cumulatively (Hermanussen *et al*, 1988). Anecdotal evidence for an inverse relationship between the two variables was reported by Seidel *et al* (1991) in a child with chronic renal failure whose LLL increased after a big weight loss following dialysis.

The cohort of healthy children in our study had a LLLv comparable to that in previous studies and their weight gain over the study period was not remarkable. In support of Hermanussen *et al* (1988), there was a weak but significant

relationship between the cumulated data LLL and BW change. However, more importantly, we have also showed a significant direct relationship in some of the individual sets of data and the absence of a negative relationship in any of the children. A failure to show any relationship in the other subjects could be due to the small individual datasets. We did not assess intra-daily variation in our cohort as this has been shown before (Valk *et al*, 1983) and would have required an extraordinary level of co-operation from the young subjects during their schooldays.

Although the LLL and BW did not change substantially from the start to the end of the study in adults, a significant amount of day-to-day fluctuation was evident in both parameters, being more pronounced in the women. Unlike previous reports of intra-daily reduction in LLL in children, the cohort of adults did not all show a consistent reduction in LLL over the day while following a normal daily routine. Like the children in Group A, none of the Group B adults gained a substantial amount of weight over the duration of the study. A significant reduction in LLL was, however, seen in the subject who became pregnant and this was accompanied by a large weight gain.

Magnetic resonance imaging of the intervertebral discs in adults has revealed diurnal changes in size related to

changes in the water content of the discs (Paajanen *et al*, 1994). Hydration is a major determinant of short-term changes in weight and would affect subcutaneous tissue and can affect cartilage/epiphyseal thickness. It is quite likely that the positive relationship between changes in weight and lower leg length seen in some of the children in this study was due to alteration in hydration level. The failure to see such changes, as well as the absence of universal intradaily reduction is most likely due to a relative lack of cartilaginous tissue in the lower leg of adults. In a previous study, we were unable to show a temporal relationship between body weight and lower leg length changes in adults. Our finding of a higher amount of day-to-day fluctuation in LLL and BW in menstruating female subjects as compared to the male subjects could be due to changes in tissue fluid composition. Recent evidence showing consistent patterns of perimenstrual growth hormone secretion (Thakore & Dinan, 1995) raises the possibility that some of these changes seen on knemometry might reflect subtle alterations in skeletal remodelling. This, however, can only be confirmed by further studies of short-term growth with simultaneous measurement of markers of growth hormone secretion and bone turnover.

In summary, changes in LLL are likely to be positively related to changes in body weight when the latter are only

modest in magnitude. However, greater sustained increases in weight are likely to have an opposite effect on LLL due to direct compression of the lower leg. Due consideration of body weight is essential in longitudinal studies of LLL changes, specially in conditions which are associated with significant changes in body weight.

Table 3.3. Intradaily changes in Lower Leg Length(mm) in adults in Group B. Subjects marked with an asterisk are female.

Subject	AM (1SD)	PM (1SD)	WSR
A *	405.5 (0.57)	404.7 (0.59)	p=0.009
B	439.5 (0.59)	439.6 (0.69)	NS
C	444.9 (1.09)	445.4 (0.59)	NS
D	424.6 (0.49)	425.1 (0.33)	p=0.04
E	445.2 (0.36)	445.4 (0.38)	NS
F	413.5 (0.63)	413.3 (0.24)	NS
G *	383.1 (0.29)	382.7 (0.29)	NS

Fig.3.2 LLLV(mm) and Body Weight(kg) changes in some individuals in Group A

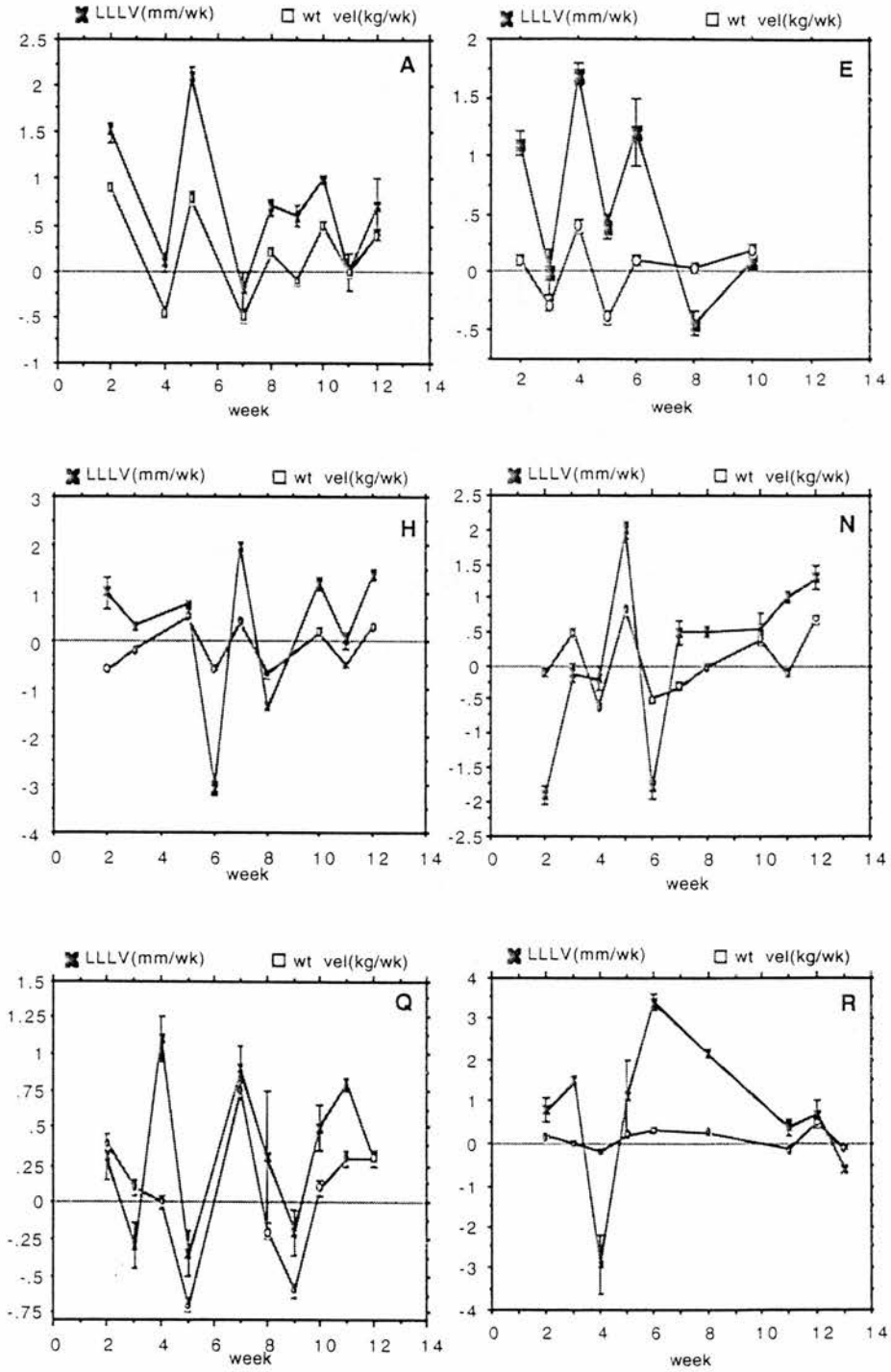


Fig.3.3 Correlation coefficients of the variables LLL(mm) and Weight(kg) in individual subjects A-Z in Group A. Rear darkly shaded bars represent value of the correlation coefficient(r^2) and front bars represent probability(p) value. The r^2 value of subjects with asterisk have a p-value less than 0.05.

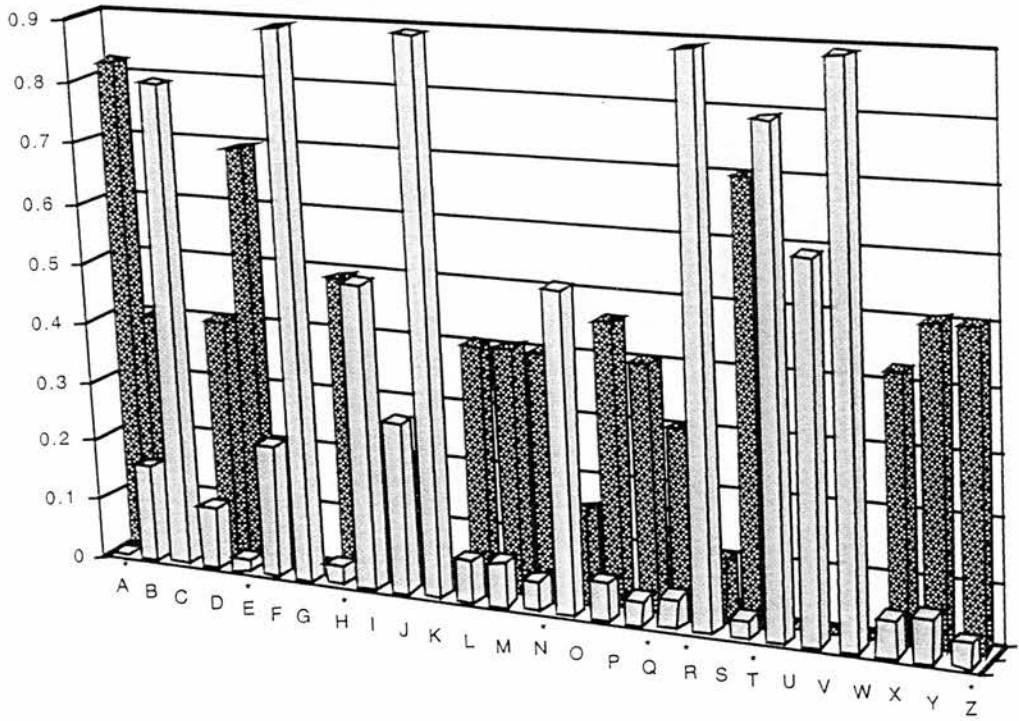


Fig.3.4 Scattergram of cumulative data from Group A showing the relationship between LLL(mm) and Body Wt (kg).

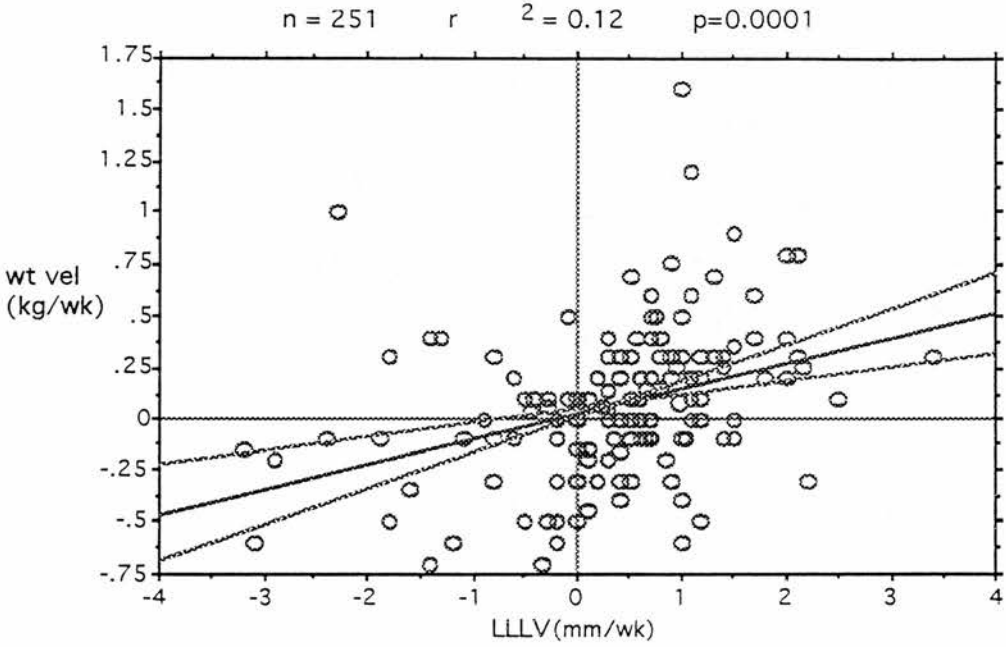
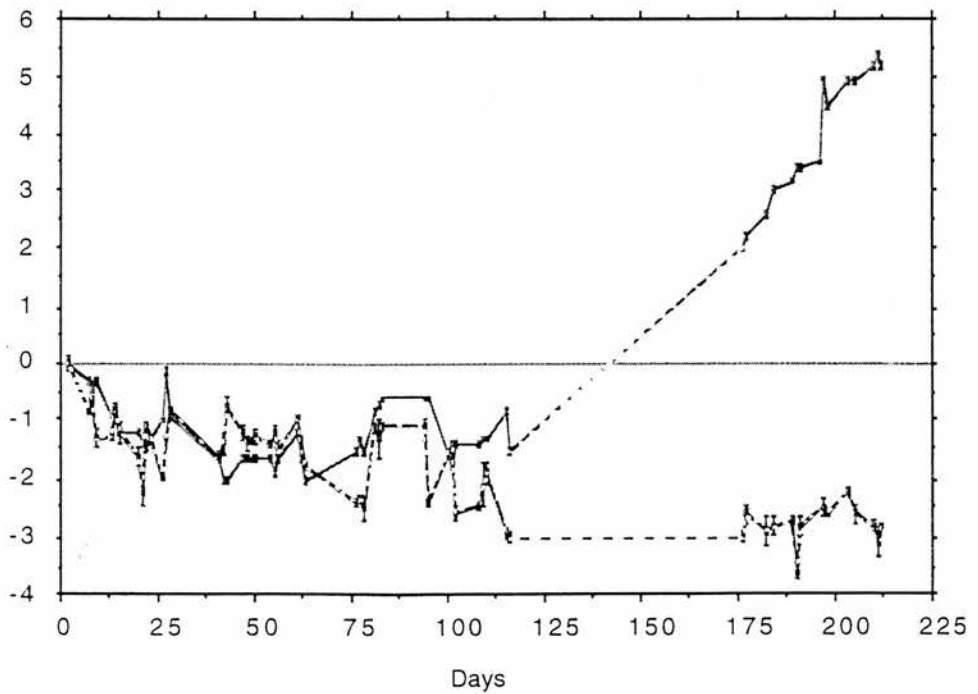
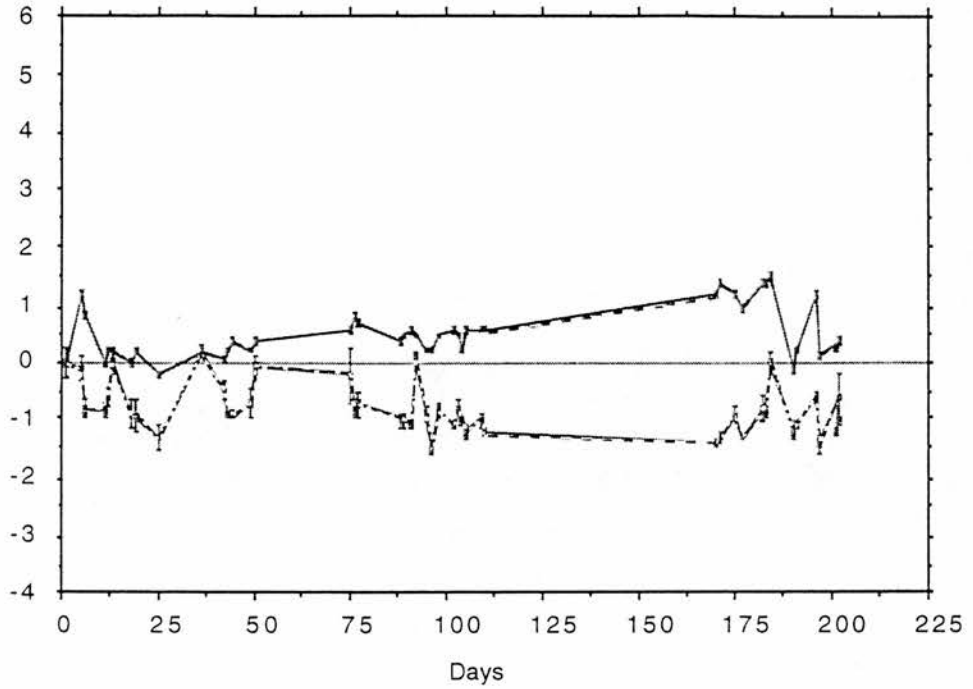


Fig 3.5 Changes in LLL(mm,broken line) and Body Weight(kg)from baseline seen in the pregnant (bottom panel)and the non-pregnant(top panel) subject in Group C.



Section 3. The Relationship of Short-term Changes in LLL and Urinary Growth Hormone Excretion

A. Introduction

Short-term growth studies have revealed an intradaily rhythm in LLL changes where the length is maximum in the morning and falls over the first half of the day reaching a plateau subsequently (Valk *et al*, 1983). This variability has been shown to relate to the duration of maintaining the erect posture and the reduction of LLL is felt to be due to weight-bearing on the compressible components of the lower leg (Hermanussen *et al*, 1988). Another possible factor contributing to this intradaily rhythm could be a diurnal variation in skeletal growth at the epiphyseal level related to GH secretion which is maximal during the night. Osteoblastic activity such as osteocalcin and PICP (carboxyterminal propeptide of Type I procollagen) display a circadian variation in serum concentration dependent on GH secretion (Saggese *et al*, 1994). uGH measurements may have a role in studying the physiology of growth and it has recently been shown that nightly uGH excretion can also vary in a pulsatile fashion (Thalange *et al*, 1996).

The aim of this study was to investigate whether the changes in LLL corresponded with changes in GH production as assessed by overnight uGH excretion.

B. Subjects & Method

Over a period of 4 weeks in June 1994, measurements were performed at the Royal Hospital for Sick Children, Edinburgh on every weekday in a group of 4 healthy children from a neighbouring school.

LLL was measured with the Valk childhood knemometer as described before (Chapter Two: Section 1(b)) by the Random Zero Method. Measurements were all performed by one measurer (SFA).

Subjects were instructed to refrain from any vigorous physical activity for 2 hours before the measurements and were allowed to miss any planned physical exercise at school. Careful record of their bedtime, rising time and general well-being was kept. All 4 subjects were recruited for a 4 week period with an option for carrying on for a further 4 weeks.

Urine for uGH assay was collected as a timed overnight sample over a 12 hr period. 0.1% BSA was added to the sample at the end of the 12 hr collection period. 1% Thiomersal was added to the sample at midday prior to freezing at -20°C . Samples were

analysed in subject batches at the end of the study. uGH was assayed using the Novo Nordisk Novoclone amplified enzyme immunoassay (between-batch CV <12% from 9-28ng/l).

C. Statistical Analysis

LLL was expressed in millimetres (mm) and LLLV was derived from the change in length from the previous day of measurement and expressed as mm/day (LLLVD^d). LLLV was also derived for the overall study period from the LLL at baseline and at the end of the study and expressed as LLLV^w in mm/wk. The precision of knemometric measurements was expressed as the TE representing 1SD from the mean of 3 repeated estimates of LLL obtained as described above. The total body height of the subjects was standardised for age and sex and expressed as Standard Deviation Scores (SDS). Considerable controversy exists as to the best method of standardising uGH levels (Moreira-Andres *et al*, 1993) and in this study they were expressed as total uGH excreted (uGH^t) over 12 hours (ng/12 hours) as well as the concentration of uGH (uGH^c) excreted (ng/l). Relationship between the two continuous variables of uGH excreted & LLLVD^d was assessed by calculating the correlation coefficient and the significance of any difference between groups was assessed by the Wilcoxon Signed Rank Test.

D. Results

Details of the 4 subjects are presented in Table 3.4. Two children were from the same family and two were in the same class at school. The children remained generally well during the study period and their bedtime and rising time in the morning did not vary by more than 1 hour. All 4 children declined the offer to continue for a further 4 weeks and the primary deterrent was the restriction of physical activity.

Knemometry was performed on every weekday and, therefore, on 20 occasions in each child. The mean TE of the measurements was 0.18mm (95%CI,0.16-0.22). The mean LLL fluctuation which represents the amount of change of LLL irrespective of whether it is an acceleration or a deceleration was 0.37mm (95%CI, 0.29-0.45) and significantly higher than the mean TE ($p=0.0001$).

Overnight samples for uGH assay were collected on 18 occasions by subject A and on 17 occasions by B, C & D. As a group the children had a mean LLLV^w of 0.52mm/wk (range,0.38-0.78) and a mean LLLV^d of 0.08mm/day (r,0.01-0.18). Subject B who was the tallest of the four subjects (Table 3.4) had the highest LLLV^w as well as LLLV^d but this was not significantly different from the rest of the group (Fig.3.6). Mean uGH^c for the group was 8.9ng/l (r,3.9-13.3) and the mean uGH^t was 1.8ng (r,1.1-2.4). uGH excretion varied widely between, as

well as within, the subjects (Fig.3.7). Subject B had the lowest uGH excretion and his excretion pattern was the most consistent as suggested by the small confidence limits in Fig.3.7.

Although the pattern of variation of uGH excretion between the subjects was similar when excretion was expressed as concentration or absolute amount of uGH, the differences between children were more significant when the former method was employed (Fig.3.7). The mean day-to-day Coefficient of Variation of uGH^C for the whole group was 55% (r,32-93) and 56% (r,38-89) for uGH^t.

Fig.3.8 shows the temporal relationship between uGH^t and LLLV^d. Although there was no significant correlation between these two variables, visual examination of the patterns shows a possible temporal association at times in Subjects A, C & D. The mean 12hr uGH^C amounted to 1.5ng (95%CI,1.2-1.8) when the LLLV^d was negative and 1.9ng (95%CI,1.4-2.4) when the LLLV^d was positive in nature; these differences did not reach significance.

E. Discussion

This preliminary study investigating the relationship of short-term growth patterns to overnight GH secretion as assessed by uGH levels was limited to 4 weeks primarily

because the children felt that restriction of their physical activities for the first half of the day before knemometry was a major hindrance. Nevertheless the study provided some important information about the parameters assessed.

The knemometer can be used to assess daily fluctuations of LLL in healthy children as significant LLL changes were evident from day to day in all four subjects. However, no discernible pattern of change was evident over the four weeks. As the leg incorporates soft-tissue as well as bone, one can speculate that the changes seen in LLLV^d are most likely to represent a combination of changes of which some, such as hydration, are transient and some, such as epiphyseal growth, are permanent. It is for this reason some researchers have advocated the use of the term "fluctuations" rather than "velocity" to describe the changes in LLL when assessed over a short period (Hermanussen, 1988).

Our study reinforces the view that healthy children can excrete a highly variable amount of uGH from day to day. This variability was also evident in Thalange *et al*'s study (1996) but interestingly they only rarely encountered a value below their cut-off value for differentiating normal children from those with GH deficiency. Previous studies at our centre using the same uGH assay have shown that timed overnight uGH^c reflected nocturnal plasma GH profiles (Stirling *et al*, 1993).

These studies have also provided a cut-off value of around 9ng/l (Stirling, personal communication) with some overlap as encountered in previous studies (Moreira-Andres *et al*, 1993). It is, therefore, interesting to see that Subject B who was growing the fastest had consistently low levels of uGH excretion and was constantly below our present cut-off value for "normality"; subjects A and D also had a substantial number of uGH values below 9ng/l (Fig.3.7).

Serial values of uGH can be highly variable and although this may be due to true biological variability in GH secretion or bioavailability controlled by proteins such as the IGFbps (Blum & Ranke, 1993), the variability can also be artefactual due to alterations in renal clearance of GH (Skinner *et al*, 1993) or inconsistencies in handling the urine sample. All the subjects and their parents were instructed on the collection method and had instruction sheets with the collection kits. Following the uGH assays the participants were again contacted to check their technique and this was recalled correctly by all four. Urinary creatinine was not measured; although there was some variation of body height amongst the subjects and Subject B with the lowest uGH levels was the tallest, all the children were prepubertal.

Expression of uGH data as concentration or as absolute mass did not alter the coefficient of variation of repeated

samples in our group of subjects. Expression as mass did, however, reduce the degree of intersubject variability.

There was a temporal relationship between uGH excretion and the nature of LLL fluctuation (positive/negative) but there was no correlation between daily LLLV and uGH levels. The former might be purely due to chance as both indices vary considerably from day to day. A conclusive evaluation of this relationship as well as detection of any rhythms requires a much longer and, therefore, a more demanding study with about 90 pairs of data-points per subject.

Renal handling of GH which can be assessed by measuring urinary proteins such as beta-2 microglobulin was not assessed in this study. It is unlikely that our group of subjects would have had any significant abnormalities of renal function but day-to-day variation of function might explain some of the variation in uGH excretion. The study of other markers such as urinary IGFI could also shed more light on the observed LLL changes. This study has shown that there is considerable day-to-day variability in LLL as well as overnight uGH excretion and the magnitude of uGH excretion does not correspond to the magnitude of LLL fluctuation. uGH values should be interpreted with considerable caution and always in the context of clinical data.

Table 3.4. Description of Subjects

Subject	Sex	Age (years)	Ht SDS	Comments
A	M	10.5	1.5	-
B	M	11.2	1.9	In same class as D
C	M	8.3	1.3	Brother of D
D	F	11	1.5	Sister of C

Fig.3.6. Scattergram of the mean LLLV^d of the 4 subjects (A,B,C,D). Error bars denote 95%CI.

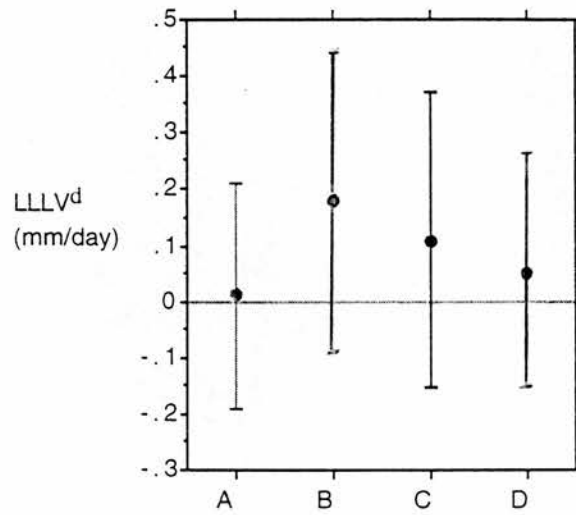


Fig 3.7 Scattergram & mean uGH values of the 4 subjects (A,B,C,D). Error Bars denote 95%CI.

*- $p < 0.05$, **- $p < 0.005$. On the left, uGH excretion is expressed as ng/l & on the right as ng/12hrs.

