Karin Blijdorp was born in Leiden on January 8th, 1984. She studied medicine at the Erasmus University Medical Centre and received her medical degree in May 2009. During her studies she was a board member (treasurer) of the Dutch Student Orchestra for one year. After finishing her studies, she started working as a resident in paediatrics at the Maasstad hospital. In January 2010, she started her PhD project on the departments of Endocrinology and Paediatric Oncology/ Haematology.
The studies performed during this project are described in this thesis. Currently, she is working as a resident and in January 2014 she will start her training in internal medicine - endocrinology.

K. Blijdorp

DETERMINANTS & SEQUELAE OF ALTERED BODY COMPOSITION IN CHILDHOOD CANCER SURVIVORS

K. Blijdorp

DETERMINANTS & SEQUELAE OF ALTERED BODY COMPOSITION IN CHILDHOOD CANCER SURVIVORS

K. Blijdorp

This thesis describes late effects of childhood cancer treatment in a large cohort of adult survivors, focusing on body composition changes.

1st
The first aim was to study the value of current obesity markers, such as body mass index and waist-hip ratio in survivors of childhood cancer.

2nd
Secondly, we aimed to determine treatment-related factors as well as other long-term effects that may contribute to or follow alterations in body composition.

DETERMINANTS & SEQUELAE OF ALTERED BODY COMPOSITION IN CHILDHOOD CANCER SURVIVORS

Determinanten & gevolgen van veranderde lichaams-samenstelling in overlevenden van kinderkanker

K. Blijdorp

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Determinants & Sequelae of Altered Body Composition in Childhood Cancer Survivors

Determinanten & gevolgen van veranderde lichaamssamenstelling in overlevenden van kinderkanker

Proefschrift

Ter verkrijging van de graad van doctor aan de
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Contents

CHAPTER 1

General introduction

1.1 Childhood cancer & survival

In the Netherlands, approximately 600 children are diagnosed with cancer every year (Figure 1.1). Due to improvement of treatment, combining surgery, multi-agent chemotherapy, and radiotherapy, in addition to remarkable advances in supportive care, survival has increased substantially over the last decades, leading to a rapidly growing cohort of childhood cancer survivors. Approximately 7000 long-term survivors of childhood cancer are living in the Netherlands nowadays[1(p134)].

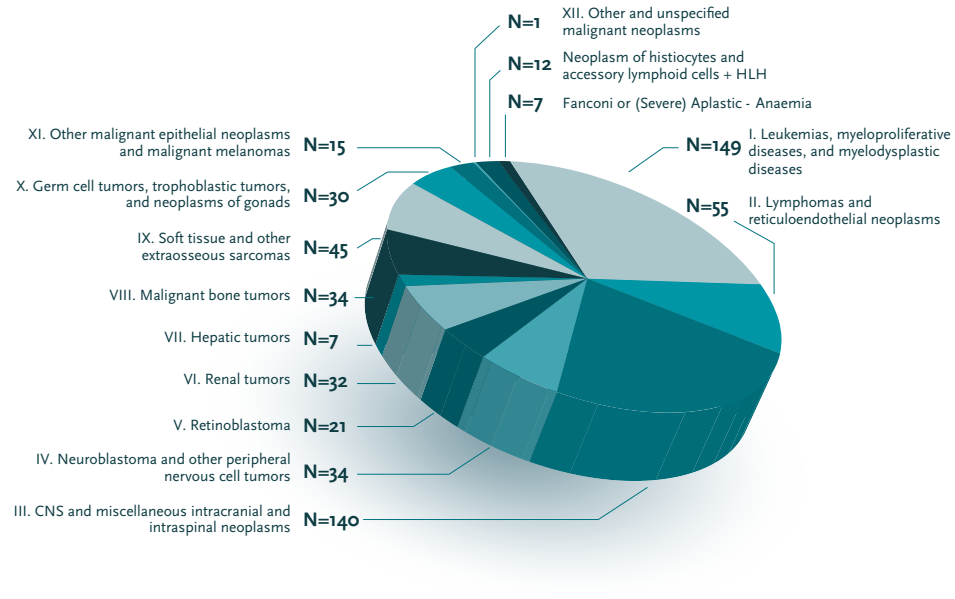

Figure 1.1 Incidence of childhood cancer types in the Netherlands in 2010.
SKION Basisregistratie 2010

Mortality and morbidity after childhood cancer treatment

In the increasing population of long-term survivors, morbidity and mortality rates are high as a consequence of damage caused by surgery, chemotherapy, radiation and stem cell transplantation. Mortality among young adults surviving cancer is higher than that of the normal population, and significantly increases over time until 18.1% at 30 years after diagnosis (Figure 1.2). This increasing mortality is mainly caused by recurrence of the original cancer, development of second neoplasms and cardiac morbidity[2, 3(p134)]. Independent risk factors associated with excess mortality rates are younger age at diagnosis and initial diagnosis of leukaemia or brain tumour[2(p134)]. Increased mortality in these diagnosis groups is contributable to

high-risk protocols and stem cell transplantation in leukaemia patients and localization of the primary tumour and high irradiation dosages in brain tumour patients. Additionally, former treatment with irradiation, particularly high dose cranial radiotherapy and total body irradiation as well as combination chemotherapy containing anthracyclines and alkylating agents contribute to high mortality rates[2, 4(p134)].