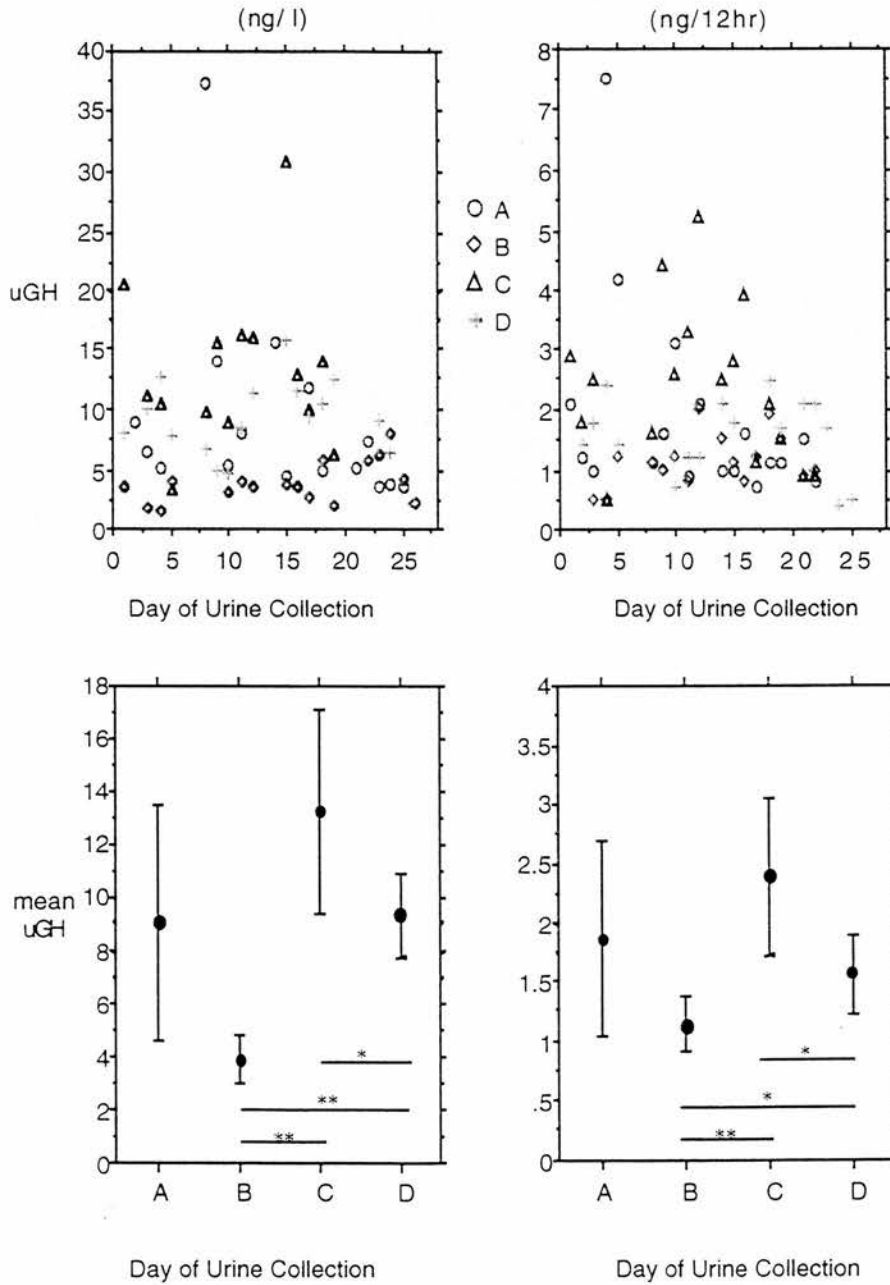
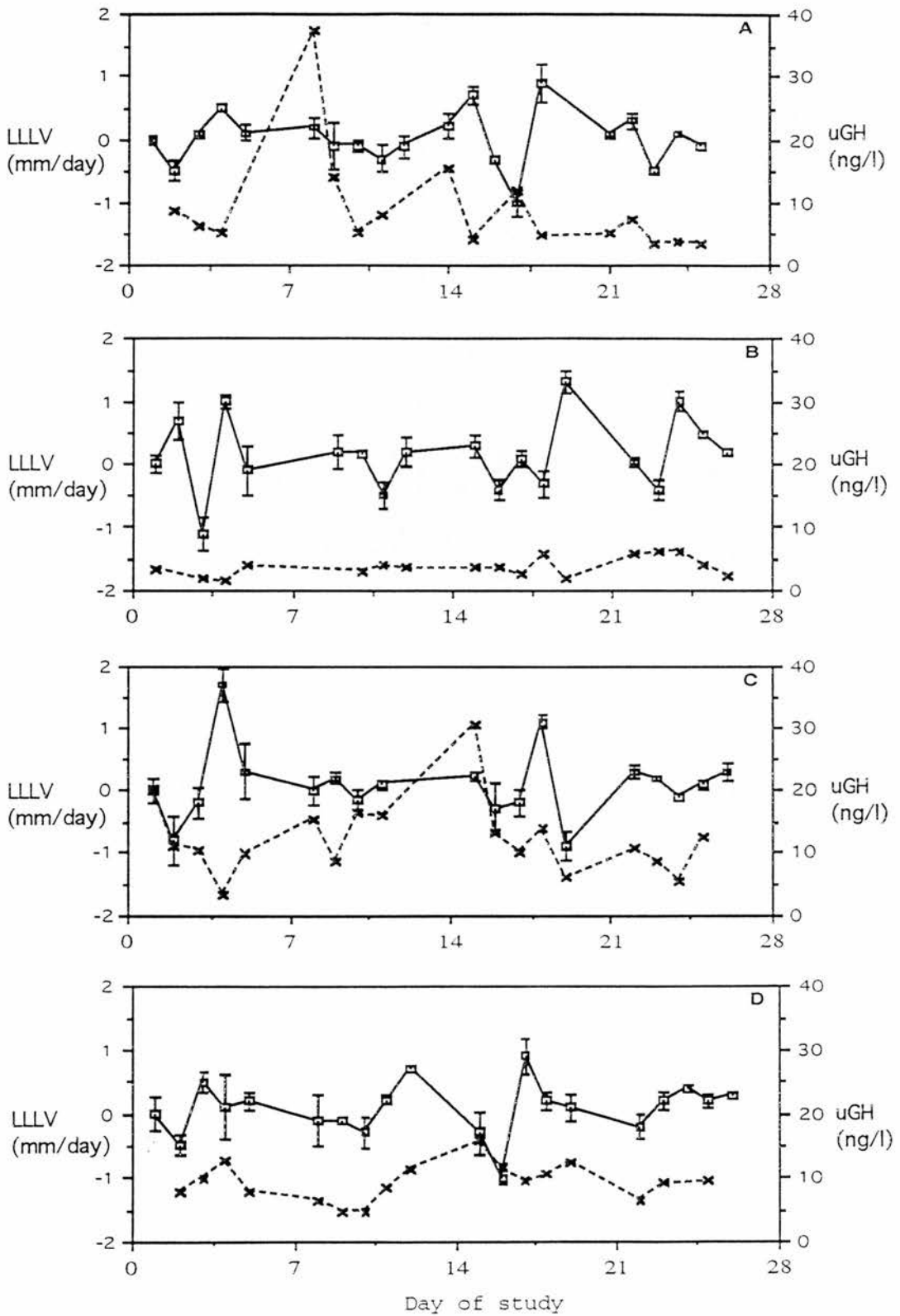


Fig.3.8 The temporal relationship between LLLV^d(solid line) and uGH values(broken line). LLLV^d error bars denote TE(mm)



Section 4 Pattern of Growth in Healthy Children

A. Introduction

In the area of growth patterns there are four different hypotheses under debate. Firstly, there is the model of saltation and stasis, which is pulsatile growth on a background of variable durations of stasis (Lampl et al, 1992, Lampl et al, 1995). Secondly, a model of smooth continuous growth that proceeds by approximately equal daily increments has been proposed (Preece & Baines, 1978). The third model proposes that growth occurs periodically by constant amplitude increments after equal stasis durations (Klein et al, 1994, Heinrichs et al, 1995). The final model hypothesises that growth occurs in a mathematically chaotic pattern (Wales & Gibson, 1994). Studying the pattern of short-term growth in healthy children would allow better interpretation of patterns encountered in disease states and following potentially growth promoting or suppressive effect of drugs. As attention is now being paid to short bursts of hGH treatment in some cases (Kelnar & Tanaka, 1994), time-intensive studies might help to direct such treatment to appropriate phases of growth or stasis.

B. Subjects & Methods

Between August 1993 and June 1994, 30 healthy pre-pubertal children aged 4.2-10yrs (M:F,12:14) had lower leg

measurements by one observer at the RHSCE. 26 children were measured at the same time of the day, on a weekly basis for 12 weeks (Group A). 4 children were also measured daily (except weekends) for a period of 4 weeks (Group B). LLL and weight was measured as described in Chapter Three, Section 2 (B).

C. Statistical Analysis

LLL changes were expressed as LLL velocity (LLL_v) which represent the relative amount of change that can be detected between consecutive measurements. In Group A, the changes were expressed over weekly intervals and in Group B changes were expressed over daily intervals. Some Group A measurements were performed at 2-weekly intervals. In Group B data analysed for assessing change consisted only of measurements performed on consecutive days. Precision of knemometric measurements was expressed as TE. Normally distributed data was described by mean and 95% Confidence Interval (95%CI) whereas skewed data was described by median value and 5th and 95th Percentile values (P₅,P₉₅). Patterns of growth were assessed by polynomial regression analysis as well as frequency distribution of growth velocities.

D. Results

All the children studied remained well over the duration of the study. The median age of the children was 8.7 (range,

4.2-10). Number of measurements performed in Group A was 223; the mean LLLV was 0.39mm/wk (95%CI,0.26-0.52). Number of measurements performed in Group B was 57; the mean LLLV was 0.5mm/day (95%CI,-0.13-0.20). The median TE was similar in both groups at 0.15mm (P10-0.06,P90-0.35). In both groups LLLV had a normal distribution around a mean value (Fig.3.9).

There was considerable variation between subjects in the pattern of growth observed with clear periods of slow and fast growth (Fig.3.10) as well as times when "negative growth" or shrinkage was evident. In Group B, who had daily LLL measurements, the pattern of growth was similar to that in Group A (Fig.3.11 & Fig.3.12).

To check for any seasonal variation the LLLV of all the children were grouped according to the month of measurement (Fig.3.13). Although there was substantial variation, none of the differences were significant. When the LLLV for the months September to May were grouped into 3-monthly "seasons" (autumn,winter,spring) there was still no statistically significant difference between the groups although the median LLLV was lower in winter at 0.5mm/wk (P5,P95,-0.6,1.4) compared to 0.6mm/wk (P5,P95,-1.4,1.9) and 0.6mm/wk (P5,P95,-1.3,1.2) in autumn and spring respectively (Fig.3.14).

E. Discussion

It was clear from this study that the pattern of growth is highly variable in healthy children. The children in Grp A did not follow the same pattern of variability. Daily measurements in a small group of children for a 4 week period did not make the patterns any clearer or provide any uniformity. It was, however, interesting to note that the cumulative LLLV values for the respective groups were normally distributed around a mean; the mean LLLV was comparable to that cited in previous studies (Valk *et al*, 1983).

The wide variability in the pattern of LLL changes in the process of linear growth emphasises it's non-uniformity. Some of the patterns could be classified as saltatory and others as continuous but there was no consistency and a large number could not be classified into either. Evaluation of the chaos model of growth (Wales & Gibson, 1994) would have required a greater number of consecutive measurements in the same individual. Patterns of growth are strongly dependent on the method of analysis as recently suggested by Johnson *et al* (1996) who found that data collection frequency, measurement error and total study duration all determine the frequency distribution of growth velocities. The debate regarding the pattern of growth is based on studies which have been performed not only in children of

different ages but also in different species. Evaluation of growth patterns can only occur when these factors have been standardised.

There was no clear evidence of seasonal variation amongst this group of subjects. However, the slight reduction in median LLLV in the winter is in accordance with previous studies investigating seasonal variation in growth (Gelander *et al*, 1994).

It was, however, clear from this study that non-uniformity is the rule in the normal growth process and predicting periods of stasis and growth in a healthy child is not possible. It also makes it more likely that any changes which are observed to occur in a uniform fashion in a group of children could be attributed to coincident external factors such as disease states or drugs.

Fig.3.9. Frequency Distribution of LLLV in Group A (top panel) and in Group B (bottom panel).

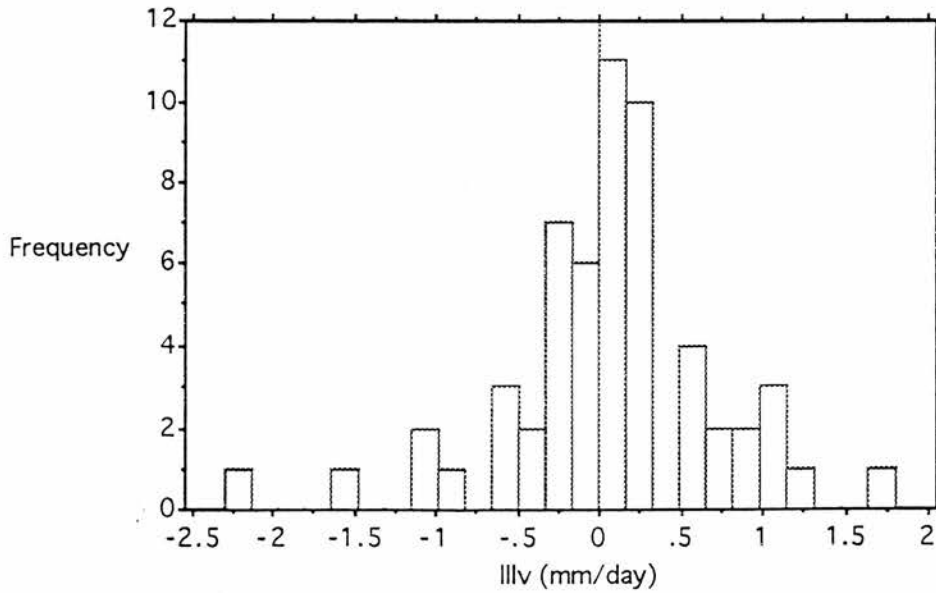
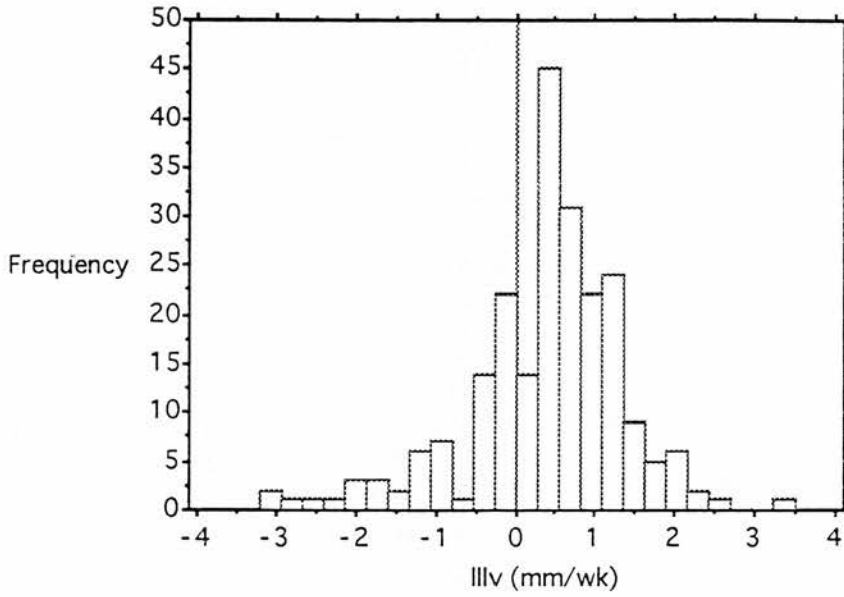


Fig 3.10 Pattern of LLL growth in 18 subjects (A-R). Horizontal Axis-Weeks of Study; Vertical Axis-LLL(mm); Error Bar-Technical Error of measurement.

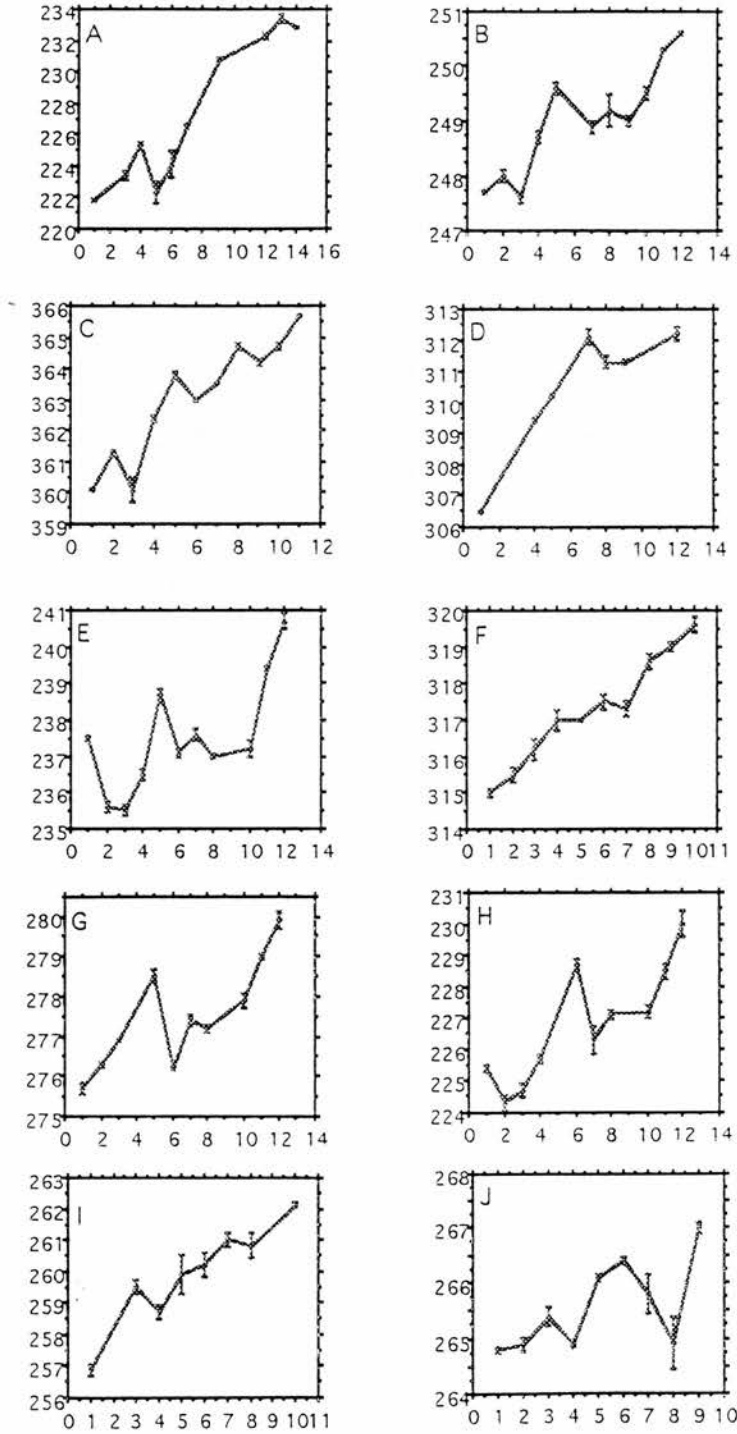


Fig 3.10 Pattern of LLL growth in 18 subjects (A-R). Horizontal Axis-Weeks of Study; Vertical Axis-LLL(mm); Error Bar-Technical Error of measurement.

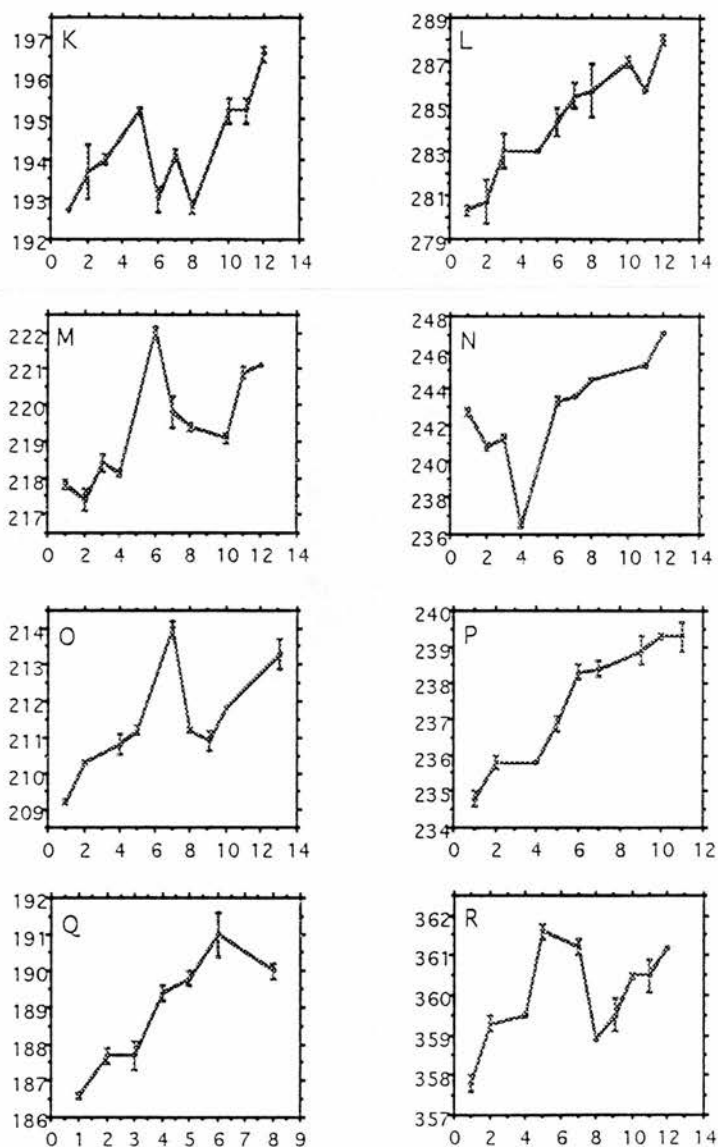


Fig.3.11 Pattern of LLL growth in subjects in Group B. Horizontal axis-week of study; vertical axis-LLL(mm); error bar-TE.

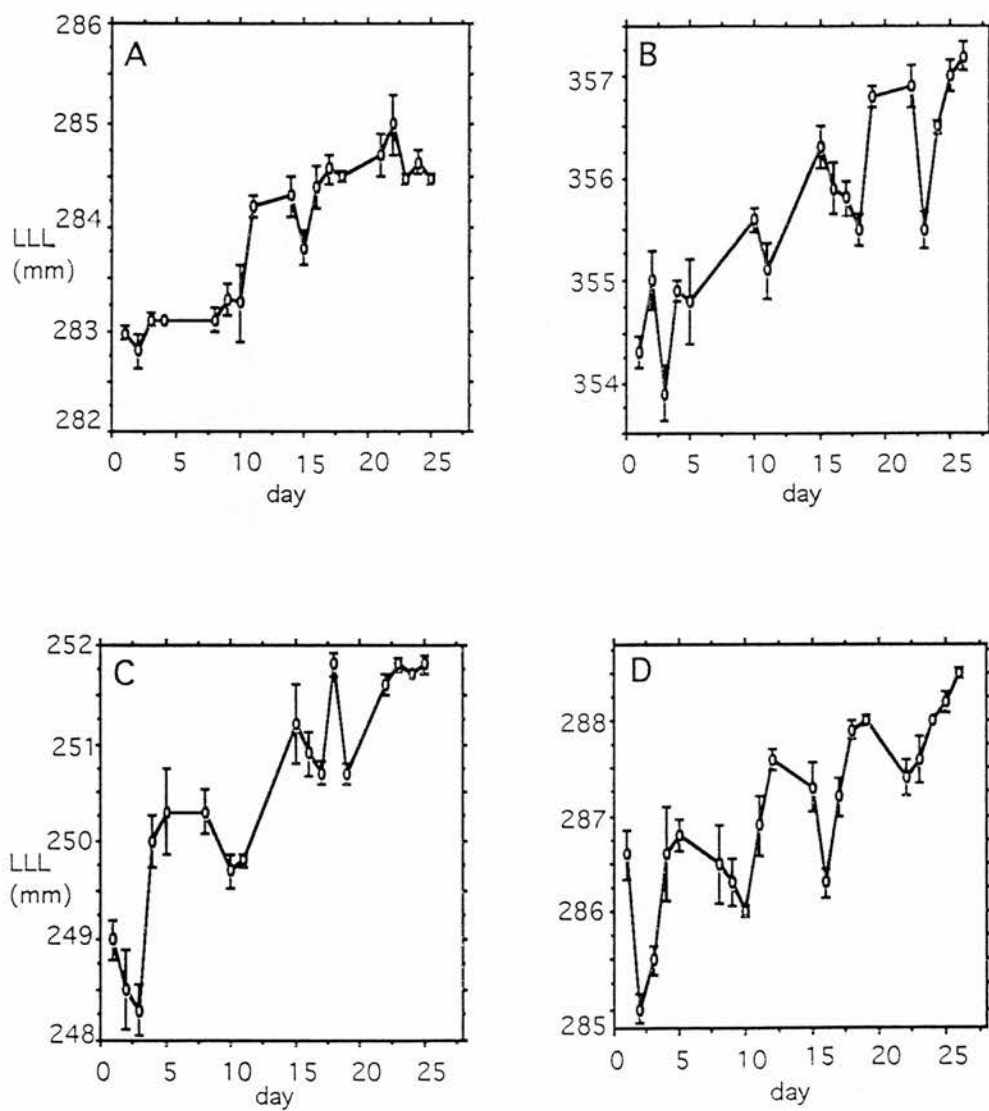


Fig.3.12 Growth Curve of best fit for subjects in Group B using third/fourth order polynomial regression.

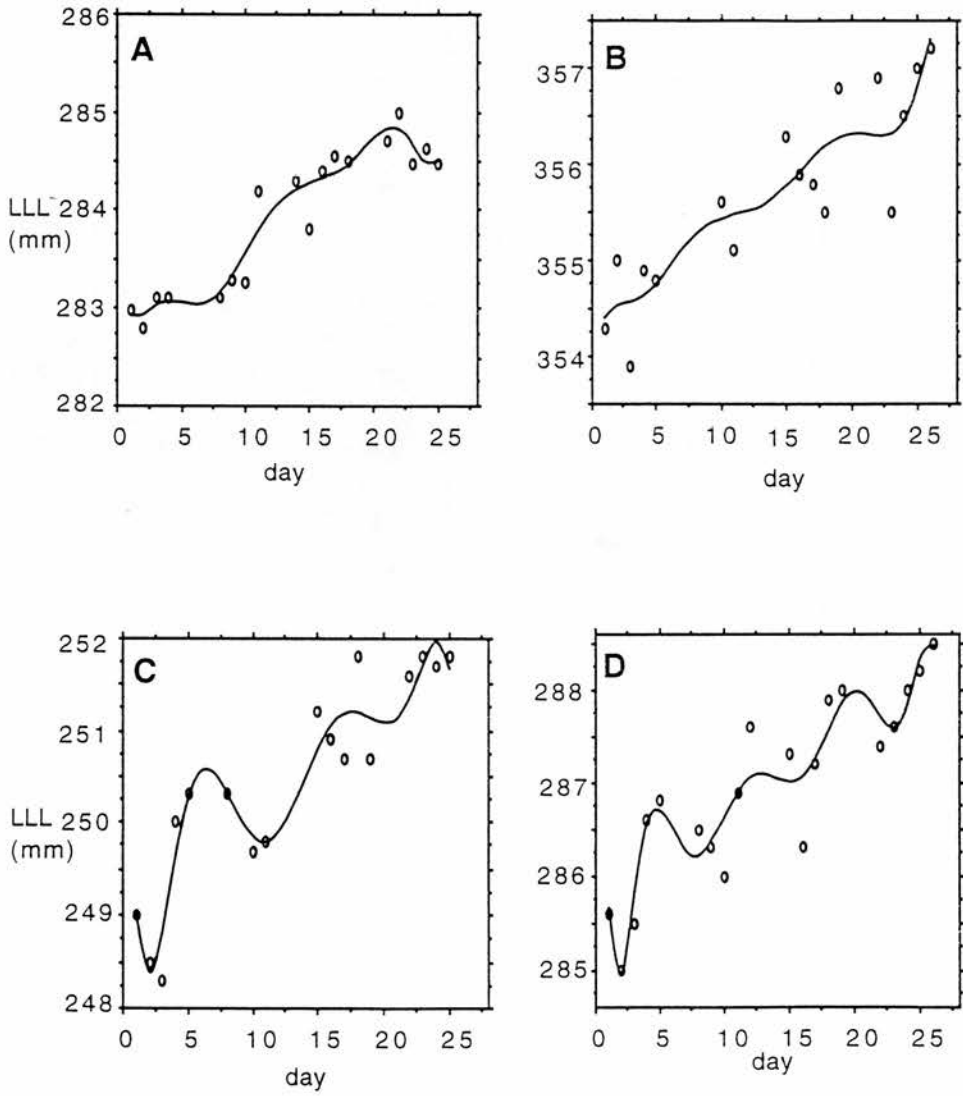


Fig.3.13 Median LLLV with 95th percentile values in Group A according to month of measurement.

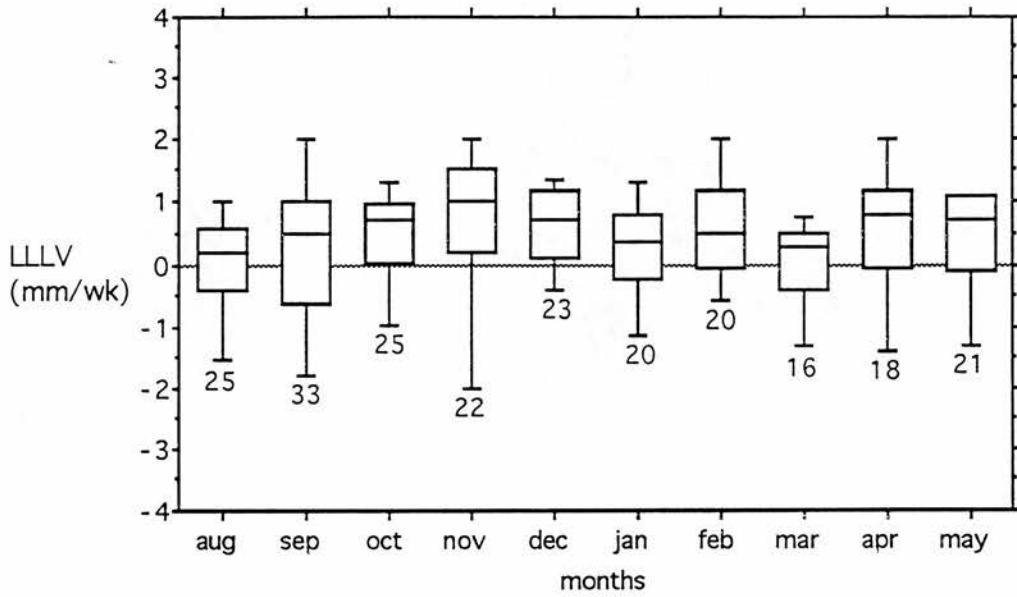
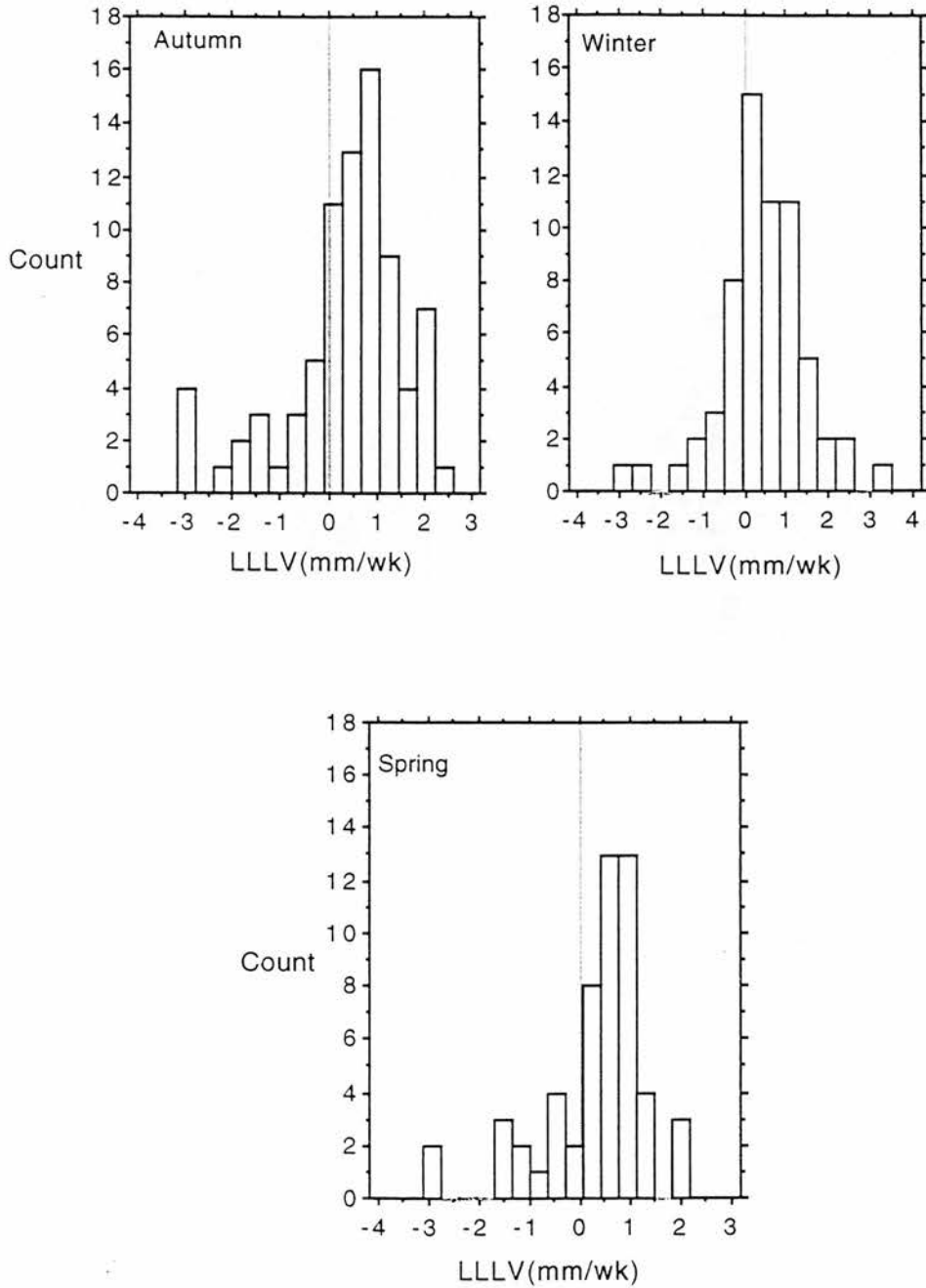


Fig.3.14 Frequency distribution of LLLV according to season in Group A.



CHAPTER FOUR

STUDIES OF SHORT-TERM GROWTH IN CHILDREN ON
CYTOTOXIC CHEMOTHERAPY

This chapter will first present a study performed to investigate the changes in Height, Weight and Sitting Height in a group of children receiving cytotoxic chemotherapy as per the present Medical Research Council(UK) protocol for treatment of Acute Lymphoblastic Leukaemia(UKALLXI)(Section 1); Section 2 presents the changes in LLL in a sub-group over the whole period of chemotherapy. As it is possible that some of the effects on short-term growth are due to the anti-mitotic effects of chemotherapy; this section will also investigate whether there is a relationship between lower leg growth and the neutrophil count. The final section will present a study looking at changes in markers of bone and collagen turnover and growth hormone secretion in relation to each other as well as to LLL changes.

Section 1. Anthropometric study of children during intensive chemotherapy for acute lymphoblastic leukaemia

A. Introduction

The effects of cranial irradiation on growth in children requiring treatment for ALL have been extensively studied and are well documented (Wallace & Shalet, 1992). In UKALLXI, CNS-directed therapy is delivered in the form of repeated intrathecal methotrexate with or without high-dose intravenous methotrexate (itMtx & ivHDMtx) for the majority of patients. There is evidence that CT per se has an adverse effect on growth (Clayton *et al*, 1988); however, it is still not clear whether these effects are temporary and whether recovery from the growth suppressive effects can occur during the period of CT (Holm *et al*, 1994, Hokken-Koelega *et al*, 1993, Katz *et al*, 1991, Moell *et al*, 1988, Wells *et al*, 1983, Hakami *et al*, 1980, Sunderman & Pearson, 1969). There have been no reported studies of the growth profile of children on UKALLXI. Concern has also been raised about altered differential skeletal growth (Makiperna *et al*, 1990, Davies *et al*, 1994) and body disproportion, as well as obesity (Odame *et al*, 1994) in children following completion of CT which has included cranial irradiation.

This study prospectively documents anthropometric changes which occurred in a group of children treated by a chemotherapy-only regimen for ALL.

B. Subjects and Methods

31 children (M:F-20:11) were on UKALLXI over the study period spanning January 1993-December 1995. From this group 2 pubertal boys, 1 boy with Downs Syndrome and 2 girls and 1 boy who received cranial irradiation were excluded. By the end of the study 16 children had completed the 2-year course of CT and remained pre-pubertal. Median age at diagnosis was 4.4yrs (range,1.1-14). Median duration of study was 18 months (r,3-24). The treatment regimen which the 25 children received is detailed in Table 4.1.

C. Statistical Analysis

Ht and SH were measured monthly(at the beginning of each month) by one of two observers with a stadiometer. The SH:Ht ratio was also calculated for each pair of measurements to assess any changes in body disproportion. SH measurements are presented in only 16 children; the remainder were either too young or did not have a baseline SH measurement. Wt was measured in standardised clothing on the same Avery scales throughout the period of study. The Body Mass Index BMI was calculated as Wt/Ht^2 (kg/m^2). The data were converted into SDS and presented as median change in SDS, with 5th and 95th

percentile values, from a baseline measurement (Δ SDS, P5 & P95). Conversion of data into SDS was performed with reference to L, M & S values obtained from the Child Growth Foundation (Freeman *et al*, 1995, Cole *et al*, 1995). Changes from baseline were tested by WSR analysis for significance.

D. Results

Height - Fig.4.1(a)

Median Δ SDS-Ht reached a nadir at 6 months after the start of CT with a value of -0.35 (P5, -0.7 ; P95, -0.1) $p=0.001$. Subsequently Δ SDS-Ht remained significantly depressed until the end of the first year after which it returned to baseline values.

Weight - Fig.4.1(b)

Median Δ SDS-Wt rose significantly over the first month by a median value of 0.15 (P5, -0.15 ; P95, 0.5) $p=0.03$. After that initial rise over the period of induction, Wt-SDS was not significantly different from baseline until the second year of CT. By the end of CT, median Δ SDS-Wt was just significantly raised at 0.5 (P5, -0.3 ; P95, 1.0) $p=0.04$.

Body Mass Index - Fig.4.1(c)

Median Δ SDS-BMI increased at induction to 0.5 (P5, -0.25 ; P95, 1.64) $p=0.002$. Median Δ SDS-BMI was also elevated later in the 10th month of CT at 0.75 (P5, -0.39 ; P95, 1.55) $p=0.01$.

Sitting Height - Fig.4.1(d)

As with Ht, an initial decline is seen in SH followed by a recovery which seems to be complete by the end of the first year. Maximum decline was evident at 3 months with a median Δ SDS-SH of -0.55 (P5, -0.7 ; P95, -0.2) $p=0.02$.

Skeletal Disproportion - Fig.4.2

Maximum decline of the SH:Ht ratio was evident at 3 months with a median Δ SH:Ht of -0.01 (P5, -0.007 ; P95, 0) $p=0.009$. An increase from baseline was noticed at 8 months with a median Δ SH:Ht of 0.003 (P5, 0 ; P95, 0.01) $p=0.04$. By the end of CT there were no significant changes from baseline.

Effect of Alternative CT Regimens - Fig.4.3

No significant anthropometric differences were observed between children separated according to the form of CNS-directed therapy received or whether they received the Third Intensification block of treatment or not. The number of children in these subgroups was, however, small for adequate statistical analysis (Table 4.1).

D. Discussion

A general decline in growth was evident over the first year of CT but which improved over the second year. The initial weight gain in the face of reduced growth is most likely due to corticosteroid therapy during induction and as a

consequence leads to a significant increase in the BMI. Over the next few months, body weight is not significantly different from baseline but height does remain adversely affected after reaching a nadir at six months after which CT becomes less intensive. The slow recovery in height with a satisfactory weight gain could explain the elevation in BMI in the latter half of the first year. Although the initial decline in height is consistent with that found in other studies of children with ALL receiving CT-only regimens, few studies have shown adequate catch-up growth during the period of CT (Sunderman & Pearson, 1969, Wells *et al*, 1983, Katz *et al*, 1991).

Corticosteroids probably play an important role in the early phase of growth retardation acting, locally on bone growth (Baron *et al*, 1992), directly on the growth plate (Brown *et al*, 1990), centrally on growth hormone secretion (Giustina *et al*, 1990, Marky *et al*, 1991) as well as by affecting calcium homeostasis (Gennari, 1994). However, as discussed in Chapter One, Section 3, almost all the cytotoxic agents included in the CT protocol can to some extent penetrate the blood-brain-barrier or have indirect effects on the CNS; Mtx can have direct effects on bone as well. ivHDMtx has been associated with lesions in the CNS, the significance of which is unclear.

The poor growth after the induction and first intensification could be due to the CNS-directed therapy. Whether ivHDMtx has a more profound effect on growth than repeated itMtx alone was not clear from our data and needs further investigation. The growth retardation seems to be reversible, reflecting the results of recent studies performed by Moell & Garwicz (1995) showing short-term suppression of growth in rabbits injected with HDMtx.

Our study also revealed an initial transient deceleration in sitting height which was accompanied by a reduction in the SH:Ht ratio. This strengthens the hypothesis that the spine with its multiple growth plates plays a major part in growth deceleration when exposed directly to cytotoxic CT. Unlike Davies *et al* (1994), our group of children did not receive cranial irradiation and have not reached final height. It is, however, encouraging to note that there were no significant differences in body proportion from baseline at the end of the two year course. It is possible that more accurate and precise short-term measurements of LLL might provide a better insight into these growth patterns.

In summary, it seems that UKALLXI CT for ALL affects growth but there is evidence of recovery before the completion of treatment. Further study of this cohort of children is needed to ensure that growth remains unaffected in the long-

term and an exploration of the underlying physiological mechanisms of growth decline due to CT is also necessary.

Table 4.1. The distribution of children receiving the various forms of chemotherapy within UKALLXI.

Type of CNS-directed therapy	3rd Intensification	No 3rd Intensification	Total
Intrathecal Methotrexate only	4	5	9
Intravenous HD Methotrexate and Intrathecal Methotrexate	7	9	16
Total	11	14	25

Fig.4.1 Median change in (a)Height SDS, (b)Weight SDS, (c)BMI SDS, (d)Sitting Height SDS, from baseline in all prepubertal children receiving chemotherapy only. Boxes denote interquartile ranges *-p<0.05, **-p<0.005 (WSR) compared to baseline. Figures below the boxes represent number of measurements.

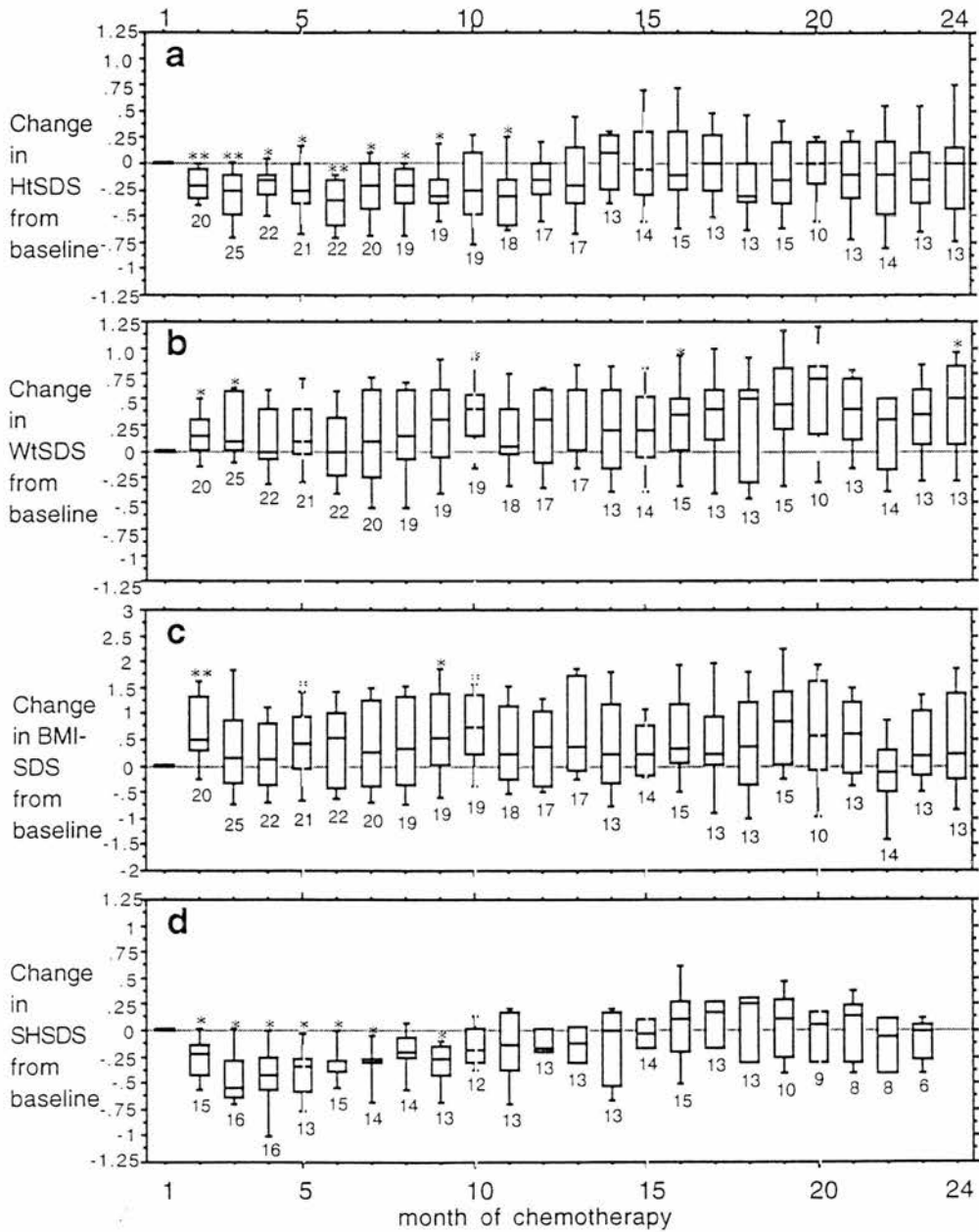


Fig 4.2 Median change in SH:HT ratio from baseline in all prepubertal children receiving chemotherapy only. Boxes denote interquartile ranges. *-p<0.05, **-p<0.005 (WSR) compared to baseline. Figures below the boxes represent the number of measurements.

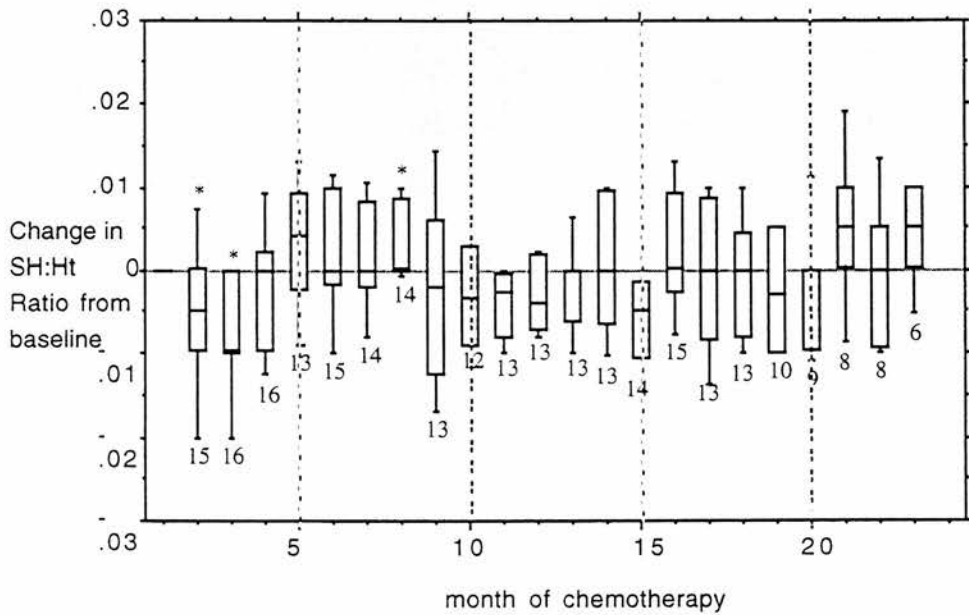
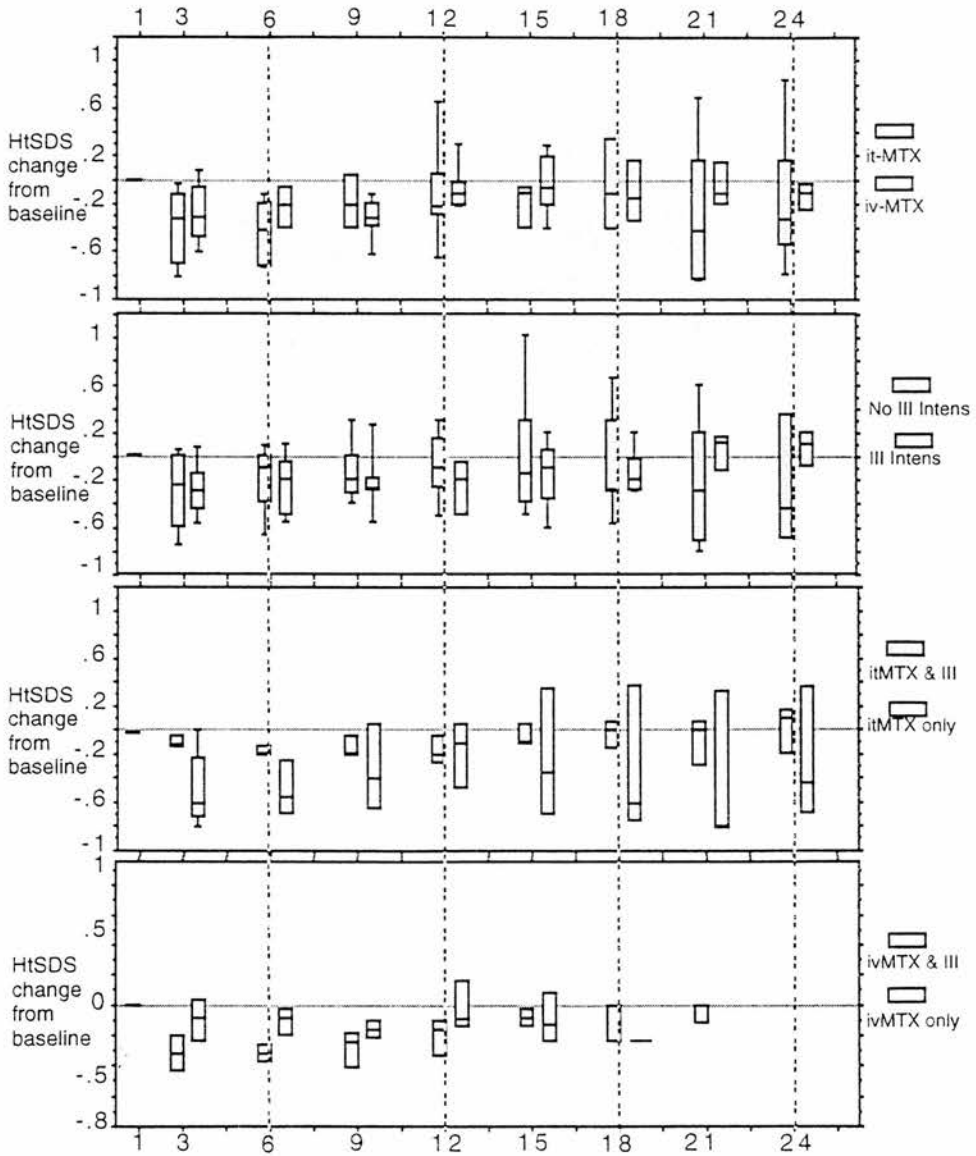


Fig 4.3 Median change in Ht-SDS from baseline in children according to type of CNS-directed therapy (itMTX-continuing intrathecal methotrexate; ivMTX-intravenous high dose methotrexate) and whether they received the 3rd intensification(III). Results at 3,6,9,12,15,18,21 and 24 months presented.



Section 2 LLL and neutrophil counts in children on cytotoxic chemotherapy

A. Introduction

This study documents the effect of UKALLXI on short-term growth during treatment by prospectively measuring LLL in a sub-group of children from that studied in the previous study (Chapter Four; Section 1). After initial presentation, the neutrophil count in children with ALL reflects the degree of myelosuppression while on CT and therefore acts as a readily measurable indirect indicator of the bioeffectiveness of the CT and a relationship between changes in LLL and neutrophil counts at the time of febrile neutropenia has previously been reported by Davies at the meeting of the British Society of Paediatric Endocrinology in 1994 (Davies, 1995). The fever confounds the interpretation of the growth deceleration as the latter is observed in other healthy children who have a pyrexial illness (Wales & Milner, 1987). By evaluating children longitudinally over the CT period including periods of non-febrile neutropenia this study further enhances our understanding of the immediate effects of cytotoxic therapy on growth and investigates the potential for catch-up growth.

B. Subjects & Methods

Between January 1993-December 1995, 31 children (male: female, 20:11) with a median age of 3.5yrs (range, 1.1-14)

received treatment for ALL at the Royal Hospital for Sick Children, Edinburgh. 10 children were too young to co-operate with knemometry, 3 children received cranial irradiation and were excluded, 2 children only had incomplete data over the first 6 months of CT and one child refused to participate in the study. 15 children with ALL at a median age at diagnosis of 4.5yrs (range, 2.7-14) were therefore studied. The children were treated by the UKALLXI treatment protocol as shown in Figs.2.3-2.5. Each subject was randomised to receive either intrathecal methotrexate (itMTX) alone or high-dose intravenous methotrexate with intrathecal methotrexate (ivMTX) as CNS-directed therapy and to receive or not a third intensification block at Week 35 of treatment. Table 4.2 shows the outcome of the randomisation of the children in this study.

Knemometry was performed by the random-zero method by three trained individuals with a median TE of 0.15mm (5th & 95th percentiles, 0.06-0.35). In order to coincide the measurements with the clinic visits, knemometry was performed in the morning and at approximately the same time at every visit.

The children were divided into five groups according to when measurements were performed during their 24-month chemotherapy protocol. Group A (n=9) had knemometry during

months 1-6; Group B (n=6), months 1-12; Group C (n=4), months 1-24; Group D(n=9), months 21-27, i.e. 3 months before and 3 months after end of CT (Fig.4.4). Group E (n=11) consisted of children who had measurements performed anytime within the last 18 months of CT.

C. Statistical Analysis

LLLV was derived from either weekly or 2 weekly measurements of LLL; any data with an interval greater than 2 weeks were excluded from analysis. LLLV data on each child during any one month was averaged to provide a median LLLV of the child for that month of CT. Values are presented as medians with their 5th and 95th percentile values (P5,P95); within groups comparison was performed with the Wilcoxon Signed Rank (WSR) test. Neutrophil counts were transcribed from records held locally. Evaluation of any relationship between the neutrophil counts and LLLV was assessed by Spearman's Correlation Coefficient calculation. Neutrophil counts associated with febrile episodes were omitted from the data analysis.

D. Results

LLLV-All Subjects

A total of 681 measurements of LLL were performed; 67 measurements were omitted from the data analysis as they were performed at an interval longer than 2 weeks. The median LLLV

over the first 6 months of CT was 0.20mm/wk (P5,P95:-0.9,1.0). In Grp A, there was a significant rise in LLLV following induction of remission when median LLLV rose from 0mm/wk (P5,P95:-1.6,0.11) during Month 1 to 0.38mm/wk (P5,P95:-0.04,0.81) during Month 4 (p=0.01) (Fig.4.5). There was no further significant change in LLLV during CT although it tended to fall in Month 5 (2nd Intensification) (Fig.4.5). After CT, median LLLV rose from 0.46mm/wk (P5,P95:-0.02,0.79) in Month 23 to 0.84mm/wk (P5,P95: 0.72,1.12) (p=0.03) in month 25.

LLLV-itMTX vs ivMTX and Third vs No Third Intensification

There were no significant differences in median LLLV observed during treatment when the subjects were analysed according to the mode of CNS-directed therapy or according to whether they received the 3rd Intensification or not (Fig.4.6).

Neutrophil Counts and LLL

The median neutrophil count reached a nadir immediately following the 1st and 2nd Intensification blocks (i.e.Wk7 and Wk21) at $0.05 \times 10^9/l$ (range, 0 to $0.13 \times 10^9/l$) and 0 (range, 0 to $1.5 \times 10^9/l$), respectively. Although marked changes in neutrophil counts and LLLV were observed during the first 6 months of treatment we could find no significant relationship between the two parameters over that period. Over the following 18 months, which consisted mainly of continuation

CT but also included the period of Third Intensification there was a weak but significant positive correlation between the neutrophil count and LLLV ($r^2=0.2$, $p=0.0001$). During this period median LLLV was 0.2mm/wk (P5,P95: -0.15,0.5) when the neutrophil count was less than $1.0 \times 10^9/l$ and 0.65mm/wk (P5,P95: 0.1,1.0) when the count was above $1.0 \times 10^9/l$ ($p=0.01$) (Fig.4.7).

E. Discussion

Recent anthropometric studies performed during chemo-therapy only regimens for children treated for ALL have shown evidence of growth retardation occurring early during treatment. However, the point when recovery starts and whether this recovery is complete seems to be controversial. The previous study which examined height-SDS as well as Wt-SDS of a larger cohort of children (Chapter Four; Section 1), which included the subjects studied here, showed that UKALLXI had a temporary adverse effect on growth and improvement occurred within the chemotherapy period.

The study presented in Chapter 3 looking at LLL changes in healthy children did not reveal any recognisable pattern. Significant reductions in LLLV were seen over the Induction and First Intensification periods; although the LLLV was also low in the Second Intensification period this fall was not statistically significant. Some of these changes can be

related to changes in weight and body composition and the study subjects did gain weight over the period of induction; besides deceleration actual shrinkage in LLL was also documented in some of the subjects over this phase and some of this might be due to direct physical compression of the compressible parts of the lower leg.

It is likely, however, that part of the deceleration, as well as shrinkage, is due to the effect of CT. In this study the period of CNS-directed therapy did not seem to cause any immediate abnormalities in LLLV and no clear differences in growth were evident between the itMtx and hdMtx groups. The short-term suppression of growth in rabbits injected with hdMtx in the study by Moell & Garwicz (1995) did not fully recover over the study period. One previous study in children did not show any significant reduction in lower leg growth (Tammings *et al*, 1992).

The degree of catch-up growth documented in this group of children was impressive and emphasises the transitory nature of the impairment of normal growth mechanisms. Following the end of CT, the LLLV reached a supraphysiological level which would have been consistent with a period of catch-up growth but only if it had been preceded by a period of impaired growth (the classical definition of catch-up growth (Williams *et al*, 1974)). However, during the second year of chemotherapy

the LLLV was similar to that recorded for other healthy children. It is possible that different parts of the skeleton grow at different rates and that, although the lower leg was growing at a normal rate during continuation treatment, other parts such as the spine were not. Non-invasive methods of measuring vertebral spinal length and studies of bone & collagen turnover as well as GH secretion might shed further light on the changes seen in this study.

While surgery and some intercurrent illnesses have been shown to affect growth of the lower leg (Wales & Milner, 1987) no correlation between LLL changes and any specific parameters of illness have been described. We excluded possible LLL changes due to severe intercurrent illness by omitting knemometry data in children admitted with febrile neutropenia. The degree of neutropenia during continuation chemotherapy depends on the doses administered of 6mp and mtx and outcome of treatment appears more favourable when the combination is administered to the limits of tolerance as indicated by the development of neutropenia (Hale & Lilleyman, 1991, Pearson et al, 1991). While the retardation of LLLV in association with neutropenia could be due to the coexistence of a sub-clinical state of catabolism, it is also possible that 6mp and Mtx have a direct effect on growth. Mtx has previously been associated with osteopathy (Schwartz and Leonidas, 1984) and in-vitro studies have shown that 6mp can

alter hepatic IGF-I metabolism (Morris, 1981). Groot-Loonen et al (1995) recently suggested that the effect of 6mp and Mtx on growth may be independent of steroids. Considering the wide intra and inter-patient variability in drug disposition and bioavailability of these agents (Teresi et al, 1987, Lennard & Lilley, 1989), the neutrophil count most accurately reflects drug activity.

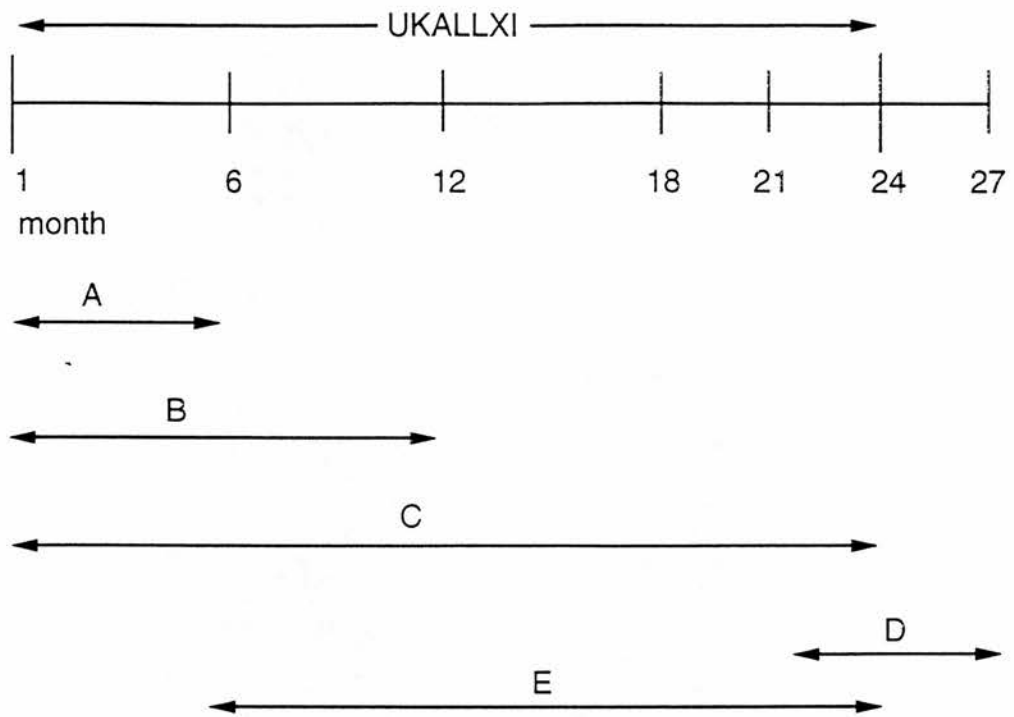
The positive relationship between LLLV and neutrophil count during the continuation CT period was not observed over the first 6 months of treatment. Neutrophil counts at presentation vary between patients and tended to rise (unlike LLLV) over the induction period before falling after first intensification. A larger combination of drugs are used during this period and prolonged courses of drugs such as high-dose corticosteroids probably affect growth via a number of different mechanisms.

Although the adverse effect on lower leg growth seems to be transient, knemometry cannot predict long-term growth and this cohort of children needs further follow-up to ensure that growth remains satisfactory. Further in-vitro as well as in-vivo studies need to be performed to study the effects of chemotherapeutic agents on longitudinal growth and bone turnover.

Table 4.2. The distribution of children receiving the various forms of chemotherapy within UKALLXI who had knemometry

	3rd Intensification	No 3rd Intensification	Total
itMTX	4	2	6
hdMTX	4	4	8
Total	8	6	14

Fig 4.4. Description of the five periods of study and the children studied in those periods.



Group	Number	M:F	Median Age(Range)
A	9	7:2	5.2 (4.4-14)
B	6	6:0	5.2 (4.4-14)
C	4	4:0	4.8 (4.4-5.2)
D	9	6:3	4.4 (2.7-8.5)
E	12	9:3	4.6 (2.7-14)

Fig.4.5 Monthly Median LLLV(mm/wk) (95th centiles) of children studied in Groups A, B,C and D.

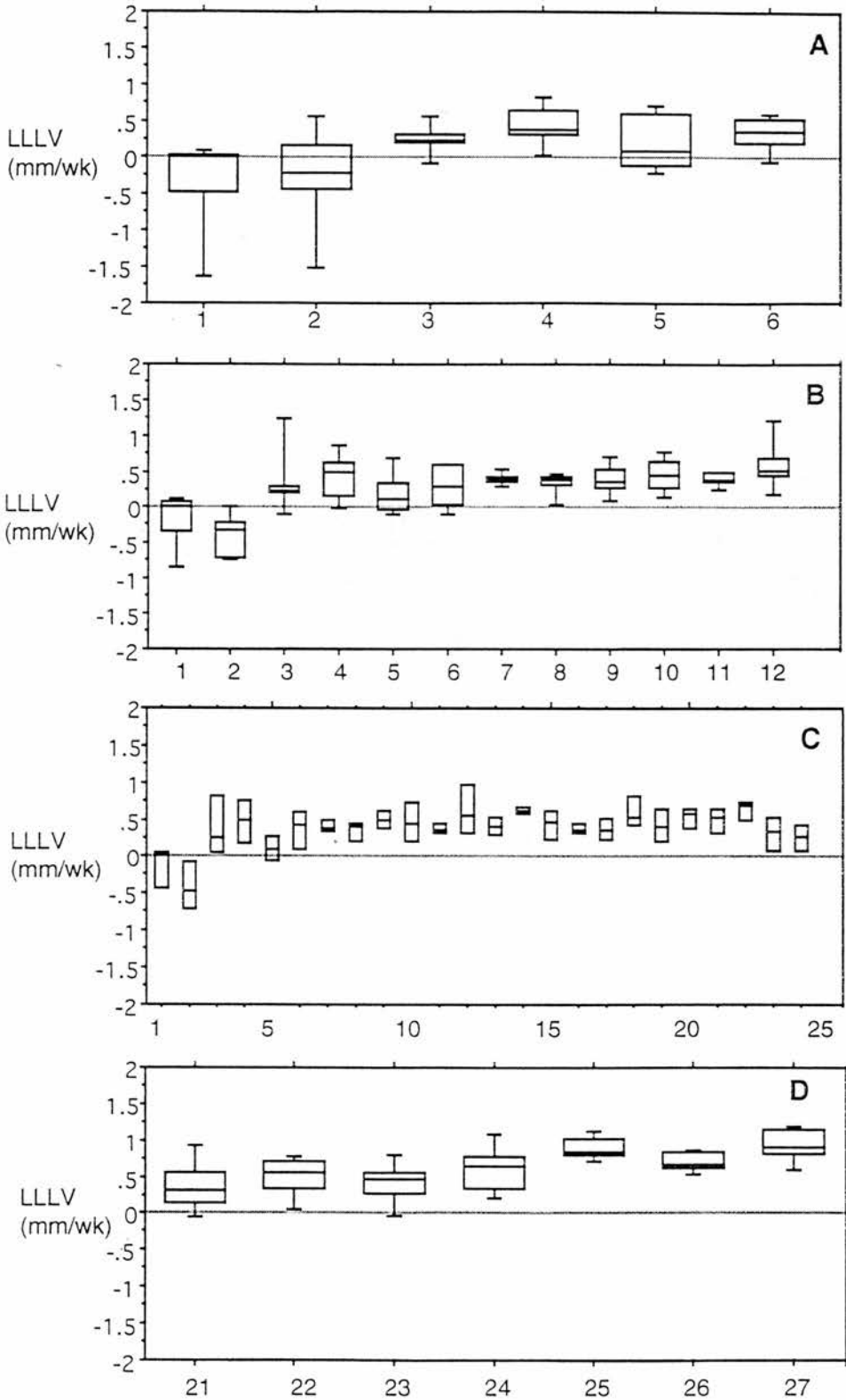


Fig.4.6 Median LLLV aggregate of each child (mm/wk) over CT. CNS-directed therapy(CNSDT) and the Third Intensification(IntIII) are marked on the figure. CNSDT was administered as either High Dose IV Methotrexate(IV) or continuous intrathecal methotrexate(IT)

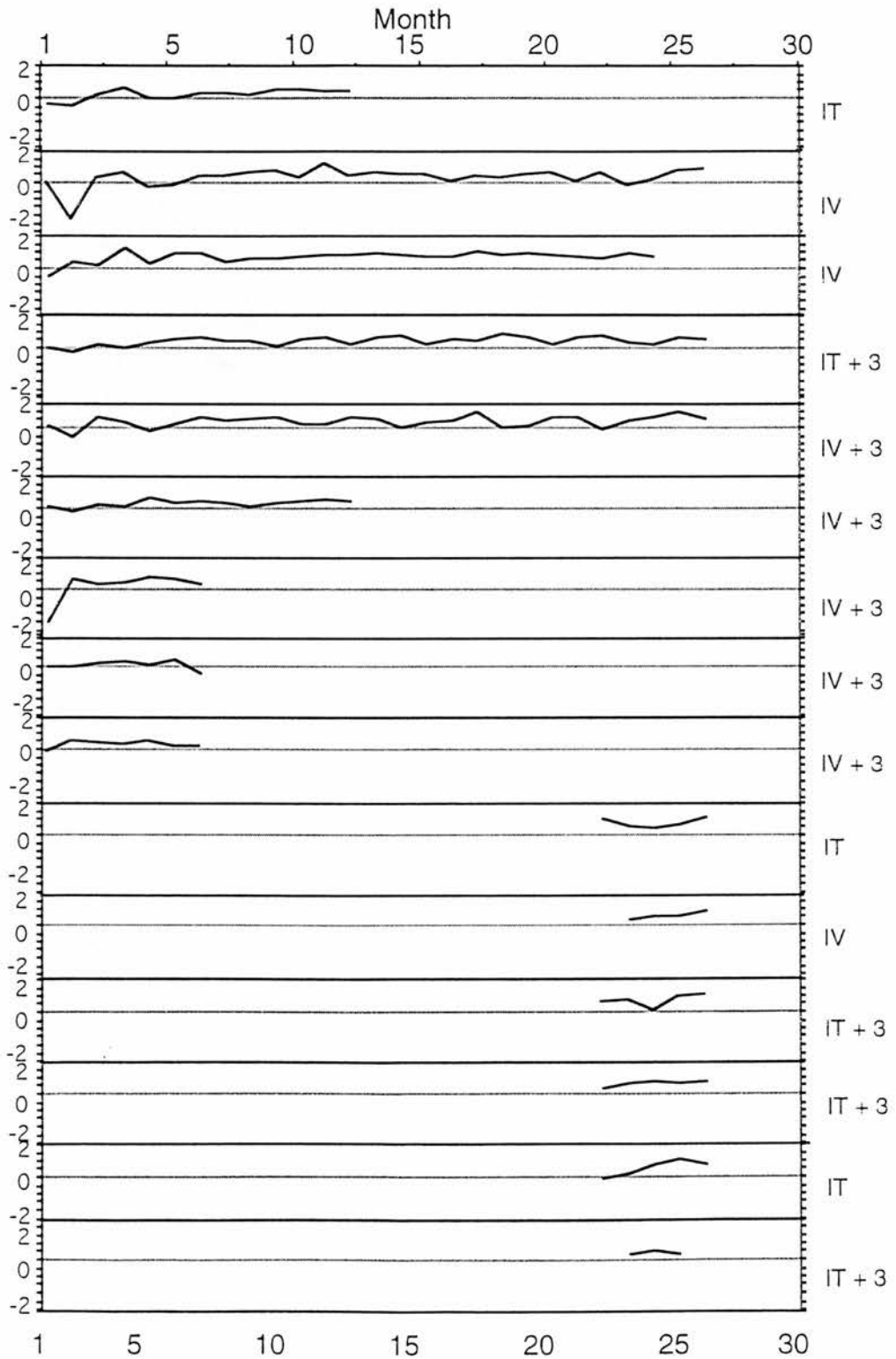
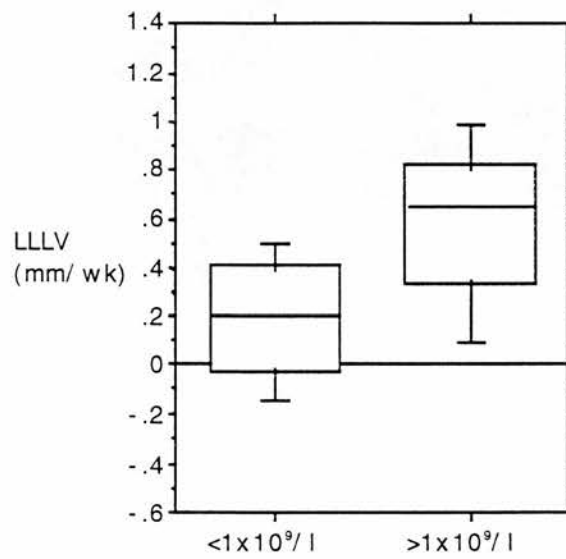


Fig.4.7 Median LLLV(mm/wk) (95th percentiles) when neutrophil count was above and below $1 \times 10^9/L$ during continuation chemotherapy.



Section 3. Changes in markers of growth hormone secretion and bone & collagen turnover

A. Introduction

The studies reported in the preceding two sections reveal a remarkable pattern of changes in stature as well as LLL in children on cytotoxic CT and especially during intensive treatment. While some knemometric changes could be artefactual and related to changes in weight and soft-tissue composition, it is probable that they also reflect altered growth and bone and collagen turnover. Physical growth has been shown to relate to markers of GH secretion, such as Insulin Like Growth Factor-1 (IGF1) and IGF Binding Protein-3 (IGFBP3). Although there have been some studies of knemometry and markers of GH secretion and bone and collagen turnover in children with asthma, these studies have not extended beyond eight weeks (Wolthers *et al*, 1994, Wolthers *et al*, 1997). The aim of this study was to compare knemometry more comprehensively with these markers of growth. Furthermore, studying the patterns of changes would provide some insight into abnormalities in growth pattern seen in children on cytotoxic chemotherapy and investigate any disorders of bone and collagen turnover.

B. Method

Between June 1993 and December 1994, 16 children on UKALLXI with a median age of 4.5yrs (range,1.1-14), (M:F, 11:5), who were anthropometrically monitored until the end of the second intensification, had blood and urine samples collected for the measurement of total Alkaline Phosphatase (tALP), bone Alkaline Phosphatase (bALP), Carboxy-terminal Propeptide of TypeI Collagen (PICP), Aminoterminal Propeptide of TypeIII Collagen (PIIINP), the Cross-linked Telopeptide of TypeI Collagen (ICTP) as well as urinary Growth Hormone (uGH), IGF1 and IGFBP3. Samples were collected at specific time points in relation to the CT, as shown in Fig.4.11.

Because of the technique's age restriction, Valk knemometry was only performed in 8 out of the 16 children enrolled above. Urine collection for uGH assay was performed in only these subjects whereas blood sampling which coincided with blood sampling related to the CT regimen was performed in all the subjects between 10am and midday. Bone and collagen marker results were available from all the samples collected from the 16 subjects, whereas GH marker results were only available in 8 subjects. Details of the subjects are presented in Table 4.2; in addition to the 14 subjects, 2 subjects who received XRT were studied upto the end of the First Intensification before they received XRT as part of CNS-directed therapy.

C. Analytical Methods

tALP activity was measured at 37°C, using *p*-nitrophenylphosphate as substrate in diethanolamine buffer. Its reference range in children was 250-800U/l and its interassay CV were 2.1 and 2.4% at 240 and 506U/l respectively. ALP isoenzymes were quantified using a modified wheat germ lectin affinity electrophoresis method (Crofton, 1992). The reference range of the bone isoform (bALP) in children using this method was 180-700U/l and its inter-assay CV (in plasma from children without liver or bone disease) were 2.2, 3.5 and 1.9% at 251, 349 and 435U/l.

PICP and ICTP were both measured by radioimmunoassay (Orion Diagnostica, Espoo Finland); their reference ranges in children were 200-460 μ /l and 3.5-18.0 μ g/l respectively. The inter-assay CV for PICP were 5.8, 4.1, 6.6, and 4.0% at 52, 105, 216 and 435 μ g/l respectively. The interassay CV for ICTP were 7.9, 5.7, 6.5, 4.9, 5.2 and 4.1% at 3.3, 6.2, 10.5, 18.8, 26.9 and 31.8 μ g/l.

PIIINP was also measured by radioimmunoassay (RIA-gnost P-III-P, Behringwerke AG Diagnostica, Marburg, Germany); its reference range in children were 0.6-2.1U/ml and its interassay CV were 6.9, 6.9, 4.6, 8.6 and 5.1% at 0.89, 0.96, 1.47, 1.57 and 3.98U/ml respectively.

IGF-I was measured by acid ethanol extraction and cryoprecipitation of its binding proteins, followed by a radioimmunoassay using a polyclonal rabbit antiserum raised against purified human IGF-I. Assays were performed in duplicate and there was negligible interference by IGFBPs 1,2,3. Half maximal displacement occurred at 1.1 μ g/l and the intra and interassay CV at 40% B/B₀ including extraction, were less than 6% and 9%, respectively.

IGFBP-3 was measured by a radioimmunoassay using polyclonal rabbit antiserum raised against human glycosylated IGFBP-3. Assays were performed in duplicate and there was undetectable cross-reactivity with IGFBP-1 and 2. Sensitivity of assay was 0.291 μ g/l. Interassay CV at 48% and 78% B/B₀ was 10.7% and 7.6% respectively. Intra-assay CV at 30%, 40% and 80% B/B₀ was 2.3%, 2.4% and 5.9%.

D. Statistical Analysis

Results are presented as median values with their 95th percentiles. While LLLV and the bone & collagen markers are presented as absolute values with reference to a normal range, IGF-I and IGFBP-3 are presented as SD scores (SDS). The normal ranges as well as the SDS are derived from healthy children in the laboratories where the assays were performed (tALP, bALP, IGF1, IGFBP3) or when the assays were performed by the same method (PICP, P3NP, ICTP). For uGH, the normal range

has been defined on the basis of the results of the study of healthy school-children in Chapter Three; Section 3. Inter-group comparison was performed by the Wilcoxon Signed Rank test and a relationship between any two variables was assessed by the Spearman's correlation coefficient test.

E. Results

Markers of Bone and Collagen Turnover

Results for the subgroup who had knemometry are shown separately (Fig.4.9) as well as with the rest of the group (Fig.4.10). It is clear from the figures that the trends remain in the smaller knemometry sub-group but the changes are less significant.

bALP and tALP

At presentation both forms of ALP were below the normal range and tended to rise initially during induction (Wk3) before reaching a nadir at Wk6 following the first intensification. Compared to a median tALP of 321U/L (P5,P95:159,466) at Wk3, median tALP at Wk6 was 201U/L (P5,P95:120,274), $p=0.003$. Corresponding values for bALP were Wk3-214U/L (P5,P95:89,301); Wk6-391U/L (P5,P95:82, 252), $p=0.04$. In between First and Second Intensification and over the period of CNS-directed therapy (Wk8-Wk18), a gradual rise in ALP was observed. Median tALP at Wk18 was 315U/L (P5,P95:259,458), significantly higher than at Wk6 ($p=0.003$). Median bALP was

also higher than at Wk6 (med bALP, 295U/L (P5, P95: 203, 408) $p=0.02$). ALP values then fell to below normal following the Second Intensification period before rising again. Neither of the two isoforms ever reached supranormal levels. The correlation coefficient of tALP and bALP in this group of children was 0.8, $p=0.0001$ (Fig. 4.11). The ratio of bALP to total ALP was, however, not constant throughout the six months (Fig. 4.12); at times of intensive CT the bALP isoform was relatively low and the ratio of bALP to tALP was highest just before second intensification when LLLV was also high.

PICP

Median PICP at 172 μ g/L (P5, P95: 99, 503) was also low at presentation but, unlike ALP, did not show an initial rise but fell throughout Induction and First Intensification reaching a nadir at Wk6 of 101 μ g/L (P5, P95: 62, 262) (Fig. 4.9, Fig. 4.10). Median PICP increased more quickly and reached a peak (supranormal) level at Wk18 of 699 μ g/L (P5, P95: 366, 1226) $p=0.008$. Significant reduction in PICP occurred over the Second Intensification reaching a second nadir at Wk21 with a median PICP of 239 μ g/L (P5, P95: 93, 274, $p=0.04$) before tending to rise again.

PIIINP

At a median level of 7.9 μ g/L (P5, P95: 5.9, 28.3), PIIINP at presentation were well within the normal range but like PICP

showed a gradual decline reaching a nadir at the lower limit of normality at Wk6 (Median P3NP of 3.8µg/L (P5,P95:2.4,6.3) p=0.003) (Fig.4.9, Fig.4.10). PIIIINP reached a peak at Wk14 (Median PIIIINP-11.4µg/L (P5,P95:6.4,12.3) p=0.005); in the knemometry-only subgroup the peak level was reached later at Wk20. PIIIINP then tended to decline but no significant changes were seen over the Second Intensification period.

ICTP

Median ICTP at 8.4µg/L (P5,P95:4.3,15.9) was within normal limits at presentation and a reduction in it's levels was seen at Wk6 when it fell to 6.4µg/L (P5,P95:3.9,15.1) (NS) (Fig.4.9, Fig.4.10). Subsequently, there was firstly a significant increase in ICTP from Wk6 to Wk8 (Median ICTP,18.4µg/L (P5,P95: 9.0,33.1) p=0.004) followed by a fall between Wk8 and Wk14 (Median ICTP,14.2µg/L (P5,P95:9.6,25.9) p=0.04). ICTP fell over the second intensification and showed a significant rise following the end of that period.

Markers of Growth Hormone Secretion

As the groups are smaller, the results of this study are presented *in toto*; the group has not been divided into those who did and who did not have knemometry (Fig.4.13).

uGH

uGH was generally higher than that observed in a group of healthy prepubertal children studied earlier (Fig.4.13). Median uGH level at presentation (Wk1) was skewed because of one very high value of 90.3ng/12hrs in Subject E; subsequent levels in this subject ranged between 3.6-8.5ng/12hrs. Despite excluding this subject uGH excretion continued to remain above the arbitrary range set in this study. Although there was a gradual decline of uGH excretion over the first 8 weeks, this change was not significant.

IGF1 and IGFBP3

These two markers of GH secretion/activity showed similar changes (Fig.4.13). Throughout the study period IGF1-SDS and IGFBP3-SDS remained within 2SDS of the mean value. Over the period of induction, systemic levels of IGF1 rose significantly from median IGF1-SDS of -0.9SDS (P5,P95;-2.7,0.4) at Wk1 to 0.2SDS (P5,P95;-1.9,1.1) at Wk5 (p=0.03). Both median IGF1 and IGFBP3 reached a peak at Wk20 with values of 0.5SDS (P5,P95;-0.6,1.6) and 1.9SDS(P5,P95;-0.3,3.1) respectively.

Correlation between LLL and Biochemical Markers (Table 4.3)

Besides a strong relationship between tALP and bALP, a moderate positive relationship was found between PICP and the two forms of ALP assayed. There was a weak positive relationship between most markers of bone turnover as well as IGF-

related markers of GH secretion. The positive relationship between IGF-I and IGFBP3 was strong ($r^2=0.7$, $p=0.0001$).

Discussion

This study of the IGF1/GH axis and markers of bone and collagen turnover over the first six intensive months of chemotherapy showed that marked changes occurred in these biochemical markers although the extent of these changes varied from one to another.

Although the general pattern of changes of the markers (specially, bone and collagen markers) was similar to that of the LLL changes, the relationship was not statistically significant. This is not surprising as changes in the markers represent all growing bones whereas knemometry reflects growth of a limited part of the skeleton as well as changes in soft-tissue composition.

Previous studies have shown that plasma IGF-I and IGFBP3 levels correlate with the diagnosis of GH deficiency and increase following GH administration (Blum & Ranke, 1990). In this study of the first 6 months of ALL chemotherapy, IGF-I and IGFBP3 levels remained within 2SD scores of the mean; some of the changes seen in IGF-I were significant and the changes seen in both parameters correlated with some of the markers of bone formation. The catch-up growth observed in

the form of an acceleration of LLLV was in the presence of normal IGF-I and IGFBP3 levels. uGh levels measured over the first few weeks of chemotherapy were supraphysiologically high despite normal IGF-I and IGFBP3 levels and a slow LLLV. These changes could be explained by a transient GH insensitivity during the intensive chemotherapy period as suggested by Nivot *et al* (1994). Both IGF-I and IGFBP3 (to a lesser extent) can be lowered by malnutrition (Jackson *et al*, 1995) and the subjects could have been suffering from this at presentation although this was not apparent from their BMI which was within the normal range (Chapter Four; Section 1). Alkylating agents can induce hepatic dysfunction and, therefore, some of the chemotherapy itself could affect IGF-I/BP3 synthesis leading to GH insensitivity (Scharf *et al*, 1996); consistent changes in the ratio of the bone isoform of ALP to the total ALP which occurred over the chemotherapy period probably reflect changes in hepatic ALP metabolism. Although total IGF-I levels have been shown to correlate well with free IGF-I levels (Hasegawa *et al*, 1996), they probably do not fully reflect IGF-I activity at the cellular level; IGFBP3 is thought to modulate the bio-availability of IGF-I by increasing the biological half-life (Guler *et al*, 1989) and by decreasing the transendothelial transport of IGF-I (Bar *et al*, 1990). IGFBP3 levels in children with leukaemia when measured by radioimmunoassay have been previously found to be in the normal range and significantly higher than that

measured by western ligand blotting; this was accompanied by an increase in IGFBP3 protease activity which is most likely due to the disease process itself (Muller *et al*, 1994).

bALP which is produced by the mature osteoblast and reflects bone mineralisation and PICP, produced by the immature proliferating osteoblast and which reflects synthesis of type I collagen (Risteli & Risteli, 1993) were both below normal levels at presentation, demonstrating the growth suppressive effects of the disease itself. PIIINP which reflects the synthesis of type III collagen in connective tissues throughout the body was within the normal range. ICTP, which is felt to reflect degradation of type I collagen was within the normal range at presentation and the combination of the above findings suggest that children with leukaemia seem to have an imbalance of bone turnover at presentation which favours bone and collagen degradation.

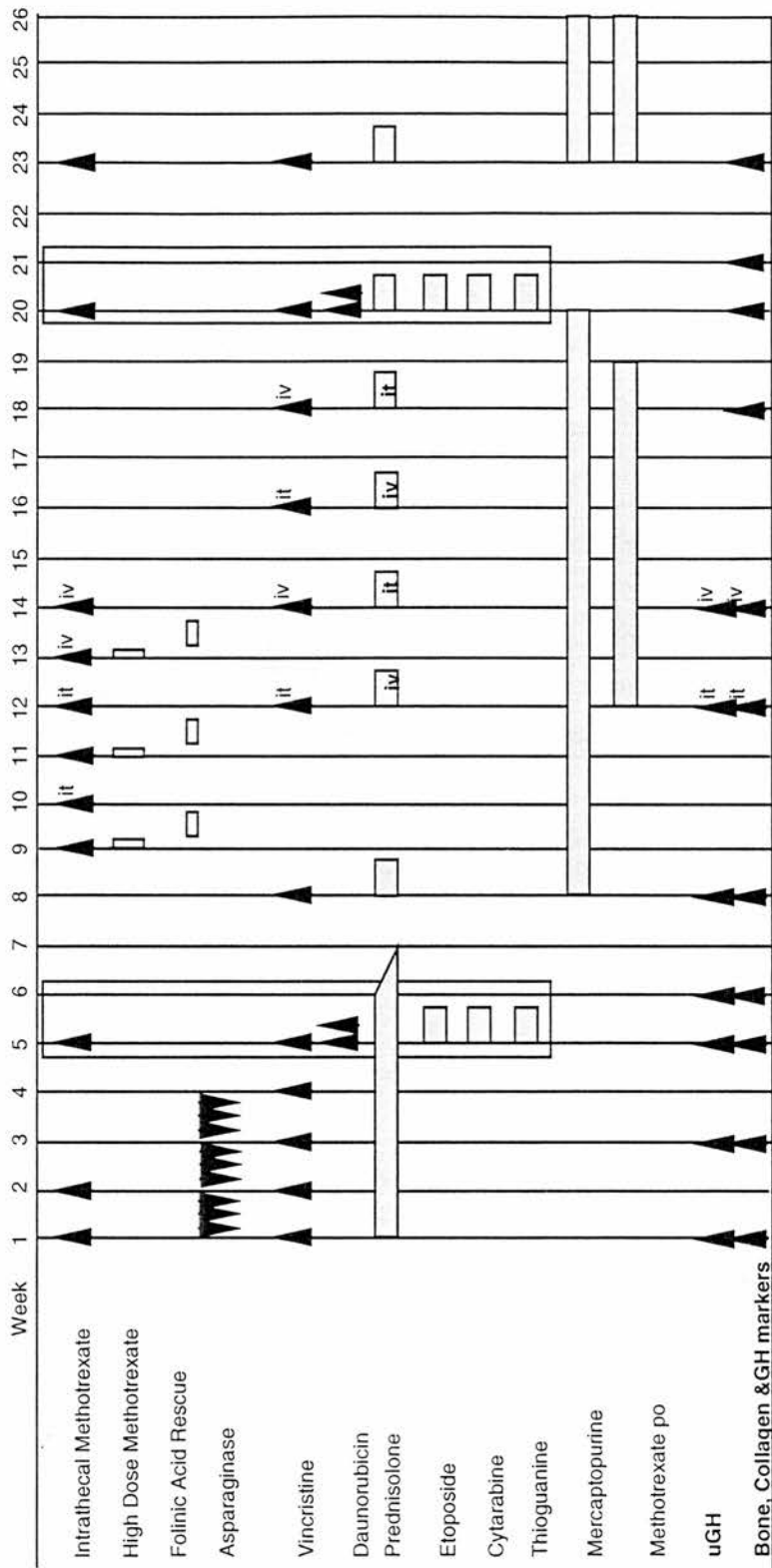
During the intensive phases of CT within the first 6 months, i.e. induction, first intensification and second intensification, all markers of bone formation and degradation showed reductions which are consistent with the known suppressive effects of steroids on growth, osteoblast function and collagen turnover (Chapter One; Section 3(C)). Unlike the other markers, bALP showed a paradoxical early increase over the first two weeks of induction followed by

subsequent suppression. There was a corresponding increase in LLLV over this period following which there was a deceleration. Immediately following the intensive periods of chemotherapy, bone and collagen markers showed a dramatic rise with PICP as well as ICTP levels rising above the normal range. Although ICTP levels rose to a peak at an early stage following the first 6 weeks of intensive chemotherapy, PICP and bALP continued to rise and reached a peak at a later stage. This high level of turnover was mirrored in the acceleration observed in LLLV following the intensive chemotherapy. The phase of CNS directed chemotherapy administered in between First and Second Intensification periods did not seem to have any apparent detrimental effect on bone turnover or LLLV. Furthermore, the imbalance in bone and collagen synthesis during these periods of recovery favoured bone formation.

Measurement of biochemical markers of bone & collagen turnover as well as the GH/IGF-I axis has provided some insight into the growth patterns observed by anthropometry in children with ALL in Chapter 4; Sections 1 & 2. Knemometry, as a technique for assessing short-term growth has, to an extent, been validated by the concurrent changes in the markers of bone and collagen turnover which can be useful adjuncts to anthropometric techniques in the assessment of the risk of growth suppressive effects of drugs. Although

this study has shown that children receiving cytotoxic chemotherapy do not show persistent abnormalities of GH/IGF-I axis and can mount a good recovery of bone and collagen turnover following intensive chemotherapy, it would be important to follow this group of children over a longer-term to ensure adequate growth. Bone mineralisation of adults who have had cranial irradiation as well as chemotherapy has been shown to be reduced based on single photon absorptiometry (Atkinson *et al*, 1989), dual x-ray absorptiometry (Nussey *et al*, 1994) as well as quantitative computed tomography (Gilsanz *et al*, 1990) and preliminary studies of children who have received chemotherapy-only regimens also suggests a possibility of reduced mineralisation (Warner *et al*, 1996); the present cohort should, therefore, have long-term studies of bone mineralisation to document their outcome.

Fig.4.8 Timing of blood and urine samples in the first 6 months of UKALLXI (92)



it - only administered in standard and continuing intrathecal methotrexate limb of CNS-directed therapy
 iv - only administered in High Dose Methotrexate iv and continuing intrathecal methotrexate limb of CNS directed therapy

Fig.4.9 Median bALP(U/l), tALP(U/l), PICP($\mu\text{g/l}$)
 PIINP($\mu\text{g/l}$), ICTP($\mu\text{g/l}$) and LLLV(mm/wk) over the
 first 6 months of UKALLXI(92) with 95th centile
 values in the group of children who had
 knemometry.*- $p < 0.05$, **- $p < 0.005$, WSR. Shaded area
 represent approximate reference ranges

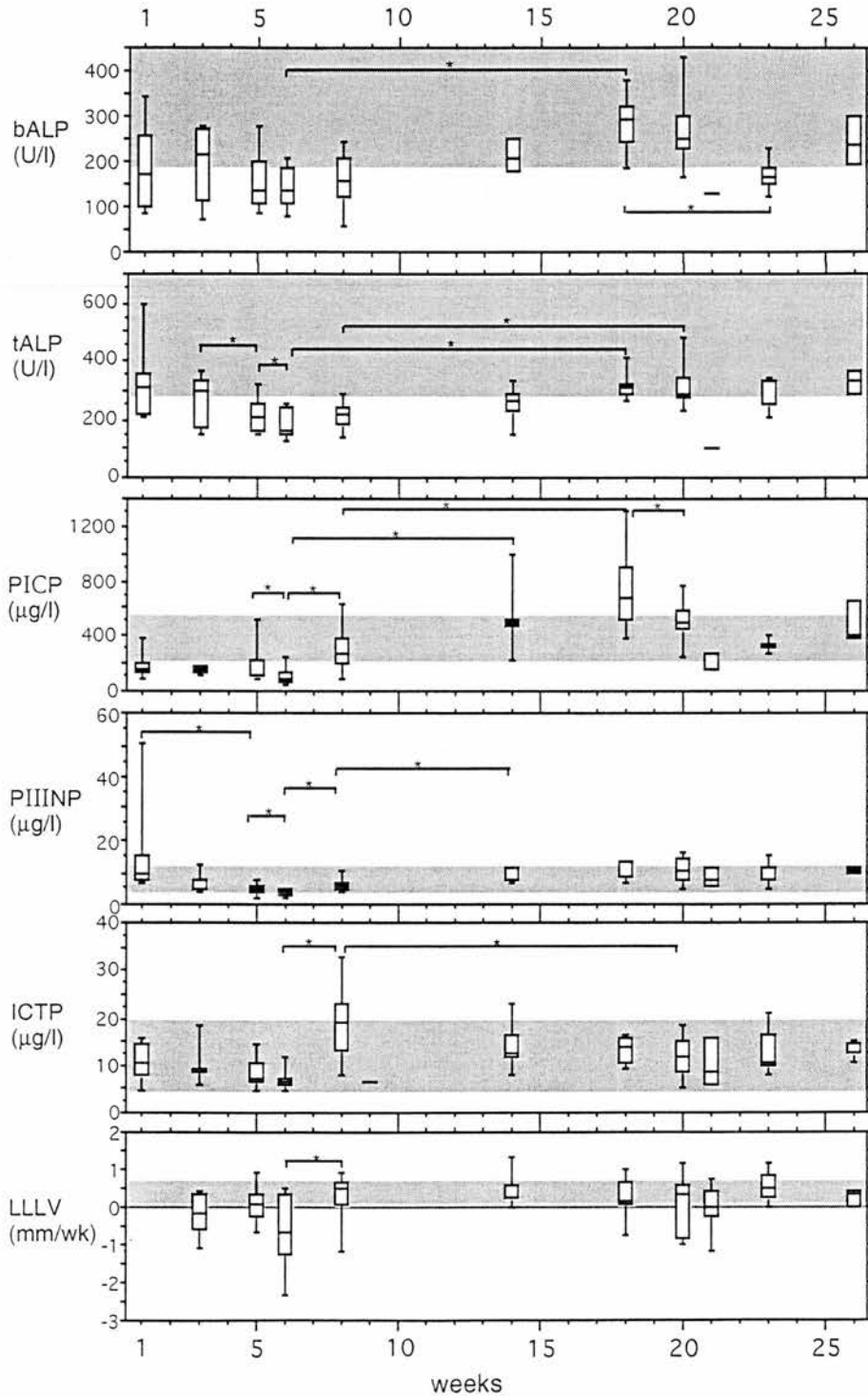


Fig.4.10 Median bALP(U/l), tALP(U/l), PICP($\mu\text{g/l}$)
 PIINP($\mu\text{g/l}$), ICTP($\mu\text{g/l}$) and LLLV(mm/wk) over the
 first 6 months of UKALLXI(92) with 95th centile
 values in all the children studied. *-p<0.05,
 **-p<0.005,WSR. Shaded area represent
 approximate reference ranges.

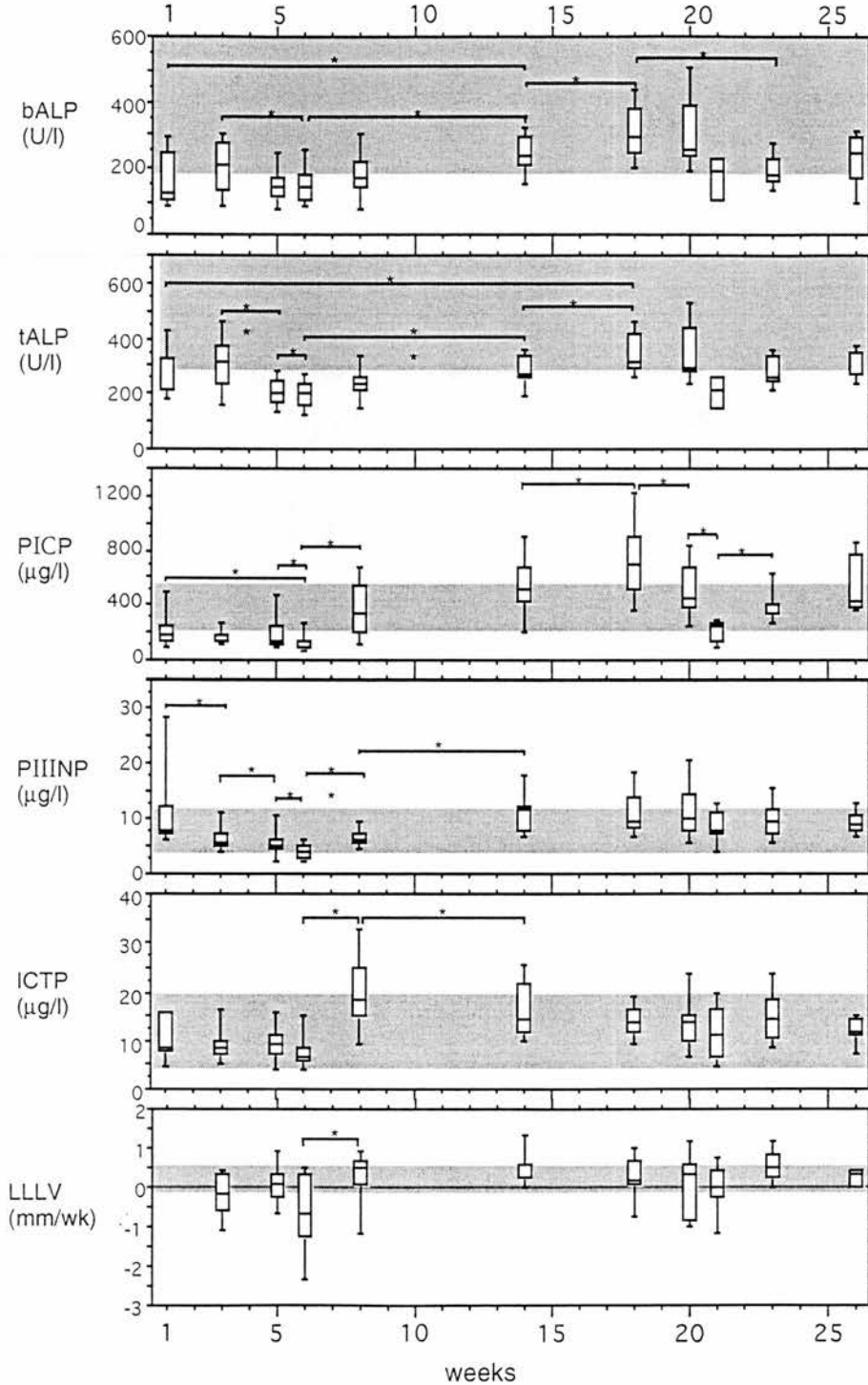


Fig.4.11 Scattergram of bALP(U/l) versus tALP (U/l).

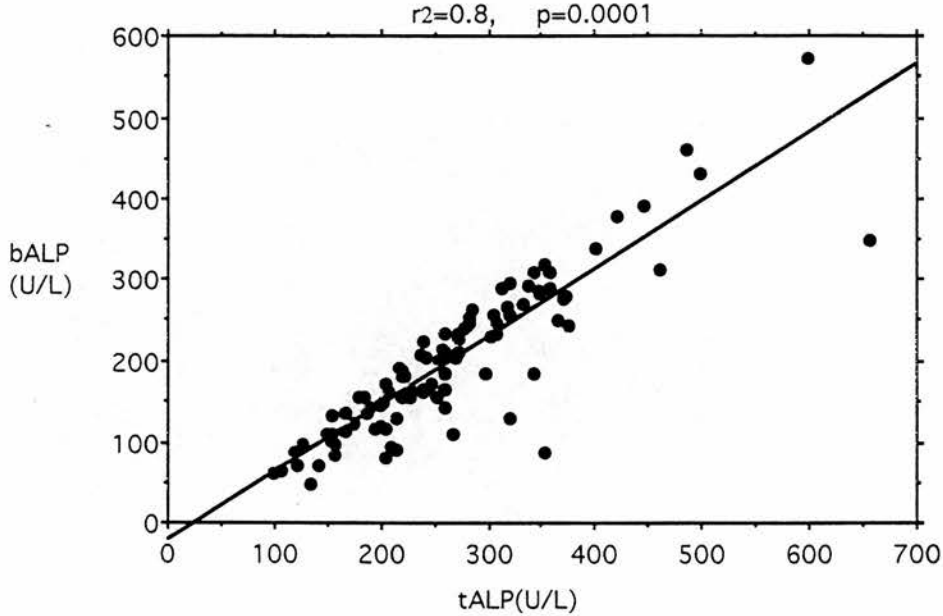


Fig.4.12 Median bALP:tALP ratio(95th centiles) over the first 6 months of UKALLXI(92).*-p<0.05, WSR.

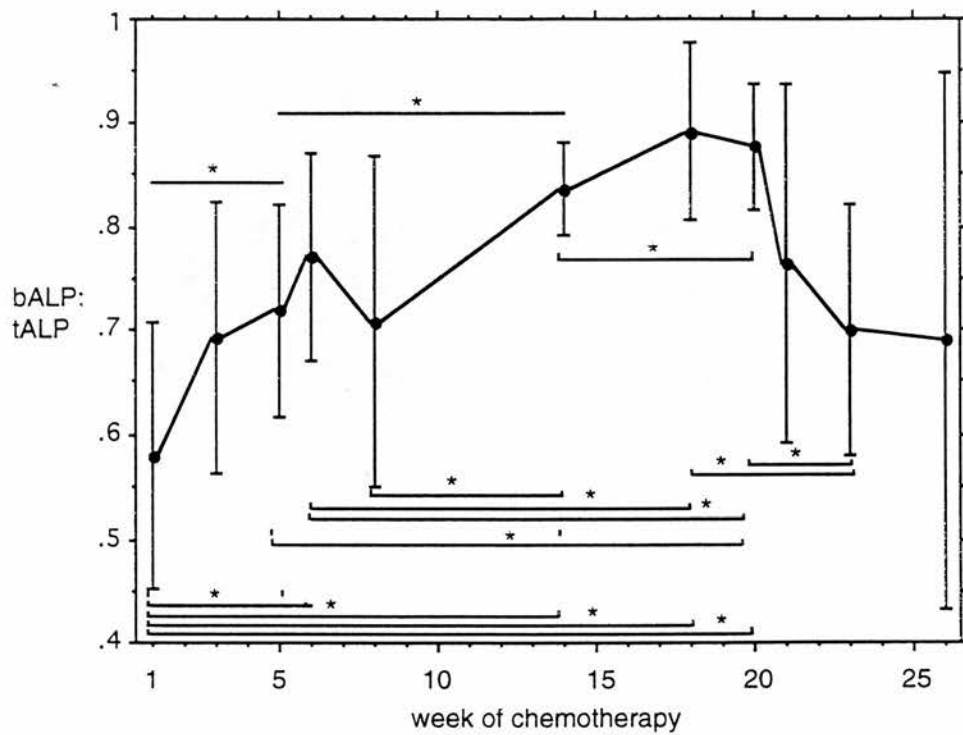


Fig.4.13 Median uGH(ng/mmol creat and ng/12hrs), IGF-ISDS,IGFBP3SDS & LLLV(mm/wk) over the first six months of UKALLXI(92)in all children studied.Shaded area represent approximate reference ranges

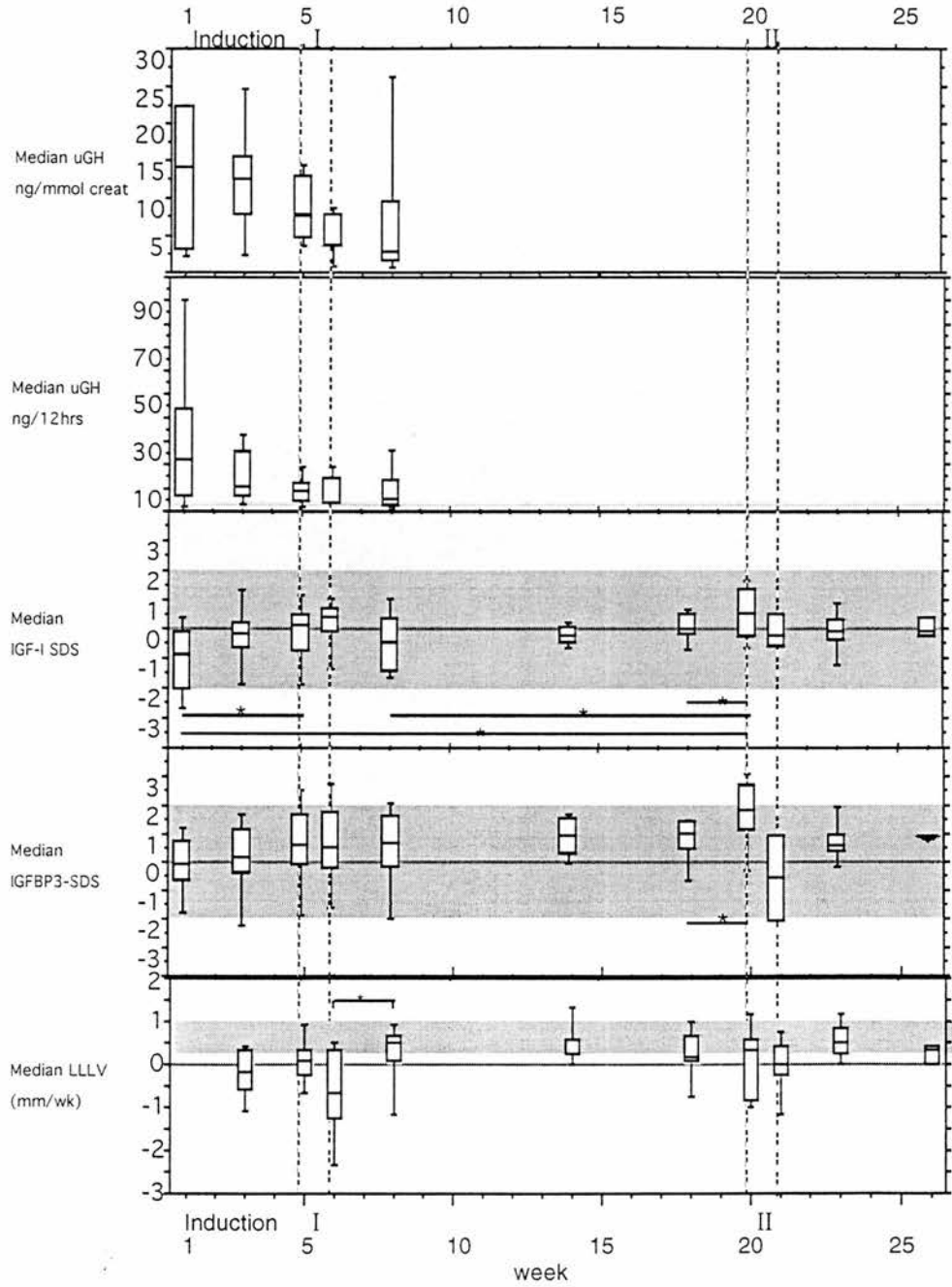


Table 4.3 Correlation coefficients (r^2) and probability value (p) of markers of bone and collagen turnover, markers of GH secretion and LLLV

	tALP	bALP	PICP	PIIINP	ICTP	IGF1	IGFBP3	uGH
LLL	ns	ns	ns	ns	ns	ns	ns	ns
tALP		$r^2=0.8$ $p=0.0001$	$r^2=0.2$ $p=0.0001$	$r^2=0.1$ $p=0.0005$	ns	$r^2=0.1$ $p=0.02$	$r^2=0.1$ $p=0.005$	ns
bALP			$r^2=0.3$ $p=0.000$	ns	ns	$r^2=0.1$ $p=0.01$	$r^2=0.1$ $p=0.005$	ns
PICP				ns	$r^2=0.1$ $p=0.003$	$r^2=0.1$ $p=0.02$	$r^2=0.1$ $p=0.002$	ns
PIIINP					$r^2=0.1$ $p=0.0001$	ns	ns	ns
ICTP						ns	ns	$r^2=0.2$ $p=0.01$
IGF1							$r^2=0.7$ $p=0.000$	ns
IGFBP3								ns
uGH								

CHAPTER FIVE

CONCLUSION & FUTURE DIRECTIONS

Anthropometry is defined as the systematized art of measuring and taking observations on man, his skeleton, his brain and other organs by the most reliable means for scientific purposes (Cameron,1986). This interface between art and science is a constant theme throughout all fields of clinical practice and research.

Proficient anthropometry of somatic growth, auxological anthropometry, is still a form of art but it is the cornerstone of childhood growth monitoring. It is only studies based on sound auxology, using techniques such as stadiometry, that have highlighted adverse long-term effects of a variety of illnesses and their consequent treatment. Following such studies, attempts have been made to study the basis of the harmful effects and subsequently changes have been introduced to the treatment regimen. The evolution of the treatment of childhood ALL typifies the above sequence of events where initial auxology in survivors not only showed an adverse effect on growth but also paved the way for research into diverse late effects of childhood cancer treatment. Besides allowing an improvement in therapy, such studies have also improved our understanding of the process of growth.

As the 5-year survival rate for childhood ALL is now over 70% (Boring et al, 1994), the major challenge is to sustain the significant improvement in survival rates while at the same time minimizing the treatment induced late effects and, therefore, continued reliance on close monitoring of growth and other possible late effects remains necessary. Accurate and reliable detection of short-term variations in growth rate has long been an objective of auxological anthropometry. The knemometer, described just over ten years ago, and with its low measurement error has been hailed as the method of choice for such short-term studies. Experience with this technique is, however, generally limited. At the outset of the project it was apparent that studies of lower leg length changes in normal healthy subjects were scarce and most of the studies which followed subsequently studied knemometry as a tool for predicting long-term response to GH therapy. The failure of lower leg length changes to act as a predictor is no doubt due to the non-linear pattern of short-term growth. However, my studies show that short-term growth studies, have the potential to enhance our understanding of abnormal growth which occurs in a variety of diverse childhood illnesses and their related treatment.

The studies of short-term growth in healthy children and adults in this thesis were performed to evaluate the

technique of knemometry and learn about the pattern of LLL changes seen in healthy individuals. Knemometry was a very sensitive method for studying short-term changes in LLL and it was not particularly difficult to learn. Measurements were highly reproducible; some of this precision was artefactual due to intra-observer bias and having eliminated this in the first study described in this thesis, the technique still remained highly precise. By introducing the Random Zero Method into knemometry, new personnel could be trained more objectively in the technique and individual measurers could compare the precision of their technique with minimal observer bias.

The study of LLL in young adults was particularly valuable in that they (and especially the pregnant subject) acted as "negative controls" and showed clearly that some of the changes seen in LLL are unrelated to real growth.

The longitudinal studies in the healthy children have not solved the debate regarding the pattern of childhood growth. There is a possibility that the model of growth advocated in the literature depends on the method of mathematical or statistical analysis of the data. Although it is highly likely that there is a form of biological control on the process of growth, it might be too simplistic to expect that this "control" is reflected in a

distinct pattern of growth when the optimal model of childhood growth should take environmental as well as genetic influences into consideration. In the words of Tanner (1989) "we may write $G \cdot E \rightarrow (GE)$ where G stands for genetic and E for environment factors. The product (GE) develops through time, and the way G and E are related is shown by a star, not a plus sign nor even a multiplication. We say that the interaction of genetics and environment is non-linear, meaning that the effects do not in general sum". As our knowledge of the biology of growth improves, it would be appropriate to add that the term "environment" not only refers to that of the growing child but also to the growing bones and related tissues. The periods of acceleration, stasis and deceleration seen in the healthy group of children occurred without any detectable pattern. They did not occur in tandem with changes in other children who were being measured at the time and were not temporally associated with any obvious external events. This is important to realise as it implies that reproducible patterns of lower leg length changes which occur uniformly at the time of an external stimulus in a group of children are probably causally related to that stimulus. It is also important to stress that the terms, LLL "change" or "fluctuations" rather than growth is preferable as only a proportion of these changes are due to real changes in somatic growth.

Standard anthropometry in this group of children showed a pattern of growth deceleration which has been seen before in children during the early phase of intensive treatment with CT-only regimens. This group of children, however, showed catch-up growth during the period of CT; this has not been a universal finding previously. In addition, significant changes in vertebral growth, body disproportion and body mass index were seen over the initial phases of CT; none of these were sustained but emphasize the fact that CT by itself can significantly affect somatic growth. The transient alteration in SH:Ht ratio following induction of remission could have been due to the direct effect of CT on the numerous vulnerable growth plates in the vertebral spine.

The studies of short-term growth in children on CT for ALL have indicated the limitations as well as the advantages of knemometry over conventional stadiometry. The ability of the children to show catch-up growth following a period of significant deceleration as well as real shrinkage (in LLL) could be observed more closely by knemometry. Whereas, stadiometry showed that these children reached a nadir in growth at about six months into therapy before any signs of improvement, it was clear from knemometry that the lower leg showed significant catch-up growth very early after

induction and which was sustained during periods of less intensive CT over the first six months of treatment. The delay in the recovery of height could be explained by a delay in the recovery of the rest of the axial skeleton or the imprecision of the conventional forms of measurement. The positive relationship between changes in LLL and neutrophil counts (which acted as a marker of cytotoxicity during the period of continuation CT) was interesting and suggested that the physical changes seen on measurement were perhaps related to a direct effect of cytotoxic, myelosuppressive CT on leg growth.

The increase in weight which was related to high dose steroids during intensive therapy could have contributed to lower leg length deceleration and shrinkage and the final study reported in this thesis was performed in order to investigate as well as relate the changes seen in bone and collagen turnover and GH secretion. Over this period changes in markers of bone and collagen turnover were related to the periods of intensive CT and showed some temporal association with the changes in LLL. These changes were not always statistically significant, partly due to the small sample size but probably also due to the fact that the markers reflected the general process overall rather than just the lower leg. The changes in the bone and collagen markers did suggest some imbalance in turnover

favouring resorption over the induction period and formation over the period of catch-up growth following induction. These findings of changes in bone turnover further support the notion that the LLL changes seen over these phases of CT relate to real changes in bone growth.

The study of markers of GH secretion suggested a possibility of partial GH resistance at presentation and over the first few weeks of CT but this needs to be validated further. The most remarkable finding was the presence of marked catch-up growth with no accompanying increase in the markers of GH secretion supporting the hypothesis that part of catch-up growth might be GH independent (Boersma & Wit,1996).

Future Directions

Knemometry is a useful tool for assessment of short-term growth provided its limitations are clearly understood. As new forms of growth promoting, as well as potentially growth suppressive therapy are introduced, knemometry could play an important role in the evaluation of any immediate effect that therapy might have on growth. As knemometry cannot predict long-term growth, it is important that subjects involved in studies of short-term growth are followed up over longer periods. The advantage of knemometry over biochemical markers of bone turnover in studying growth is it is non-invasive and with modifications to the device it is possible to make the knemometer more portable as well as practicable for younger age groups. As methods of assessing short-term growth improve, it might become possible to perform studies of short-term growth on a larger sample of healthy children over a longer period to look more conclusively at the physiology of normal as well as catch-up growth.

Biochemical markers are a useful adjunct in the assessment of short-term growth; future research in this field should concentrate in developing new forms of markers which could be assayed in urine or saliva and therefore become more easily accessible; at the present time only degradation

fragments of type I collagen can be measured in the urine. Besides providing information on somatic growth, bone and collagen markers provide an insight into mechanisms of altered bone turnover.

The auxological studies of children on cytotoxic chemotherapy for ALL in this thesis show that growth is certainly affected in the short-term; whether there is any long-lasting effect on growth will only be answered by a longer study of this cohort as well as results from studies of other groups of children (for example, those treated for solid tumours with or without the CNS) on the same and differing chemotherapy regimens. The abnormalities in bone turnover seen in these children during treatment raise concern about whether bone turnover, density and structure are normal in these children in the long-term and this again will be answered as this group as well as other comparable groups are studied in the future. As these changes in bone turnover coincided with changes in lower leg length, knemometry could be employed as an indicator of short-term effects on physiological systems other than just linear growth.

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Short-Term Changes in Urinary Growth Hormone Excretion and Lower Leg Length in Healthy Children

Key Words

Urinary growth hormone
Knemometry
Lower leg length

Abstract

To investigate any association between changes in lower leg length (LLL) and urinary growth hormone (uGH) excretion, 4 prepubertal children supplied daily 12-hour overnight urine samples and had daily knemometry performed for 4 weeks. The mean daily and weekly LLL velocity (LLLV) of the group was 0.08 mm/day (95% CI 0.01–0.18) and 0.52 mm/week (range 0.38–0.78), respectively. The mean uGH excretion was 8.9 ng/l (CI 3.7–13.3), and the mean intrasubject coefficient of variation of uGH was 55% (range 32–93). The tallest subject who also had the highest LLLV excreted the least amount of uGH (mean 3.7 ng/l, CI 2.9–4.5). No temporal relationship was evident between daily uGH excretion and LLL changes. There was no evidence of any association between amount of uGH excreted and LLLV. There remains some doubt on the usefulness of uGH measurement as the sole predictor of normal GH production.

Introduction

The process of human growth can be studied in detail using the knemometer which can non-invasively measure the lower leg length (LLL) very accurately and precisely [1]. In children short-term growth of the lower leg is felt to progress in a saltatory fashion, and there is evidence to suggest that the periods of increased growth rates occur in distinct phases [2].

Knemometry has also revealed an intradaily rhythm in LLL variability where the length is maximum in the morning and falls over the first half of the day, reaching a plateau subsequently [1]. This variability has been shown to relate to the duration of maintaining the erect posture, and the reduction of LLL is thought to be due to weight bearing on the compressible components of the lower leg [3]. The growth hormone (GH) secretion, which is maximal during the night, could be another factor contributing

to this intradaily rhythm. This is supported by the finding that serum markers of osteoblastic activity such as osteocalcin and carboxyterminal propeptide of type I procollagen display a circadian variation in serum concentration dependent on the GH secretion [4].

Timed overnight collection of urine for measuring urinary GH (uGH) provides a non-invasive integrated measure of GH secretion [5–7] and may be used as a more accurate predictor of normality than GH provocation tests [8]. uGH measurements also have a role in studying the physiology of growth, and it has recently been shown that the nightly uGH excretion can also vary in a pulsatile fashion [9].

The aim of this study was to investigate whether there is any relationship between changes in LLL as evidenced by knemometry and GH secretion as assessed by overnight uGH excretion.

Subjects and Methods

Over a period of 4 weeks in June 1994, measurements were performed at the Royal Hospital for Sick Children, Edinburgh, on every weekday in a group of 4 healthy children from a neighbouring school. The LLL was measured with the Valk childhood knemometer as described before [1], but with a simple modification (random zero method) designed to reduce operator bias [10, 11]. Measurements were performed by one measurer (S.F.A.) who had been performing knemometry for a period of 10 months before the onset of this study.

The subjects were instructed to refrain from any vigorous physical activity for 2 h before the measurements were performed and were permitted to miss any planned physical exercise at school. Careful record of their bedtime, rising time, and general well-being was kept. The subjects were given an option to carry on for a further 4 weeks.

Urine for uGH assay was collected as a 12-hour overnight sample. 0.1% bovine serum albumin was added to the sample at the end of the collection by the subjects. 1% thiomersal was added to the sample by the investigators at midday prior to freezing at -20°C . Samples were analyzed in subject batches at the end of the study with the Novo Nordisk Novoclone amplified enzyme immunoassay (between-batch coefficient of variation $<12\%$ from 9 to 28 ng/l).

The study was approved by the local ethical committee, and informed consent was given by all subjects and/or their parents.

Statistics

The daily LLL velocity (LLLV^d) was derived from the change in length from the previous day of measurement and expressed as millimetres per day. LLLV was also derived for the overall study period from the LLL at baseline and at the end of the study and expressed as LLLV^w in millimetres per week. The precision of knemometric measurements was expressed as the technical error (TE) and represented 1 SD from the mean of three repeated estimates of LLL. The uGH levels were expressed as total uGH excreted (uGH^t) over 12 h (ng/12 h) as well as the concentration of uGH (uGH^c) excreted (ng/l). The relationship between uGH excreted and LLLV^d was assessed by calculating the correlation coefficient, and the Wilcoxon signed-rank test was used to compare the groups.

Table 2. Details of LLL changes and uGH excretion in the 4 subjects

Subject	Number of knemometric measurements	Mean LLLV ^d mm/day (95% CI)	Mean LLLV ^w mm/week	Number of uGH measurements	Mean uGH	
					ng/l (95% CI)	ng/12 h (95% CI)
A	20	0.01 (-0.19 to 0.21)	0.45	18	9.1 (4.6-13.6)	1.9 (1.0-2.7)
B	20	0.18 (-0.09 to 0.45)	0.78	17	3.9 (3.0-4.8)	1.1 (0.9-1.4)
C	20	0.11 (-0.16 to 0.37)	0.47	17	13.3 (9.4-17.1)	2.4 (1.7-3.1)
D	20	0.01 (-0.14 to 0.26)	0.38	17	9.6 (7.8-11.3)	1.6 (1.2-1.9)

Results

Details of the 4 subjects who were all prepubertal are shown in table 1. The children remained generally well during the study period, and their bedtime and rising time in the morning did not vary by more than 1 h. All 4 children declined the offer to continue for a further 4 weeks, and the primary deterrent was the restriction of physical activity.

Knemometry was performed on every weekday (20 occasions in each child). The mean TE of the measurements was 0.18 mm (95% CI 0.16-0.22). Overnight samples for uGH assay were collected on 18 occasions by subject A and on 17 occasions by subjects B, C, and D.

As a group, the children had a median LLLV^w of 0.52 mm/week (range 0.38-0.78) and a median LLLV^d of 0.08 mm/day (range 0.01-0.18). Subject B who was the tallest of the 4 subjects (table 1) had the highest mean LLLV^w as well as LLLV^d, but this was not significantly different from the rest of the group (table 2). The mean uGH^c for the group was 8.9 ng/l (range 3.9-13.3), and the mean uGH^t was 1.8 ng (range 1.1-2.4). The uGH excre-

Table 1. Description of subjects

Subject	Sex	Age years	Height SDS	Comments
A	male	10.5	1.5	
B	male	11.2	1.9	in same class as D
C	male	8.3	1.3	brother of D
D	female	11.0	1.5	sister of C

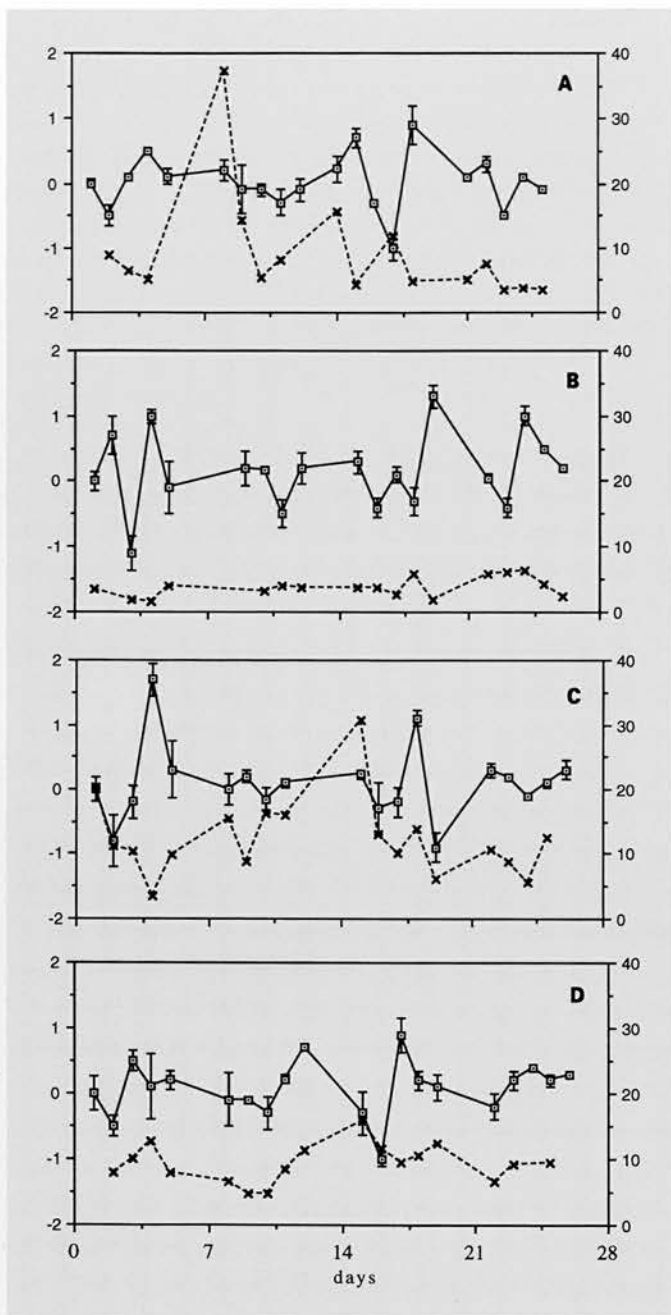


Fig. 1. Temporal relationship between LLLV^d (solid line, mm/day, left-hand axis) and uGH values (broken line, ng/l, right-hand axis). LLLV^d error bars denote TE (mm).

tion varied widely between as well as within the subjects (table 2). It was interesting to note that subject B had the lowest uGH excretion, and his excretion pattern was the most consistent as suggested by the small confidence limits.

Although the pattern of variation of uGH excretion between the subjects was no different when excretion was expressed as concentration or absolute amount of uGH, the differences tended to be more significant when the former method was employed (table 2). The mean day-to-day coefficient of variation of uGH^c for the whole group was 55% (range 32–93) and 56% (range 38–89) for uGH^l.

Figure 1 shows the temporal relationship between uGH^l and LLLV^d. Although there was no significant correlation between the two variables over the whole duration of the study, close examination of the patterns does shown a temporal relationship at times in subjects A, C, and D. The mean 12-hour uGH^c amounted to 1.5 ng (95% CI 1.2–1.8) when the LLLV^d was negative and to 1.9 ng (95% CI 1.4–2.4) when the LLLV^d was positive (NS).

Discussion

Although significant LLL changes were evident from day to day in all 4 subjects, no discernible pattern of change was evident over the 4 weeks. As the leg incorporates soft tissue as well as bone, the changes seen in LLLV^d are most likely to represent a combination of changes, of which some, such as hydration, are transient and some, such as epiphyseal growth, are permanent. It is for this reason some researchers have advocated the use of the term 'fluctuations' rather than 'velocity' to describe the changes in LLL when assessed over a short period [12].

Our study reinforces the view that healthy children can excrete a highly variable amount of uGH from day to day. This variability was also evident in the study performed by Thalange et al. [9], but, interestingly, these authors only rarely encountered a value below their cutoff value for differentiating normal children from those with GH deficiency [personal commun.]. Previous studies at our centre using the Novoclone uGH assay have shown that timed overnight uGH^c reflected nocturnal plasma GH profiles [13]. These studies have also provided a cutoff value of around 9 ng/l [personal commun.]. It is, therefore, interesting to see that subject B who was growing the fastest had consistently low levels of uGH excretion and was constantly below our present cutoff value for 'normality', subjects A and D also had a substantial number of uGH values below 9 ng/l. Expression of uGH data as concentration or as absolute mass did not alter the coefficient of variation of repeated samples in our group of subjects. Expression as mass did, however, reduce the degree of intersubject variability.

Serial values of uGH can be highly variable, and although this may be due to true biological variability in GH secretion or bioavailability controlled by proteins such as the insulin-like growth factor binding proteins [14], the variability can also be artefactual due to alterations in renal clearance of GH [15] or inconsistencies in handling the urine sample. All the subjects and their parents were instructed on the collection method and had instruction sheets with the collection kits. Following the uGH assays, the participants were again contacted to check their technique which was recalled correctly. The urinary creatinine concentration was not measured; although there was some variation of body height amongst the subjects, and although subject B with the lowest uGH levels was the tallest, all the children were prepubertal.

With the limited size of the data it was evident that at times there was a temporal relationship between uGH excretion and nature of LLL fluctuation. However, there was no correlation between daily LLLV and uGH levels. The former might be purely due to chance, as both indices vary considerably from day to day [16]. Conclusive evaluation of this relationship as well as detection of any rhythms requires a more demanding study with about 90 pairs of data points per subject.

We did not control for renal tubular handling of GH which can be assessed by measuring urinary proteins such as β_2 -microglobulin [17]. It is unlikely that our group of subjects would have had any significant abnormalities of glomerular or tubular function, but the day-to-day variation of function might explain some of the variation in uGH excretion. The study of markers of bone turnover and perhaps other indicators of a sustained GH secretion, such as urinary insulin-like growth factor I [18], could shed more light on the short-term changes observed through knemometry.

In conclusion, there is considerable day-to-day variability in LLL as well as overnight uGH excretion. The magnitude of uGH excretion does not correspond to the magnitude of LLL change. uGH values should always be interpreted in the context with clinical data. The evaluation of a temporal relationship between uGH excretion and LLL changes requires a study at a much larger scale.

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The relationship between short-term changes in weight and lower leg length in children and young adults

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Summary. As the knemometer is increasingly being used to study changes in lower leg length in conditions associated with weight changes it is important to clearly delineate the relationship between these two variables. Lower leg length and weight were measured in 26 children and nine adults including one pregnant woman. There was a weak but positive relationship between lower leg length and weight fluctuation in children. Daily fluctuations in weight as well as lower leg length were higher in women than men; median lower leg length fluctuation: women, 0.16 mm (P5-0, P95-0.7); men, 0.1 mm (P5-0, P95-0.48) $p \sim 0.02$, Wilcoxon signed-rank test. Median weight fluctuation: women 0.15 kg (P5-0, P95-0.54); men, 0.1 kg (P5-0, P95-0.5) $p = 0.94$ (Wilcoxon signed-rank test). Sustained weight gain in pregnancy led to a reduction in lower leg length followed by an increase which was coincident with the appearance of dependent oedema. Lower leg length changes are likely to be positively related to changes in weight when the latter are only modest in magnitude. However, greater sustained increases in weight are likely to have an opposite effect on lower leg length due to direct compression of the lower leg. Due consideration of weight is essential in longitudinal studies of lower leg length changes, especially in conditions which are associated with significant changes in weight.

1. Introduction

With its high level of accuracy and precision, knemometry has revealed significant intra- and inter-daily changes in lower leg length in children and adults (Valk, Langhout-Chabloz, Smals, Kloppenberg, Cassorla and Schutte 1983, Ahmed, Wardaugh, Duff, Wallace and Kelnar 1995(a)). As the knemometer measures the complete lower leg, it is possible that these changes may represent alterations in the composition of subcutaneous tissue, as well as changes in bone and cartilage turnover. Previous studies have shown anecdotal evidence of an inverse as well as a direct relationship between lower leg length (LLL) and Weight (Wt) (Hermanussen, Geiger-Benoit, Burmeister and Sippel 1989, Seidel, Schaefer, Walther and Scharer 1991).

The knemometer is increasingly being used to study short-term changes in LLL in conditions associated with substantial weight changes (Wales and Milner 1988, Ahmed, Crofton, Wade, Wallace and Kelnar 1995b) and in this study we have attempted to delineate the relationship of Wt to LLL by documenting these changes in a group of healthy children and adults.

2. Method

Between August 1993 and April 1994, 26 prepubertal children (Group A) aged 4.2-10 years (M:F, 12:14) and nine healthy adults aged 29-39 years (M:F, 5:4) had LLL and Wt measurements. Individual children were measured at the same time of the day, on a weekly basis for 12 weeks. In seven adults (Group B) knemometry was performed twice daily, about 8 hours apart for 6 weeks; in the remaining two (Group C) who were both women, measurements were performed once, 3 days a

week, for a period of 8 months. During the study one of the Group C subjects became pregnant.

Knemometry was performed by an observer-independent method, as previously described (Ahmed, Wallace and Kelnar 1995c). Subjects in Group A and B were measured by one observer (S.F.A.) whereas those in Group C were measured by three observers (S.F.A., B.W., J.D.). Weight was recorded with standardized clothing on the same Avery scales with an accuracy of 0.05 kg and a standard deviation of 0.05 kg from the mean for three repeated measurements. Standardization for ingestion, micturition and defaecation were not instituted, in order to facilitate the enlistment of subjects into the study.

3. Mathematical and statistical analysis

LLL and Wt changes were expressed as *fluctuations* (LLLf and Wtf) which represent the amount of absolute change (as opposed to relative change) that can be detected between consecutive measurements. In Group A the changes were expressed over weekly intervals and in Groups B and C the changes were expressed over daily intervals. Some Group A measurements were performed at 2-weekly intervals. In Groups B and C, data analysed for assessing change consisted only of measurements performed on consecutive days. Precision of knemometric measurements was expressed as technical error (TE) and represented 1 SD from the mean of three estimates of LLL. Normally distributed data were described by 95% confidence interval (95% CI) whereas skewed data were described by 5th and 95th percentile values (P5, P95). Spearman's correlation coefficient was used to assess any association between variables. Inter-group differences were assessed by the Wilcoxon signed-rank (WSR) test.

4. Results

4.1. Measurements and technical error

The number of measurements performed in Group A was 241; 110 pairs of measurements were performed in Group B and 123 measurements in Group C. The median TE was 0.13 mm (P5-0, P95-0.58).

4.2. LLL and Wt changes in Group A—children

Mean LLLf was 0.83 mm/week (95% CI, 0.74-0.91) and mean Wtf was 0.26 kg/week (95% CI, 0.23-0.29). In six out of the 26 children there was a direct relationship between Wt and LLL values (data not shown); none of the children had an inverse relationship. Cumulative data for the whole of Group A also showed a weak but highly significant positive relationship ($r^2 = 0.12$, $p = 0.0001$).

4.3. LLL and Wt changes in Group B—adults

Significant intra-daily variation was seen in only two individuals; both these individuals were female subjects. For longitudinal fluctuation analysis, the mean of each day's measurements in every individual was used. Median LLLf was higher in women at 0.16 mm/day (P5-0, P95-0.7) as compared to 0.1 mm/day (P5-0, P95-0.48) in men ($p = 0.02$). This was also true for median Wtf, which was 0.15 kg/day (P5-0, P95-0.54) as compared to 0.1 kg/day (P5-0, P95-0.5) in men ($p = 0.04$).

4.4. LLL and Wt changes in Group C—Pregnancy data

Figure 1 displays the changes in LLL and Wt seen in the two subjects in this group. The interval with no measurements enables comparison between pre-

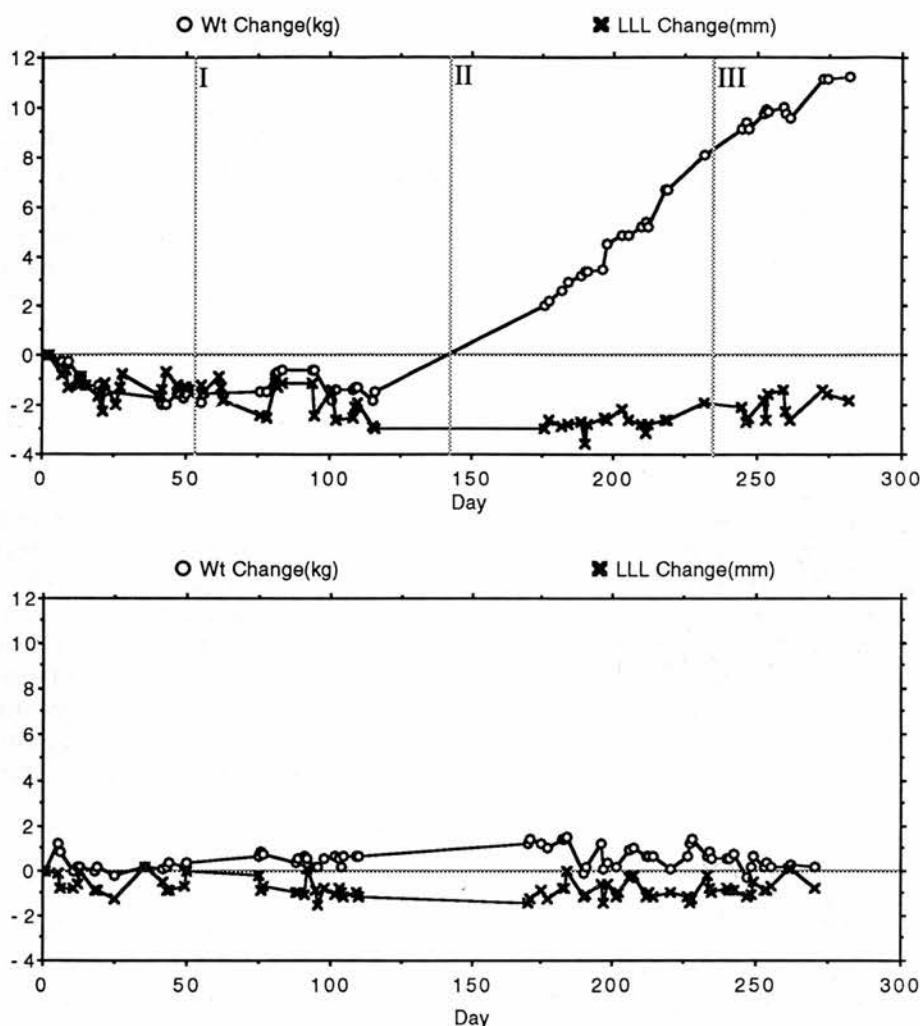


Figure 1. Changes in lower leg length (mm) and body weight (kg) from baseline seen in the pregnant (top panel) and the non-pregnant (bottom panel) subject in Group C. The Roman numerals denote the trimester.

confinement and peri-confinement data. As evident in Group B individuals, there was no significant relationship between day-to-day variation in Wt and LLL in either individual. However, over the duration of the pregnancy LLL reduced while Wt increased until a point was reached (beginning of third trimester, in this case) when LLL started to increase; this increase coincided with the appearance of leg oedema associated with the pregnancy.

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Zusammenfassung. Da das Knemometer zunehmend häufig benutzt wird, um Veränderungen in der unteren Beinlänge in Situationen zu analysieren, die mit Gewichtsveränderungen assoziiert sind, ist es sicherlich von großer Bedeutung, die Beziehung zwischen diesen beiden Variablen zu schildern. Die untere Beinlänge und das Gewicht wurden an 26 Kindern und neun Erwachsenen, darunter eine schwangere Frau, gemessen. Bei Kindern ließ sich ein schwacher aber positiver Zusammenhang zwischen der unteren Beinlänge und Gewichtsveränderungen beobachten. Tägliche Fluktuationen im Gewicht und in der unteren Beinlänge waren bei Frauen deutlicher ausgeprägt als bei Männern; die medianen Fluktuationen in der unteren Beinlänge betragen: 0.16 mm (P5-0, P95-0.7) bei Frauen, 0.1 mm (P5-0, P95-0.48) bei Männern, $p = 0.02$ im Wilcoxon signed-rank Test. Die medianen Fluktuationen im Gewicht betragen: 0.15 kg (P5-0, P95-0.54) bei Frauen und 0.1 kg (P5-0, P95-0.5) bei Männern, $p = 0.04$ (Wilcoxon signed-rank Test). Eine gleichmäßige Gewichtszunahme während der Schwangerschaft führte zu einer Abnahme der unteren Beinlänge, gefolgt von einer Zunahme, die mit dem Auftreten von Ödemen koinzidierte. Veränderungen in der unteren Beinlänge sind wahrscheinlich positiv mit Gewichtsveränderungen assoziiert, wenn letztere nur von moderater Größenordnung sind. Größere gleichmäßige Gewichtszunahmen haben jedoch vermutlich wegen einer direkten Kompression des unteren Beins einen entgegengesetzten Effekt auf die untere Beinlänge. Eine gebührende Betrachtung des Gewichts in Längsschnittstudien zur Veränderung der unteren Beinlänge ist wichtig, insbesondere in Situationen, die mit signifikanten Gewichtsveränderungen verbunden sind.

Résumé. Etant donné que le knémomètre est de plus en plus utilisé pour étudier les changements de longueur de la jambe dans des conditions associées avec les changements du poids, il est important de circonscrire clairement la relation existant entre ces deux variables. La longueur de la jambe et le poids ont été mesurés chez 26 enfants et 9 adultes dont une femme enceinte. Il y avait une association faible mais significative entre la longueur de la jambe et la fluctuation du poids chez les enfants. Les fluctuations quotidiennes en poids aussi bien qu'en longueur de la jambe, étaient plus élevées chez les femmes que chez les hommes; fluctuation médiane de la longueur de la jambe: femmes 0.16 mm (P5-0, P95-0.7); hommes 0.1 mm (P5-0, P95-0.48), $p = 0.02$ (Wilcoxon signed-rank test); fluctuation médiane du poids: femmes 0.15 kg (P5-0, P95-0.54); hommes 0.1 kg (P5-0, P95-0.5) $p = 0.04$ (Wilcoxon signed-rank test). Des gains de poids soutenus au cours de la grossesse conduisant à une réduction de la longueur de la jambe, suivie d'un accroissement coïncidant avec l'apparition d'un œdème associé. Les changements de la longueur de la jambe paraissent être positivement associés aux changements en poids lorsque ceux-ci sont de magnitude modérée. Cependant, de grands accroissements prolongés de poids sont susceptibles de produire un effet opposé sur la longueur de la jambe, par suite de la compression directe de la jambe. Une prise en considération du poids est essentielle dans les études longitudinales des changements de la longueur de la jambe, en particulier dans les conditions qui sont associées à des changements significatifs du poids.

Knemometry in childhood: a study to compare the precision of two different techniques

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Summary. Knemometry is an accurate and non-invasive method of quantifying lower leg length changes. It reveals multiple fluctuations in leg length velocity over a short period of measurement, and on the basis of this it has been proposed that short-term growth is saltatory rather than a continuous phenomenon. The technical error (TE) of the technique which is generally employed, and which is subject to observer bias, ranges from 0.09 to 0.16 mm. This study was undertaken to compare the original method (OM) to a modified technique which involved measuring from a baseline value of which the operator was not aware; this technique is referred to as the random zero method (RM). Over a period of 10 months, 58 subjects were measured on 413 occasions. Overall, median TE in the RM group at 0.15 mm (P5-0, P95-0.65) was higher than the median TE in the OM group at 0.11 mm (P5-0, P95-0.37). However, the median TE over the last 3 months of 0.15 mm (P5-0.05, P95-0.87) was lower than the TE in the preceding 4 months of 0.20 mm (P5-0, P95-0.55) (WSR, $p=0.04$) pointing towards the presence of an operator learning curve. The random zero method is a simple modification of the original method. It reduces observer bias but leads to a higher TE, which could explain some of the fluctuations seen between frequent knemometric measurements. Some knowledge of the length of the training period is important in the design of new studies involving knemometry; our data suggest that there should be a learning period of about 4 months if knemometry is performed as often as quoted above.

1. Introduction

Since the original description by Valk, knemometry has proven to be a very accurate and non-invasive technique of lower leg measurement (Valk, Langhout-Chabloz, Smals, Kloppenberg, Cassorla and Schutte 1983). Over a period of a few weeks it provides growth data which are non-linear, and the consensus among most investigators is that it is an inadequate tool for predicting long-term growth (Wales and Gibson 1994). Its main use seems to lie in assessing the effect of therapeutic interventions, such as steroid therapy, on short-term growth (Woithers and Pedersen 1992) and, indirectly, studying the physiology of growth.

The method of knemometry in children as described by the original investigators is now universally accepted (Valk *et al.* 1983). The technique is easy to acquire, and although some investigators have suggested that there should be a learning period (Hermanussen, Geiger-Benoit, Burmeister and Sippel 1988), there are no data available regarding how long it takes to become proficient. Using the Volk knemometer, which has an accuracy of 0.1 mm, the technical error (the mean standard deviation in a series of n independent measurements) (TE) has ranged between 0.09 mm and 0.16 mm in previous studies (Hermanussen 1988). As no modifications of the original method have been described, it is likely that the measurer in previous studies is not blind to the previous estimations in the set from which each measurement is derived.

Methods which employ 'blind' lower leg length estimations have been developed for the neonatal knemometer where the estimations are printed at some distance from

the measurer (Gibson, Pearse and Wales 1993). The TE has been reported to be higher at 0.3–0.5 mm, and a learning curve was also found using this method.

In this study we describe a simple method which further refines and validates the technique of knemometry in children. We also investigated further the learning period for the technique; information which is important for prospective users.

2. Patients and methods

Over the period between August 1993 and May 1994, 50 children aged 4.2–12.8 years, and eight healthy adults, had knemometry performed at the Royal Hospital for Sick Children in Edinburgh. The children consisted of 26 healthy volunteers from a neighbouring primary school, 18 children on cytotoxic chemotherapy and six children with cystic fibrosis. Individual children were measured at the same time of the day (within an hour's time interval from the first measurement), on a weekly basis, for periods ranging from 1 to 10 months, depending on the group. The adults were measured in the last 2 months of the study. Measurements were performed at either 1-weekly or 2-weekly intervals.

Each measurement was derived from a set of four 'estimations'; the most deviant of the four estimations was eliminated and the mean of the rest was calculated to provide 'measurement' (Wales and Milner 1987). Measurements were performed using two methods by a measurer (S.F.A.) who had been instructed by trained individuals.

The original method (OM) was performed as described previously (Hermanussen 1988). Briefly, the patient sits on a moveable and adjustable chair, the position of which is customized for each volunteer. The feet rest on a template of the feet drawn at the first visit and placed on a reference stool. A measuring platform connected to a Sony digiruler, when lowered onto the surface of the knee, measures the distance between the two reference points. The distance is displayed on the knemometer's digital analogue scale. Other controls present on the digiruler include a 'reset' button which is used for zeroing the device, and a 'preset' button which is for calibrating the digiruler.

The random zero method (RM) differed from OM in that the baseline reading on the preset meter was altered by the measurer before each estimation when the measuring platform was resting at zero. The preset meter was covered with a piece of paper when the alteration was made, so that the measurer was not aware of the baseline reading until the end of the estimation. Each estimation was recorded as a set of two readings, and deduction of the baseline from the actual digital read-out provided the estimation.

The study was approved by the local ethical committee and informed consent was given by all subjects and/or their parents.

3. Statistical analysis

The technical error (TE) for each measurement was derived from the standard deviation of the set of three estimations which make up each measurement as described in 'Methods'. The TE and coefficient of variation (CV) for each measurement were calculated and grouped with that of other measurements performed in the same month. The overall average TE and CV of the two groups (OM and RM) were presented as median values with their respective 5th and 95th percentile values (P5 and P95). The Wilcoxon signed-ranked test (WSR) test was used as a non-parametric test to compare differences between groups.

4. Results

A total of 413 measurements were performed over the 10-month period; the RM group consisted of 314 measurements and the OM group consisted of 99. The monthly breakdown of these measurements is detailed in table 1.

Table 1. Distribution of measurements according to method (random zero vs. original) and month of study. Figures in parentheses denote the number of measurements performed in adults.

	Random zero method	Original method
August	0	35
September	8	35
October	11	23
November	36	6
December	29	0
January	22	0
February	35	0
March	24	0
April	39(10)	0
May	110(60)	0
Total	314	99

Mean lower leg length velocity in the children was 0.39 mm/week (95%–CI 0.26–0.53). Median TE in the RM and OM group was 0.15 mm (P5–0, P95–0.65) and 0.11 mm (P5–0, P95–0.37), respectively. Median CV in the RM group was 0.06% (P5–0, P95–0.21), not significantly different from 0.06% (P5–0, P95–0.16) which was the CV in the OM group.

Adults were measured in the last 2 months of the study period by the random zero method only. Median TF was 0.36 mm (P5–0, P95–0.7) in April and 0.12 mm (P5–0, P95–0.58) in May.

TE and CV reduced in both groups over the period of study (table 2). To analyse any trend the values for the September and October subgroups in the RM group were excluded from evaluation as they consisted of relatively small samples, as was the November subgroup of the OM group. The TE reduced over the first 4 months when RM was used on a substantial number of occasions (table 2).

Table 2. Median monthly technical error (TE) and coefficient of variation (CV) of measurements performed by the original method (OM) and the random zero method (RM); 5th and 95th percentile values in parentheses.

	TE-RM	TE-OM	CV-RM	CV-OM
August		0.15 (0.03–0.55)		0.04 (0–0.27)
September	0.13 (0.06–0.2)	0.12 (0.03–0.31)	0.06 (0.02–0.09)	0.07 (0–0.15)
October	0.11 (0.01–0.47)	0.1 (0.04–0.28)	0.04 (0–0.06)	0.04 (0.01–0.14)
November	0.17 (0.08–1.05)	0.17 (0–0.30)	0.10 (0.02–0.4)	0.10 (0–0.10)
December	0.20 (0.08–0.36)		0.10 (0.02–0.22)	
January	0.20 (0.05–0.4)		0.10 (0.02–0.2)	
February	0.16 (0.05–1.15)		0.08 (0.02–0.40)	
March	0.13 (0.05–1.0)		0.06 (0.02–0.31)	
April	0.14 (0–0.58)		0.06 (0–0.18)	
May	0.11 (0–0.59)		0.04 (0–0.13)	

Differences of TE and CV between consecutive periods of measurement did not reach significance because of the wide distribution of the values (figure 1). However,

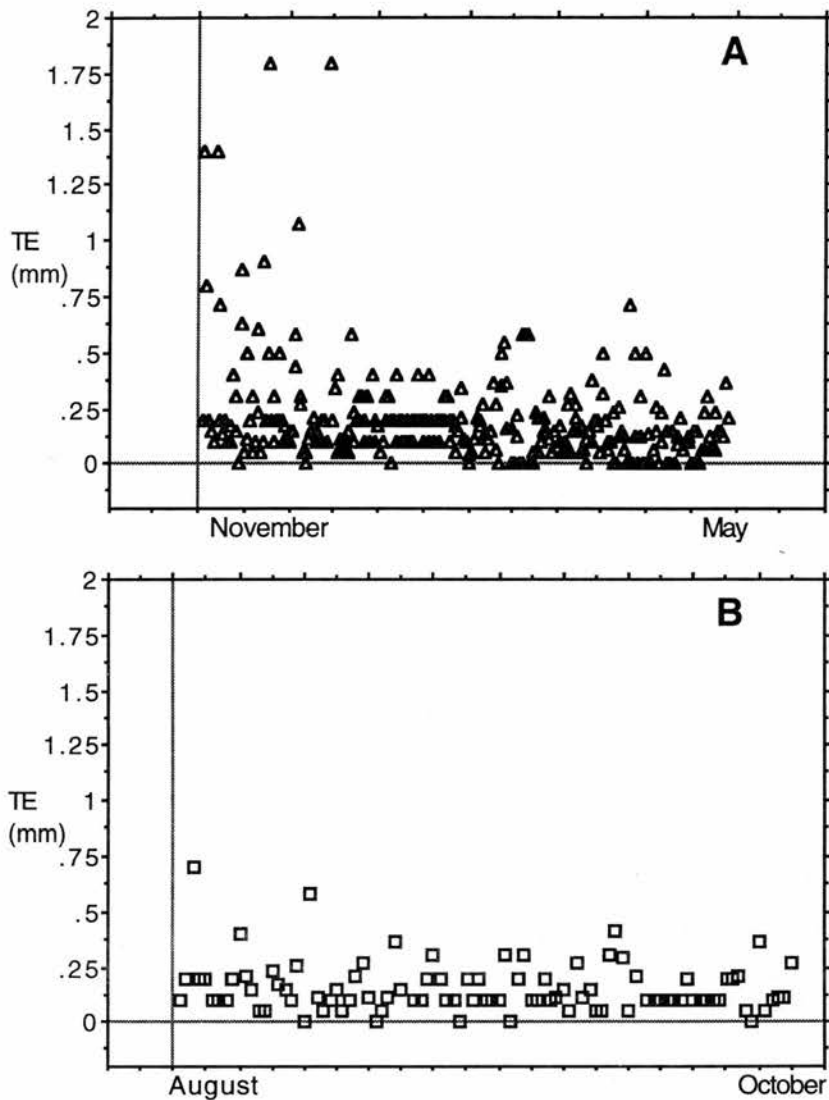


Figure 1. Scattergram of individual TE values of the random zero method (A) and the original method (B) plotted in chronological fashion.

grouping together the TE values for the first 4 months (November, December, January, February) revealed a median value of 0.2 mm (P5-0.05, P95-0.87) which was significantly higher than the TE for the following 3 months at 0.15 mm (P5-0, P05-0.55) ($p=0.04$, WSR).

5. Discussion

The physiology of short-term changes in human growth is still poorly understood. Although instruments such as the knemometer might shed light on changes not very far from the cellular level, their limitations, as well as their advantages, must be

thoroughly assessed. Knemometry, as performed traditionally, is subject to intra-observer bias which arises as the measurer is aware of the previous estimation in the series. A reported TE of 0.09 mm (Hermanussen 1988) is comparable to more precise techniques such as kyniklometry (Hermanussen, Bugiel, Aronson and Moell 1992) in which reproducible estimations are more probably due to better localization of reference points of measurement.

The RM technique in this study eliminates the observer bias very simply. It can be performed without any structural modification to the original knemometer, and the measuring process takes no longer than OM. However, there is one extra step: deducting the baseline reading from the initial estimation to provide the real estimation.

Another advantage of RM is that it provides a better guide to the length of the training period alluded to in previous studies (Hermanussen *et al.* 1988). We saw a trend towards lower TE in the RM as well as the OM groups, but this was significant only for the TE values in the former group. This trend might have become statistically significant by the high outlying values at the beginning of the study, but the fact that there were more TE values near zero by the end of the study supports the suggestion of a real learning curve (figure 1). The lower TE values in the last 2 months were not due to the introduction of adults into the study, as the TE for that specific subgroup was slightly higher than the overall value for that month. The low TE values for the first 2 months in the RM group were unrepresentative as the sample size was small and the RM measurements were also being performed with extra care, as it was a newly introduced method. Subjectively, RM took much longer initially than the latter months.

On the basis of our results we would recommend a period of 3–4 months for learning, if the knemometer is used as frequently as described above. By using RM operators can also evaluate their own technique, looking for an improvement and subsequent plateauing of the TE. To improve the measurement process further, it would be helpful for the operator if the knemometer could be electronically adapted so that the series of estimates could be stored, the most deviant estimate omitted and a TE calculated instantly. If the latter is above an arbitrary limit, then there is scope to immediately repeat the measurements.

To summarize, we have described a new but simple random zero method of performing knemometry which reduces observer bias on the part of the measurer but which does have a higher TE than reported before. This will clearly have implications on interpreting patterns of short-term growth with multiple fluctuations which might well be an artifact of measurement rather than a real phenomenon. The method allows independent evaluation of operator proficiency. This knowledge of the length of the training period would help new investigators who are designing studies involving knemometry.

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Zusammenfassung. Knemometrie ist eine exakte und nicht invasive Methode zur Quantifizierung von Veränderungen in der unteren Beinlänge. Sie läßt multiple Fluktuationen in der Wachstumsgeschwindigkeit der Beinlänge über kurze Meßintervalle erkennen. Auf dieser Basis wurde vermutet, daß Kurzzeitwachstum nicht kontinuierlich verläuft, sondern saltatorisch, d.h. in Sprüngen. Der technische Fehler (TE) dieser Technik, der generell verwendet wird und der Gegenstand des Untersucherbias ist, variiert von 0.09 bis 0.16 mm. Diese Studie dient dazu, die Originalmethode (OM) mit einer modifizierten Technik zu vergleichen, die darauf basiert, daß von einem Basiswert gemessen wird, der dem Untersucher nicht bekannt ist. Diese Technik wird als Random Zero Methode (Methode mit einem zufälligen Nullwert, RM) bezeichnet. Über einen Zeitraum von 10 Monaten erfolgten an 58 Individuen 413 Messungen. Insgesamt war der mediane TE in der RM-Gruppe mit 0.15 mm (P5-0, P95-0.65) größer als der mediane TE in der OM-Gruppe mit 0.11 mm (P5-0, P95-0.37). Der über die letzten 3 Monate gemittelte TE: 0.15 mm (P5-0.05, P95-0.87) war jedoch geringer als der in den vorhergehenden 4 Monaten: 0.20 mm (P5-0, P95-0.55) (WSR, $p=0.04$), was für die Präsenz eines Lerneffekts beim Untersucher spricht. Die RM-Methode stellt eine einfache Modifikation der Originalmethode dar. Sie reduziert den Untersucherbias, führt jedoch zu einem höheren TE was einige Fluktuationen erklären könnte, die bei häufiger Anwendung knemometrischer Messungen beobachtet werden können. Kenntnisse über die Länge der Trainingsphase sind für das Design neuer knemometrischer Studien von Bedeutung. Die vorliegenden Daten sprechen dafür, daß die Länge der Trainingsphase etwa 4 Monate betragen sollte, wenn die Knemometrie so oft, wie hier erwähnt, durchgeführt werden soll.

Résumé. La knémométrie est une méthode rigoureuse et non invasive de quantification des modifications de la jambe. Elle a révélé de multiples fluctuations dans la vélocité de croissance de la jambe sur une courte période et cela a conduit à comprendre la croissance au cours de petites périodes comme un phénomène saltatoire plutôt que continu. L'erreur technique (ET) produite par le biais inter-observateur de la méthode de mesure généralement employée est de 0.09 à 0.16 mm. Cette étude a été entreprise dans le but de comparer la méthode originale (MO) à une technique modifiée: 'méthode zéro aléatoire' (MA) qui implique des mesures à partir d'une valeur de base dont l'opérateur n'est pas informé. 58 sujets ont été mesurés à 413 reprises sur une période de 10 mois. L'ET médiane de 0.15 mm (P5-0, P95-0.65) dans le groupe MA est plus élevée que l'ET médiane du groupe MO à 0.11 mm (P5-0, P95-0.37). Cependant, l'ET médiane des trois derniers mois, de 0.15 mm (P5-0.05, P95-0.87) est plus basse que l'ET des quatre mois précédents, de 0.20 mm (P5-0, P95-0.55) (WSR, $p=0.04$), évoquant la présence d'une courbe d'apprentissage de l'opérateur. La méthode zéro aléatoire est une simple modification de la méthode originale. Elle réduit le biais du à l'observateur, mais conduit à une ET plus élevée, ce qui pourrait expliquer quelques unes des fluctuations constatées entre mesures knémométriques fréquentes. La connaissance de la durée de la période d'entraînement est importante dans l'organisation de nouvelles études de knémométrie. Nos données suggèrent qu'elle devrait être d'environ 4 mois, si la knémométrie est faite aussi fréquemment que décrit ci-dessus.

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Biochemical Markers of Bone Turnover

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Key Words

Acute lymphoblastic leukaemia
Bronchopulmonary dysplasia
Chemotherapy
Corticosteroids
Growth hormone
Preterm infants

Abstract

Three studies to evaluate procollagen type I C-terminal propeptide, type I collagen cross-linked telopeptide and bone alkaline phosphatase (BALP) in the assessment of bone turnover and growth in children are presented. (1) In 50 short normal children treated with placebo or growth hormone, Δ BALP after 3 months of treatment was highly correlated with height velocity response after 1 year ($r = 0.67$, $p < 0.0001$). (2) In 12 children with acute lymphoblastic leukaemia, marked changes in collagen peptides, BALP, and lower leg length velocity were seen during the first 6 months of chemotherapy. Suppression occurred during induction and the two intensification phases, with catch-up during the intervening phase (paired t-tests, $p < 0.001$). (3) Fourteen babies (birthweight $< 1,500$ g) treated with high-dose dexamethasone for bronchopulmonary dysplasia were compared with 25 non-steroid-treated babies $< 1,500$ g. Both collagen peptides decreased rapidly and dramatically (mean decreases 41–68%) after dexamethasone was started, accompanied by weight loss and lower leg shrinkage and followed by recovery during steroid weaning.

Introduction

Height velocity is central to growth assessment in children but is operator-dependent, insensitive, and imprecise over short time periods [1]. Knemometry overcomes these disadvantages and has attracted much interest, but it ignores spinal growth and may be influenced by the hydration state of overlying soft tissues. All anthropometric techniques require a relatively fit, co-operative child. Biochemical markers of growth are virtually free from observer bias, reflect whole-body growth rather than simply that of the lower leg, may be used over short or long sampling intervals, and have the bonus of giving insight into the mechanisms of growth variations.

Linear growth occurs predominantly at the epiphyseal growth plate of the long bones, and is accompanied by extensive modelling of bone. Osteoblastic bone formation involves deposition of type I collagen, followed by mineralization and maturation during which stable crosslinks between collagen fibrils are formed. Procollagen type I C-terminal propeptide (PICP) is released into the circulation by proliferating osteoblasts during collagen biosynthesis [2]. The crosslinked telopeptide of type I collagen (ICTP) is released into the circulation by collagen breakdown during bone modelling [2]. Bone alkaline phosphate (BALP) is found in the hypertrophic chondrocytes of the epiphyseal growth plate, in the matrix vesicles associated with bone mineralization and in mature osteoblasts [3, 4].

We have recently reported a close quantitative relationship between serial measurements of BALP and height velocity in short normal children undergoing growth hormone treatment [5].

We present here the results of three studies in which we have evaluated PICP, ICTP, and BALP in the assessment of growth and bone turnover in children. All studies were approved by the local ethical committee; informed consent was obtained from parents and, where appropriate, children.

Methods

PICP and ICTP were measured by radioimmunoassay (Orion Diagnostica, Espoo, Finland) and BALP by wheat germ lectin affinity electrophoresis [6].

Short Normal Children

Fifty short normal children were treated with placebo (6), human recombinant growth hormone (GH) alone (32), GH plus oxandralone (8), or GH plus testosterone (4). Dosages were: GH 15 or 24 IU/m²/week divided into daily subcutaneous injections; oxandralone 2.5 mg/day orally; testosterone undecanoate 40 mg orally on alternate days. All of the treatment groups except placebo showed an early significant increase in each of the markers after 3 months of treatment and in height velocity after a year: $p < 0.001$ for GH alone, $p < 0.05$ for the smaller treatment groups (paired t-tests). The increment in BALP after 3 months gave the best correlation with height velocity after 1 year ($r = 0.67$, $p < 0.0001$ compared with $r = 0.54$, $p < 0.0001$ for PICP and $r = 0.41$, $p = 0.02$ for ICTP). The timing of the 3-month sample may have influenced which markers gave the best prediction, as PICP and ICTP may respond earlier than BALP to GH treatment. This study suggested that BALP in particular might provide a tool for targeting expensive and invasive growth hormone treatment to those children most likely to respond with a medium-term improvement in growth.

Children on Chemotherapy

There is limited evidence that cytotoxic chemotherapy may have a detrimental effect on growth [7] but the precise timing, extent, and aetiology of any growth deficit are unknown. As the current national chemotherapy protocols [UKALL XI (92)] used to treat children with acute

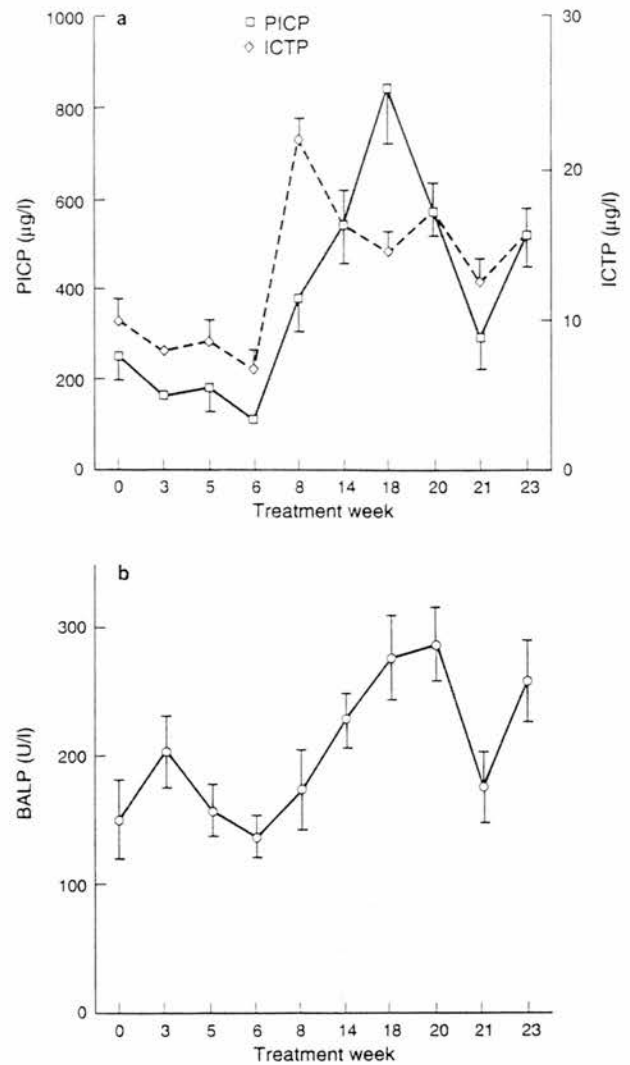


Fig. 1. Bone turnover markers (mean and SE) in 12 patients with acute lymphoblastic leukaemia during the first 6 months of chemotherapy: (a) PICP and ICTP; (b) BALP. Induction was from week 1–5, first intensification during week 5, continuing chemotherapy from weeks 6–20 and second intensification during week 20, with resumption of continuing chemotherapy from week 21. The samples from weeks 5 and 20 were each taken before intensification; the samples from weeks 6 and 21 were each taken after intensification. Reference ranges were as follows: PICP 200–550 µg/l; ICTP 3.5–20.0 µg/l; BALP 180–600 U/l.

lymphoblastic leukaemia employ more intensive chemotherapy schedules than those previously investigated [7], it is important to establish the cost of any increased survival to the children in terms of growth impairment, the phases of chemotherapy that contribute most to any

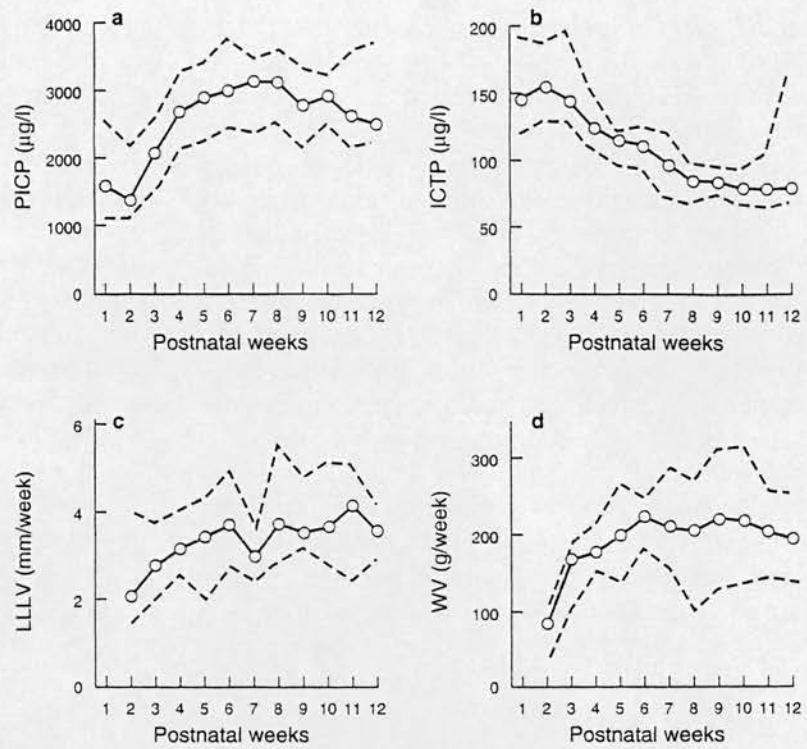


Fig. 2. Median and interquartile ranges of (a) PICP, (b) ICTP, (c) lower leg length velocity (LLV), and (d) weight velocity (WV) in 25 infants of birthweight less than 1,500 g, who never received steroids.

growth deficit and the underlying mechanisms of these growth changes. This information may be of value in planning future management protocols on a rational basis. In a prospective longitudinal study of 12 children with acute lymphoblastic leukaemia, marked and significant changes in collagen peptides and BALP were seen during the first 6 months of chemotherapy (fig. 1). At diagnosis, PICP and BALP were generally below or close to the lower limit of the reference range, suggesting impairment of bone growth by the disease process itself. During induction and first intensification, the collagen markers were progressively suppressed, whereas BALP showed initial recovery before subsequent suppression. We postulate that these changes were largely due to the prednisolone component of chemotherapy which was given continuously (40 mg/m² orally) throughout induction and first intensification. Glucocorticoids are known to have an inhibitory effect on collagen synthesis and degradation and on the cartilage zones of the growth plate [8–10]. After first intensification (when prednisolone was withdrawn), a dramatic catch-up was observed in all bone markers, with the collagen markers reaching supranormal concentrations (paired t-tests, $p < 0.001$). During second

intensification, all markers were again suppressed ($p < 0.05$), followed by a second catch-up ($p < 0.05$). This study illustrates the limitations of knemometry, as many children were too young for the technique. However, in those for whom lower leg velocity was available, it paralleled the changes in PICP and BALP.

Preterm Infants

Among babies who develop bronchopulmonary dysplasia (BPD), high-dose steroids are used with increasing frequency, but no controlled trials have been carried out to justify such high dosages. Their effect on bone metabolism and growth in these infants is unknown. Knemometry provides one tool for the investigation of short-term effects of steroids on growth but, like weight, it may also be influenced by their diuretic effects. Mineral balance studies are cumbersome, impractical, and indirect measures of bone metabolism. There is a need for simple robust biochemical markers that will provide surrogate measures for the effects of steroids on bone turnover and weighing less than 1,500 g at birth, who developed bron-

chopulmonary dysplasia and were treated with high-dose dexamethasone (500 µg/kg/day for 3 days, followed by gradually decreasing doses for various periods), were compared with 25 non-steroid-treated babies weighing less than 1,500 g over the first 15 weeks of life. All preterm babies had very high plasma concentrations of collagen markers compared with older children, indicating very rapid turnover of bone. In non-steroid-treated babies, PICP, lower leg length velocity (as measured by serial knemometry), and weight velocity all increased to a plateau over the first few weeks of life while ICTP decreased, indicating a net increase in bone formation (fig. 2). After dexamethasone was started in the steroid-treated group, both PICP and ICTP decreased rapidly and dramatically (mean decreases 41% and 68% respectively), accompanied by slowing of radial growth, weight loss, and lower leg shrinkage. Bone markers and anthropometric indices recovered during steroid weaning, followed by marked catch-up in some cases. There was evidence that both weight and knemometry were affected by marked fluid

shifts occurring during steroid administration and weaning. Under these circumstances collagen markers are likely to provide a more reliable index of growth than anthropometric measures.

Conclusions

In the first of the studies described here, BALP was helpful in giving an early prediction of growth response to GH treatment. In the other two, the markers of bone collagen turnover were of particular value in assessing acute changes in bone turnover and growth in response to clinical interventions.

Acknowledgements

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Collagen markers and bone alkaline phosphatase as predictors of bone turnover and growth

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Abstract. Three studies to evaluate procollagen type I C-terminal propeptide (PICP), type I collagen cross-linked telopeptide (ICTP), procollagen type III N-terminal propeptide (P3NP) and bone alkaline phosphatase (BALP) in the assessment of bone turnover and growth in children are presented.

Studies and Results. 1) In 50 short normal children treated with placebo or growth hormone (alone or combined with oxandalolone or testosterone), Δ BALP and Δ P3NP after 3 months were each highly correlated ($p < 0.001$) with height velocity response after 1 year and together predicted 61% of its variability (ANOVA, $p < 0.0001$).

2) In a longitudinal study, 14 babies (birth weight $< 1,500$ g) treated with high-dose dexamethasone for bronchopulmonary dysplasia were compared with 25 non-steroid-treated babies $< 1,500$ g. All collagen peptides decreased rapidly and dramatically (mean decreases 41–68%) after dexamethasone was started, accompanied by weight loss and lower leg shrinkage, followed by recovery during steroid weaning.

3) In 12 children with acute lymphoblastic leukaemia, marked and significant changes in collagen peptides and BALP were seen during the 1st 6 months of chemotherapy. Suppression occurred during induction and the two intensification phases, with catch-up during the intervening phase ($p < 0.001$).

Conclusions. Collagen peptides and BALP were of value in predicting growth response to treatment and in assessing acute changes in bone turnover and growth in response to clinical interventions.

Key words: acute lymphoblastic leukaemia, bronchopulmonary dysplasia, chemotherapy, corticosteroids, growth hormone, preterm infants.

Background

Measurement of height velocity (HV) is the mainstay of growth assessment in children but it does have limitations, being operator- and instrument-dependent, insensitive and imprecise. It provides an integrated assessment of growth over a period, but cannot be used reliably for clinical situations in which growth may fluctuate over periods of days or weeks. More recently, knemometry has allowed much more short-term assessment of growth. However, knemometry ignores spinal

growth and may be influenced by the hydration state of the soft tissues overlying the bone. Moreover, there is a gap between infancy and around 4 years of age, during which knemometry is not currently possible. All anthropometric techniques require a relatively fit, cooperative child.

Well-chosen biochemical markers of growth may theoretically overcome many of these disadvantages. They are virtually free from observer bias and their precision is independent of the frequency of sampling. They reflect whole-body growth rather than simply that of the lower leg, may be used over short or long sampling intervals and have the bonus of giving insight into the mechanisms of growth variations.

Linear growth occurs predominantly at the epiphyseal growth plate of the long bones, and is accompanied by extensive modelling of bone. Osteoblastic bone formation involves deposition of type I collagen, followed by mineralisation and maturation during which stable cross-links between collagen fibrils are formed. Procollagen type I C-terminal propeptide (PICP) is released into the circulation by proliferating osteoblasts during collagen biosynthesis [1]. The cross-linked telopeptide of type I collagen (ICTP) is released into the circulation by collagen breakdown during bone modelling [1]. Bone alkaline phosphatase (BALP) is found in the hypertrophic chondrocytes of the epiphyseal growth plate, in the matrix vesicles associated with bone mineralisation and in mature osteoblasts [2,3]. We have recently reported a close quantitative relationship between serial measurements of BALP and HV in short normal children undergoing GH treatment [4]. Type III collagen is not present in bone but is widely distributed in the interstitial fibrils of soft tissue; procollagen type III N-terminal propeptide (P3NP) is released during soft tissue growth [5].

We present here a brief outline of some of our recent studies on the relationship between these markers, clinical interventions and growth. All studies were approved by the local ethical committee; informed consent was obtained from parents and, where appropriate, children.

BALP and collagen markers as early predictors of HV response to treatment in short normal children

Introduction

Studies on small mixed groups of GH-deficient and short normal children treated with GH suggested that early measurements of PICP [6,7] or P3NP [8,9] gave good correlations with HV response after 1 year. Our aim in this study in short normal children was to compare PICP, P3NP, ICTP and BALP as early predictors of HV response to treatment with GH alone or in combination with oxandratone or testosterone.

Patients and Methods

Fifty prepubertal children (8 girls) were treated with placebo (6), human recombinant

GH alone (32), GH plus oxandratone (8) or GH plus testosterone (4). Dosages were: GH 15 or 24 U/m² per week divided into daily subcutaneous injections, oxandratone 2.5 mg/day orally, testosterone undecanoate 40 mg orally on alternate days. Fifteen children attained a testicular size ≥ 8 ml or breast stage > 1 during the 1st year of treatment. HV was calculated for the year prior to treatment and again after one year of treatment. We collected blood at baseline and again after 3 months of treatment. BALP was measured by wheat germ lectin affinity electrophoresis [10], PICP and ICTP were measured by radioimmunoassay (Orion Diagnostica, Espoo, Finland); P3NP was also measured by radioimmunoassay (Behringwerke AG Diagnostica, Marburg, Germany). Responses to treatment were expressed as the change compared with pretreatment values.

Results

All of the treatment groups except placebo showed a significant increase (paired *t* tests) in each of the markers after 3 months and in HV after 12 months: $p < 0.001$ for GH alone, $p < 0.05$ for the smaller treatment groups. Increments in all biochemical markers after 3 months were significantly correlated with HV increments after 1 year of treatment: *r* values were 0.70 for BALP ($p < 0.0001$), 0.58 for P3NP ($p = 0.0002$), 0.54 for PICP ($p = 0.003$) and 0.42 for ICTP ($p = 0.09$). Table 1 shows the discriminatory powers of the markers in predicting the HV response. Multiple linear regression with stepwise selection of variables identified ABALP and AP3NP as the only two independent variables that contributed significantly to the prediction of Δ HV (ANOVA, $p < 0.0001$). Together they predicted 61% of the variability in HV response after a year. In comparison, the interim HV response after 3 months predicted 53% of the variability.

Discussion

BALP and P3NP were highly sensitive markers that gave almost identical discrimination between "responders" and "nonresponders", defined as an HV increment of greater or less than 2 cm/year after 1 year of treatment. Lack of a 3-month increase in these markers almost certainly meant that there would be no adequate HV response by the end of the year. Wales and Gibson have discussed in detail the difficulties in

Table 1. Sensitivity, specificity and predictive values for each biochemical marker, at the optimum cut-off concentration shown, in predicting an HV increment of ≥ 2 cm/year after 1 complete year of treatment.

Marker	Cut-off	Sensitivity (%)	Specificity (%)	Predictive values (%)	
				Positive result	Negative result
ABALP	50 U/l	94	43	81	75
AP3NP	0.4 U/ml	94	50	83	78
APICP	50 pg/l	79	46	79	46
AICTP	2 pg/l	70	56	80	42

predicting long-term growth from short-term measurements, whether biochemical or auxological [11]. This study confirms that complete prediction may be an unattainable goal, but early biochemical measurements were better than auxological measurements, even when the latter were made under ideal research conditions. The timing of the 3-month sample may have influenced which markers gave the best prediction: there are grounds for suspecting that PICP and ICTP might respond sooner than BALP to GH treatment. We conclude that biochemical markers provide a tool for targeting expensive and invasive GH treatment to those children most likely to respond with a medium-term improvement in growth. No conclusions can be drawn about their value in predicting final height.

Collagen markers and growth in preterm infants

Introduction

Among babies who develop bronchopulmonary dysplasia (BPD), high-dose steroids are used with increasing frequency at ever earlier stages. No controlled trials have been carried out to justify the use of such high dosages and there are worries that their use may have widespread deleterious effects, including those on bone metabolism and growth. Knemometry provides one tool for the investigation of short-term effects of steroids on growth but, like weight, may also be influenced by their diuretic effects. Mineral balance studies have been carried out in a research setting, but are cumbersome, prone to error and unsuited to routine use. There is a need for simple and robust markers of bone turnover that will provide surrogate measures of the effects of steroids on bone turnover and growth. Once such markers have been validated, they may be used to assess the optimum dosage of steroids that will maximise therapeutic benefit and minimise adverse effects on bone metabolism. We report a pilot study designed to investigate collagen markers and growth in preterm babies treated with high-dose dexamethasone compared to control preterm babies who did not receive steroids.

Patients and Methods

Thirty-nine infants < 1,500 g at birth were studied over the 1st 15 weeks of life. Treatment decisions were based on clinical criteria. Fourteen babies who developed severe BPD were treated with the standard therapeutic dose of dexamethasone (500 µg/kg/day for 3 days, followed by gradually decreasing doses for various periods). Another thirteen babies developed BPD but did not receive dexamethasone. Twelve babies had neither BPD nor dexamethasone. Blood for collagen markers was collected weekly, except that an extra sample was taken approximately 3 days after starting dexamethasone. Weight and lower leg length (using a neonatal knemometer) were also measured weekly until dexamethasone was started, after which they were measured daily. PICP, ICTP and P3NP were all measured by radioimmunoassay (Orton Diagnostica, Espoo, Finland).

Results

Figure 1 shows the postnatal changes in the markers and in LLLV and WV in the infants who were not treated with steroids. Median LLLV and WV showed high positive correlations with median PICP ($r = 0.74$, $p = 0.01$, and $r = 0.92$, $p < 0.0001$, respectively) and negative correlations with median ICTP ($r = -0.85$, $p = 0.001$, and $r = -0.79$, $p = 0.004$, respectively). Before dexamethasone treatment was started, babies in the steroid group did not differ significantly in terms of LLLV, WV or collagen marker concentrations from those babies who developed BPD but were not treated with steroids. A precipitate decrease in all markers, LLLV and WV occurred

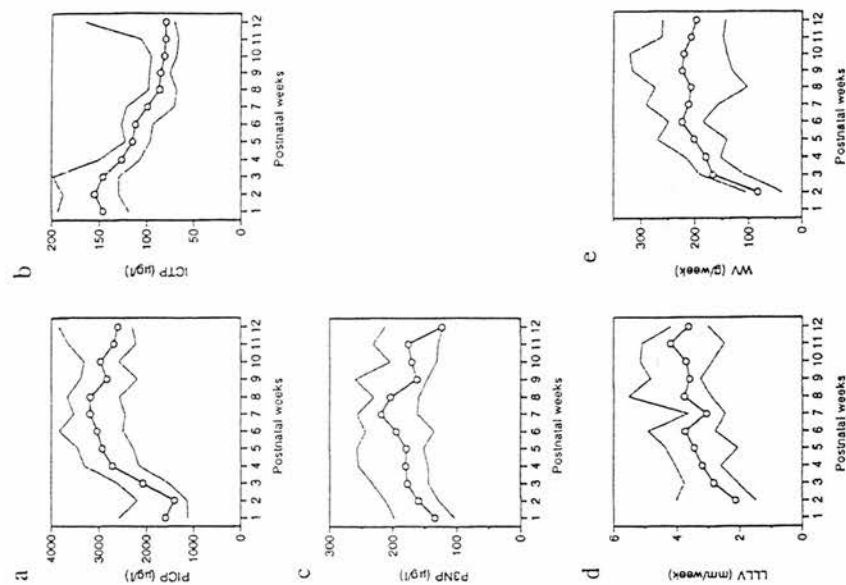


Fig. 1. Median and interquartile ranges of a) PICP; b) ICTP; c) P3NP; d) LLLV; and e) WV in 25 infants of birth weight < 1,500 g, who never received steroids.

Table 2. Initial decrease (%) in biochemical markers and anthropometric indices in response to dexamethasone administration^a.

	Mean	SD	SE	t	P
PICP	-41.1	22.2	4.8	5.49	<0.0001
ICTP	-67.5	13.2	3.0	7.50	<0.0001
P3NP	-47.7	31.9	7.0	6.60	<0.0001
LLLV ^b	-244.7	168.0	38.6	9.83	<0.0001
WV ^b	-290.0	310.6	80.2	7.11	<0.0001

^aTwenty-one separate episodes of dexamethasone administration in 14 babies. Second or third episodes were only included if they were separated from the previous episode by clear steroid-free intervals during which recovery of markers and anthropometric indices occurred. There were no significant differences between successive episodes for any of the measurements.

^bMaximum daily steroid-induced decrease.

within approximately 3 days of starting dexamethasone (Table 2). Marked day-to-day fluctuations in both WV and LLLV occurred after starting steroid treatment but overall there was dramatic early weight loss with shrinkage of the lower leg. LLLV, WV and markers began to increase while the babies were being weaned from steroids: the median dexamethasone doses at which recovery occurred are shown in Table 3. In several babies, collagen markers and anthropometric indices showed marked catch-up after stopping steroids.

Discussion

Preterm babies had very high plasma concentrations of collagen markers (an order of magnitude greater than those found in growing children) indicating that their bones

Table 3. Doses of dexamethasone ($\mu\text{g}/\text{kg}/\text{day}$) at which recovery of anthropometric and collagen measurements occurred during steroid weaning^a.

	PICP	ICTP	P3NP	LLLV	WV
Partial recovery ^b					
Median	220	180	180	270	250
Interquartile interval	173-270	88-220	138-270	200-300	185-300
Full recovery ^c					
Median	0	0	50	178	140
Interquartile interval	0-95	0-0	0-100	50-248	60-2

^aTwenty-four episodes of steroid administration in 13 babies (no significant differences between successive episodes of dexamethasone treatment).

^bPartial recovery was defined as the dose of dexamethasone during steroid weaning at which measurements began to increase after having reached their nadir.

^cFull recovery was defined as the dose of dexamethasone during (or after) steroid weaning at which measurements regained the median value for equivalent postnatal age in 25 babies (birth weight < 1,500 g) who had never been treated with steroids.

and soft tissues were turning over very rapidly. The increase of PICP and decrease of ICTP during the postnatal period suggest an increase in net bone formation and this was confirmed by parallel increases in LLLV and WV, with high correlations between the median values through time. Dexamethasone treatment resulted in a precipitate decrease in all collagen markers suggesting that bone turnover had virtually ceased, and this was confirmed by slowing of growth in the radius of these babies (data not shown). This is in agreement with the reported *in vivo* and *in vitro* inhibitory effects of glucocorticoids on collagen formation and breakdown [12,13]. Fluid shifts following steroid administration may well have made a major contribution to the dramatic and acute fluctuations seen in LLLV and WV. Under these circumstances, collagen markers are likely to provide a more reliable index of growth than anthropometric measures. During steroid weaning, the recovery of LLLV and WV generally preceded that of the collagen markers, but again this may have been influenced by fluid shifts. The partial recovery of the collagen markers as the dexamethasone dose was reduced suggests that its side-effects on bone may be dose-related. A follow-up study is planned to randomise babies to different initial dosages of dexamethasone with a standardised weaning schedule in order to determine the optimum dosage in these vulnerable infants.

BALP and collagen markers during chemotherapy in children with acute lymphoblastic leukaemia

Introduction

The majority of children with acute lymphoblastic leukaemia survive to adulthood but impaired growth has been a frequent complication of treatment. The long-term effects of cranial irradiation on growth have been extensively studied [14], and this mode of treatment is now only used in a small selected number of cases. There is limited evidence to suggest that cytotoxic chemotherapy may also have a significant detrimental effect on growth [15]. No studies to date have investigated the precise timing and extent of any growth deficit in relation to chemotherapy, nor on biochemical markers that may reflect this and give some insight into the aetiology. Since the current national chemotherapy protocols (UKALL XI (92)) employ more intensive chemotherapy schedules than those previously investigated [15], it is important to establish 1) the cost of any increased survival to the children in terms of growth impairment; 2) the phases of chemotherapy that contribute most to the growth deficit; and 3) the underlying mechanisms of these growth changes. This information may be of value in planning future management protocols on a rational basis. The present study was undertaken to evaluate the longitudinal changes in biochemical markers and growth during and after chemotherapy. Here we report the interim results on 12 children who have completed the 1st 6 months of chemo-

Twelve children (nine boys and three girls) with a mean age of 4.6 years (range 1.2–13.5 years) with acute lymphoblastic leukaemia and following the UKALL XI (92) protocol completed at least 6 months of chemotherapy (none received cranial irradiation during the period of measurement). A schematic representation of the treatment regime together with the blood sampling intervals is shown in Fig. 2. PICP, ICTP and P3NP were measured by the blood sampling intervals in Fig. 2. PICP, Finland) and BALP was quantified by lectin affinity electrophoresis [10]. In two children, weekly krenometry allowed calculation of LLLV.

Results

Figure 3 shows the changes in the biochemical markers during induction, first intensification, continuing chemotherapy and second intensification. During the "catch-up" phase following completion of first intensification at week 6, there were significant increases in PICP, ICTP, BALP and P3NP ($p < 0.001$ by paired *t* tests). Second intensification resulted in a decrease in all markers ($p < 0.05$), followed by a second catch-up ($p < 0.05$). In two children who underwent krenometry, there was a close parallel relationship between the markers and LLLV (data not shown).

Discussion

BALP and PICP at diagnosis were generally below or close to the lower limit of the reference range, suggesting impairment of bone growth by the disease process itself.

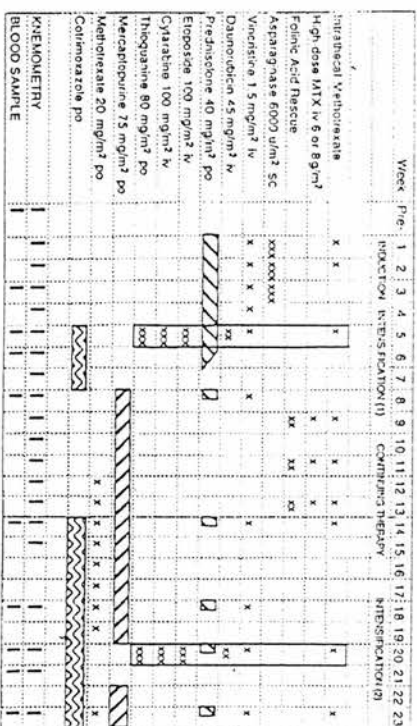


Fig. 2. Schematic representation of the high-dose methotrexate (MT) treatment group of the UKALL XI (92) chemotherapy protocol, showing blood sampling and krenometry intervals. Other treatment groups were similar during the first 6 months, except that high-dose MT and folic acid rescue were omitted.

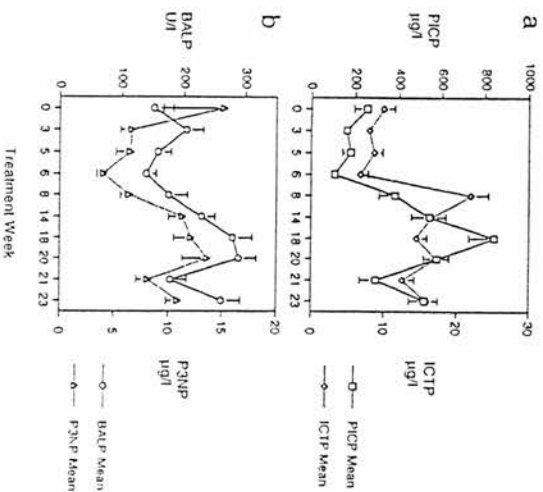


Fig. 3. Biochemical markers (mean and SEM) in 12 patients with acute lymphoblastic leukaemia during the first 6 months of chemotherapy: a) PICP and ICTP, and b) BALP and P3NP. The samples from weeks 5 and 20 were each taken before intensification; the samples from weeks 6 and 21 were each taken after intensification. Reference ranges were as follows: PICP 200–550 µg/l; ICTP 3.5–20.0 µg/l; BALP 180–600 U/l; and P3NP 4–12 µg/l.

During induction and first intensification, all three collagen markers were progressively suppressed, whereas BALP showed initial recovery before subsequent suppression. After first intensification, a dramatic catch-up response was observed in all markers, with the collagen markers reaching supranormal concentrations. During second intensification, all markers were again suppressed followed by catch-up over the next 2 weeks. This study illustrates the limitations of krenometry, as many children were too young for the technique. However, in two for whom LLLV data were available they mirrored the changes in PICP and BALP. We postulate that the suppression of biochemical markers and LLLV during the induction and intensification phases of chemotherapy was due to the prednisolone component of chemotherapy, since glucocorticoids have an inhibitory effect on collagen synthesis and degradation and on the cartilage zones of the growth plate [12,13,16]. The effect appeared to be temporary as marked catch-up in markers and growth occurred once glucocorticoids were withdrawn. It remains to be determined whether the degree of marker suppression or the adequacy of marker catch-up have any predictive value for assessing outcome in terms of height deficit or subsequent growth.

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A new model of growth prediction?

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Abstract. The question of how to select patients for growth hormone (GH) treatment and how to adapt the dose very early in treatment is still unresolved since not all children with nonendocrine-based short stature respond to growth-stimulating therapy.

This paper was prepared to give an overview regarding new markers for bone collagen turnover which have shown a clear relationship to growth velocity. We discuss a model of growth prediction by a combination of different markers: a) growth plate stimulation (insulin-like growth factor one (IGF-1) and IGF-1-binding protein three (IGF-1-BP3)), b) cartilage activity (hyaluronic acid), c) bone resorption (cross-link components and galactosylhydroxylysine), and d) bone formation (alkaline phosphatase and procollagen peptides).

Key words: biochemical markers, collagen metabolism, growth disorders, growth prediction.

Introduction

In recent years, many attempts have been made to predict response to growth-stimulating therapies. This has become increasingly necessary because of the experience that a relatively large number of patients with nonendocrine short stature, such as Ullrich-Turner syndrome (UTS), have an inadequate response to growth hormone treatment. Similar problems occur in patients, who on the basis of growth hormone-stimulation testing, are on the borderline between normal growth hormone secretion and growth hormone deficiency.

Anxiological parameters such as height and growth velocity (GV) before therapy, growth hormone levels, growth hormone-dependent factors (IGF-1 and IGF-1-BP3) and parameters of bone metabolism have been investigated [1,2]. For the evaluation of individual growth behavior, however, these parameters have proven to be inadequate. Current work investigating new biological markers of collagen metabolism have shown close relationships with growth behavior in children [3-6]. The development of methods such as the determination of bone-specific collagen components have allowed more specific description of the remodeling and modeling processes of the skeletal system. Due to the importance of these parameters in the diagnosis of osteoporosis, new methods are reported almost on a monthly basis.

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An Anthropometric Study of Children during Intensive Chemotherapy for Acute Lymphoblastic Leukaemia

Key Words

Growth
Body mass index
Acute lymphoblastic leukaemia

Abstract

In order to study the short- to medium-term effects of cytotoxic chemotherapy (CT) for acute lymphoblastic leukaemia (ALL) on growth in prepubertal children, a prospective study of weight (Wt), height (Ht), sitting height (SH) and body mass index (BMI) was performed in 31 children receiving intensive CT for ALL. Two pubertal children, 1 child with Down's syndrome and 3 children who had cranial irradiation were excluded from the analysis. Monthly measurements were presented as change in standard deviation scores from baseline (Δ SDS). The median age at diagnosis was 4.4 years (range, 1.1-14). The median duration of study per subject was 18 months (3-24). The median Δ SDS-Ht reached a nadir of -0.35 at 6 months after the start of CT ($p = 0.001$, Wilcoxon signed rank test); the median Δ SDS-SH was -0.55 at 3 months ($p = 0.02$); the median Δ SDS-Wt and Δ SDS-BMI increased at 2 months to 0.15 ($p = 0.03$) and 0.5 ($p = 0.002$), respectively. The median Δ SDS-BMI was also elevated at 10 months (0.75 , $p = 0.01$) reflecting the relative delay in recovery of Ht-SDS in comparison to Wt-SDS. The median SH:Ht ratio, a measure of disproportion, was reduced at 3 months (Δ SH:Ht ratio, -0.01 , $p = 0.009$). No significant changes in Ht-SDS, BMI-SDS or SH:Ht from baseline were evident at the end of the CT. Cytotoxic CT for ALL in childhood has a significant adverse effect on growth, but there is some evidence for recovery before the end of CT.

Introduction

The effects of cranial irradiation on growth in children requiring treatment for acute lymphoblastic leukaemia (ALL) have been extensively studied and are well documented [1]. In the present trial of ALL treatment in the UK (UKALLXI), CNS-directed therapy is delivered in the form of repeated intrathecal methotrexate (MTX)

with or without high-dose intravenous MTX for the majority of patients. There is evidence that chemotherapy (CT) per se has an adverse effect on growth [2]; however, it is still not clear whether these effects are temporary and whether recovery from the growth-suppressive effects can occur during the period of CT [3-9]. There have been no reported studies of the growth profile of children on UKALLXI. Recently, some concern has been raised

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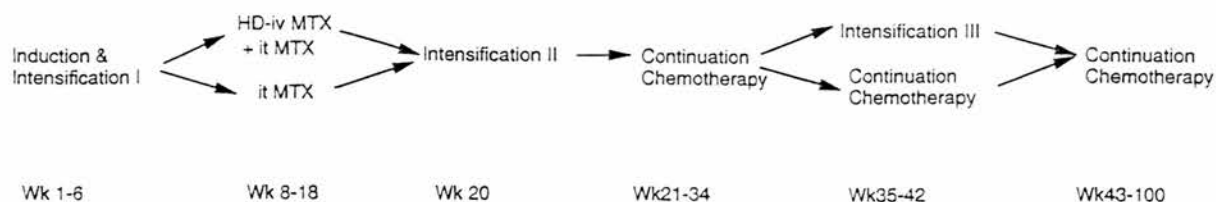


Fig. 1. The UKALLXI treatment protocol; the first 6 months includes induction, first (I) and second (II) intensification as well as CNS-directed therapy which can either be in the form of continuing intrathecal (it) MTX or high-dose intravenous (HD-iv) MTX as well as intrathecal MTX. The second 6 months may include the third intensification (III); the next year consists of continuation chemotherapy also administered between the specific treatment blocks mentioned above.

about altered differential skeletal growth [10, 11] and body disproportion, as well as obesity [12] in children following completion of CT protocols which have included cranial irradiation.

This study prospectively documents anthropometric changes which occurred in a group of children treated by a CT-only regimen for ALL.

Subjects and Methods

31 children (M:F, 20:11) were on UKALLXI over the study period spanning January 1993 to December 1995. From this group, 2 pubertal boys, 1 boy with Down's syndrome and 2 girls and 1 boy who received cranial irradiation were excluded. By the end of the study, 16 children had completed the 2-year course of CT and remained prepubertal. The median age at diagnosis was 4.4 years (range, 1.1–14). The median duration of study was 18 months (range, 3–24).

A brief description of UKALLXI and its randomisation steps are presented in figure 1. Induction CT consists of prednisolone 40 mg/m² o.d., weekly vincristine 1.5 mg/m², asparaginase 6,000 U/m² (9 doses) and intrathecal MTX (2 doses). First and second intensification periods last for a week and consist of prednisolone 40 mg/m² o.d., vincristine 1.5 mg/m² (1 dose), daunorubicin 45 mg/m² (2 doses), etoposide 100 mg/m² (5 doses), cytarabine 100 mg/m² b.i.d., thioguanine 80 mg/m² o.d. and intrathecal MTX (1 dose). High-dose MTX treatment consists of 3 pulses of intravenous MTX (8 mg/m²) as well as 3 doses of intrathecal MTX. During the third intensification period which lasts for 8 weeks, children receive dexamethasone 10 mg/m² for 14 days, vincristine 1.5 mg/m² (4 doses), asparaginase 6,000 U/m² (9 doses), intrathecal MTX (2 doses), cyclophosphamide 600 mg/m² (2 doses), cytarabine 75 mg/m² (16 doses) and thioguanine 60 mg/m² o.d. The treatment regimen which the 25 children received is detailed in table 1.

Height (Ht) and sitting height (SH) were measured monthly (at the beginning of each month) by one of two observers with a stadiom-

Table 1. The distribution of children receiving the various forms of CT within UKALLXI

Type of CNS-directed therapy	Third intensification	No third intensification	Total
MTX only	4	5	9
Intravenous high-dose and intrathecal MTX	7	9	16
Total	11	14	25

eter. The SH:Ht ratio was also calculated for each pair of measurements to assess any changes in body disproportion. SH measurements are presented in only 16 children; the remainder were either too young or did not have a baseline SH measurement. Weight (Wt) was measured in standardised clothing on the same Avery scales throughout the period of study. The body mass index (BMI) was calculated as Wt/Ht² (kg/m²). The data were converted into standard deviation scores (SDS) and presented as median change in SDS, with 5th and 95th percentile values, from a baseline measurement (Δ SDS, P5 and P95). Conversion of data into SDS was performed with reference to L, M and S values obtained from the Child Growth Foundation [13, 14]. Significance of any change from baseline was assessed by the Wilcoxon signed rank (WSR) test.

Results

Height

Median Δ SDS-Ht (fig. 2a) reached a nadir at 6 months after the start of CT with a value of -0.35 (P5, -0.7 ; P95, -0.1 ; $p = 0.001$). Subsequently Δ SDS-Ht remained significantly depressed until the end of the first year after which it

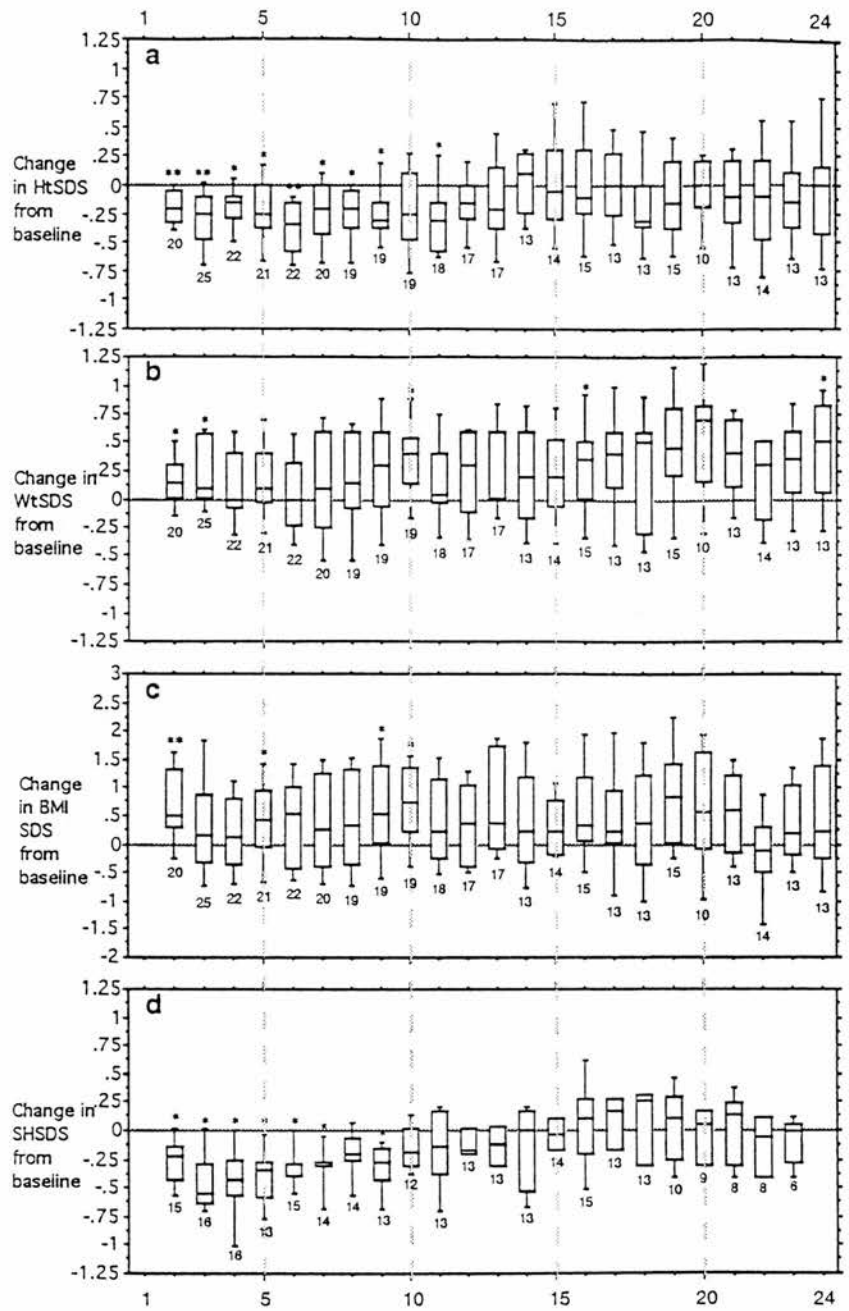


Fig. 2. Median change in Ht SDS (a), Wt SDS (b), BMI SDS (c) and SH SDS (d) from baseline in all prepubertal children receiving CT only. Boxes denote interquartile ranges. * $p < 0.05$, ** $p < 0.005$ (WSR) compared to baseline. Figures below the boxes represent the number of measurements.

returned to baseline values. These trends were also present in a small group of 14 cases where Ht measurements were available from the beginning to the end of CT (fig. 3).

Weight

Median Δ SDS-Wt (fig. 2b) rose significantly over the first month by a median value of 0.15 (P5, -0.15; P95,

0.5; $p = 0.03$). After that initial rise over the period of induction, Wt-SDS was not significantly different from baseline until the second year of CT. By the end of CT, median Δ SDS-Wt was just significantly raised at 0.5 (P5, -0.3; P95, 1.0; $p = 0.04$).

Fig. 3. Median change in Ht SDS from baseline in 14 prepubertal children receiving CT. Boxes denote interquartile ranges. * $p < 0.05$, ** $p < 0.005$ (WSR) compared to baseline. Figures below the boxes represent the number of measurements.

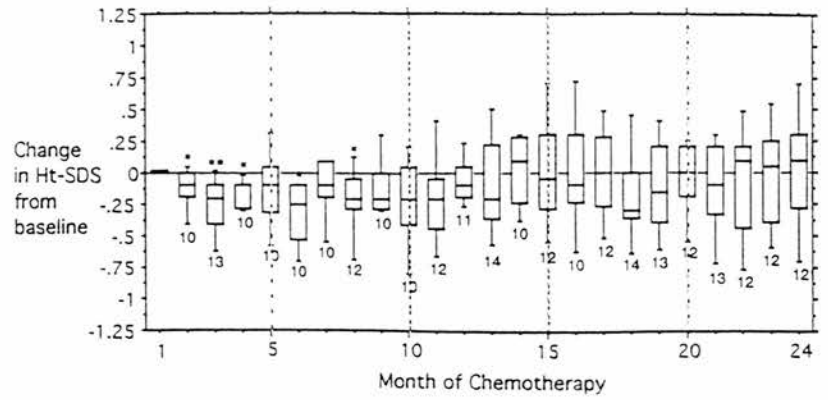
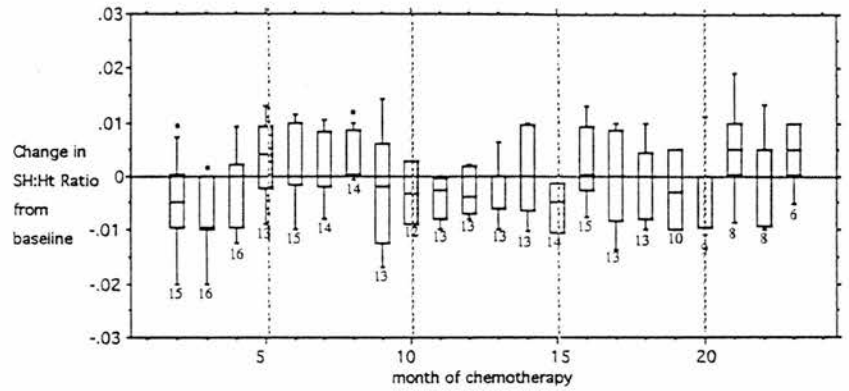


Fig. 4. Median change in SH:Ht ratio from baseline in all prepubertal children receiving CT only. Boxes denote interquartile ranges. * $p < 0.05$, ** $p < 0.005$ (WSR) compared to baseline. Figures below the boxes represent the number of measurements.



Body Mass Index

Median Δ SDS-BMI (fig. 2c) increased at induction to 0.5 (P5, -0.25; P95, 1.64; $p = 0.002$). Median Δ SDS-BMI was also elevated later in the 10th month of CT at 0.75 (P5, -0.39; P95, 1.55; $p = 0.01$).

Sitting Height

As with Ht, an initial decline is seen in SH (fig. 2d) followed by a recovery which seems to be complete by the end of the first year. Maximum decline was evident at 3 months with a median Δ SDS-SH of -0.55 (P5, -0.7; P95, -0.2; $p = 0.02$).

Skeletal Disproportion

Maximum decline of the SH:Ht ratio (fig. 4) was evident at 3 months with a median Δ SH:Ht of -0.01 (P5, -0.007; P95, 0; $p = 0.009$). An increase from baseline was noted at 8 months with a median Δ SH:Ht of 0.003 (P5, 0; P95, 0.01; $p = 0.04$). By the end of CT there were no significant changes from baseline.

Effect of Alternative CT Regimens

No significant anthropometric differences were observed between children separated according to the form of CNS-directed therapy received or whether they received the third intensification block of treatment or not. The number of children in these subgroups was, however, small for adequate statistical analysis (table 1).

Discussion

The results of our study show a general decline in growth over the first year of CT but which tends to improve over the second year. The initial weight gain in the face of reduced growth is most likely due to corticosteroid therapy during induction and as a consequence leads to a significant increase in the BMI [15]. Over the next few months, body weight is not significantly different from baseline but Ht does remain adversely affected after reaching a nadir at 6 months after which CT becomes less

intensive. The slow recovery in Ht with a satisfactory Wt gain could explain the elevation in BMI in the latter half of the first year. Although the initial decline in Ht is consistent with that found in other studies of children with ALL receiving CT-only regimens [1–10], few studies have shown adequate catch-up growth during the period of CT [3, 5, 7].

Corticosteroids probably play an important role in the early phase of growth retardation acting locally on bone growth [16], directly on the growth plate [17] and centrally on growth hormone secretion [18, 19] as well as by affecting calcium homeostasis [20]. However, almost all the cytotoxic agents included in the CT protocol can to some extent penetrate the blood-brain barrier [21–25] or have indirect effects on the CNS [26]; MTX can have direct effects on bone as well [27].

Intravenous high-dose MTX has been associated with lesions in the CNS [28], the significance of which is unclear. The poor growth after the induction and first intensification could be due to the CNS-directed therapy. Whether intravenous MTX has a more profound effect on growth than repeated intrathecal MTX alone was not clear from our data and needs further investigation. The growth retardation seems to be reversible, reflecting the results of recent studies showing short-term suppression of growth in rabbits injected with high-dose MTX [29].

Our study also revealed an initial transient deceleration in SH which was accompanied by a reduction in the SH:Ht ratio. This strengthens the hypothesis that the spine with its multiple growth plates plays a major part in

growth deceleration when exposed directly to cytotoxic CT [11]. Unlike Davies et al. [11], our group of children did not receive cranial irradiation and have not reached final Ht. It is, however, encouraging to note that there were no significant differences in body proportion from baseline at the end of the 2-year course. It is possible that more accurate and precise short-term measurements of lower leg length [30] might provide a better insight into these growth patterns.

In summary, our results show that UKALLXI chemotherapy for ALL in childhood does adversely affect growth, but there is evidence of recovery occurring before the completion of treatment. Further study of this cohort of children is needed to ensure that growth remains unaffected in the long term and further studies are also required to explore the underlying physiological mechanisms of growth decline due to CT. To understand the effects of the various forms of CT, further studies comparing the different regimens within UKALLXI would also be advantageous.

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