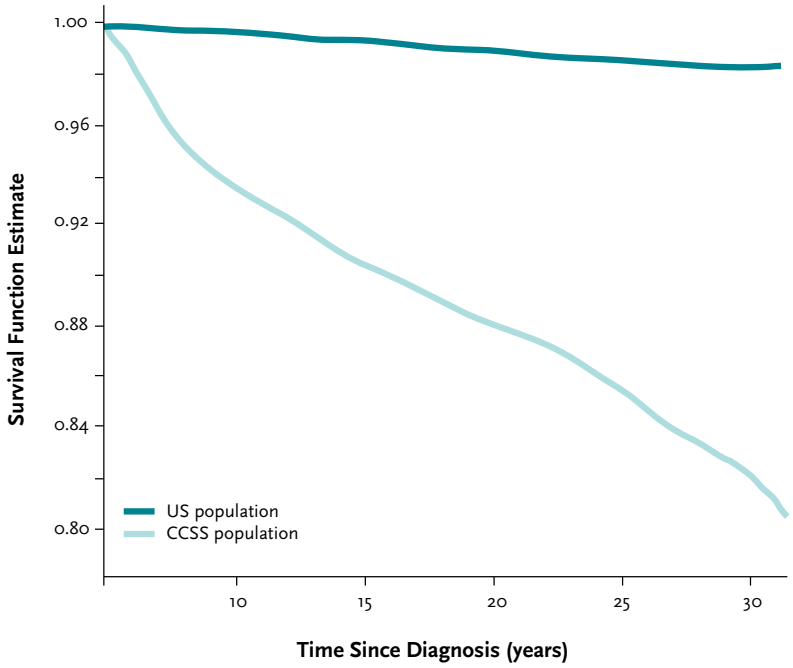

Figure 1.2 Mortality in childhood cancer survivors compared to healthy siblings.
G Armstrong et al. J Clin Oncol 2009:27:2328-2338

The risk of having a chronic health condition being a childhood cancer survivor is 3 times higher than healthy peers and 8 times higher if the condition is severe or life-threatening[5, 6(p134)]. The occurrence of this burden of disease among childhood cancer survivors is depending on former diagnosis, but more importantly on cancer treatment. Second cancers have an important contribution to this impaired health status. They are more prevalent in women than in men due to the high contribution of breast cancer occurring in Hodgkin survivors treated with mantle field irradiation[4, 7, 8(p134)], which is now replaced by involved field or even by involved node irradiation. Other treatment-induced complications include anthracycline-related cardiomyopathy and congestive heart failure, irradiation-induced pulmonary toxicity, chronic kidney disease related to nephrectomy, abdominal irradiation and nephrotoxic agents, premature gonadal failure due to former treatment with abdominal irradiation and alkylating agents, and cranial radiotherapy-related cognitive dysfunction, growth hormone deficiency and obesity[5, 6(p134)]. Yet, much has been learned about the causes and effects of life-threatening late effects, such as second cancers and cardiotoxicity, which has led to improvement of treatment protocols, followed by decreasing numbers of these sequelae.

1.2 Endocrine and metabolic disorders after childhood cancer treatment

Endocrine disorders are among the most frequently reported complications in childhood cancer survivors, affecting between 20-50% of them[9(p134)]. Especially survivors exposed to total body irradiation, abdominal radiotherapy, cranial radiotherapy or high dosages of alkylating agents, are at high risk for endocrine disorders due to their effects on the hypothalamic-pituitary axis, thyroid gland and the gonads[10(p135)]. Consequently, bone mass, body composition and glucose metabolism are affected by these treatments. Table 1.1 summarizes the endocrine and metabolic disorders associated with treatment-related risk factors.

Obesity

Obesity is a major side effect of childhood cancer treatment, and is present in 9-30% of individuals depending on former therapy[11(p135)]. The risk of obesity for an adult childhood cancer survivor is up to 3.8 times higher than that of the normal population[11(p135)]. As accelerated weight gain is associated with increased morbidity and mortality, particularly due to cardiovascular disease, obesity among childhood cancer survivors has become a considerable concern.

Measures of obesity and body composition

The most generally used obesity measure is body mass index (BMI), taking into account total body weight and height (calculated by the formula: weight (kg)/height (m)^2). Despite its worldwide use as a predictor for metabolic health risk, it is an inadequate measure for true body composition since it does not distinguish between lean and fat mass. Currently, waist circumference and waist-hip ratio are easily applicable clinical tools, and have been suggested in the definition of metabolic syndrome criteria rather than BMI, since they represent the amount of intra-abdominal fat[12(p135)]. Other frequently used methods to assess fatness are skinfold thickness and electrical bio-impedance[13(p135)]. However, the gold standard is dual energy X-ray absorptiometry (DXA), which directly measures total fat, lean body mass and bone mass with a low radiation exposure[14(p135)]. It can distinguish regional as well as whole body fat, and therefore it has been used for the measurement of intra-abdominal fat, which

is associated with insulin resistance. Hence, in addition to the amount, but more importantly the distribution of body fat, defining obesity, lean mass and bone density as well as height, are important determinants of body composition.

System	Complication	Therapy-related risks
Linear growth	Skeletal dysplasia	Spinal irradiation
	GH deficiency	Surgery
		Cranial radiotherapy
Puberty	Precocious puberty	Cranial radiotherapy
	Hypogonadotropic hypogonadism	Cranial radiotherapy
Testes	Leydig cell dysfunction	Alkylating agents
	Germ cell dysfunction	Radiotherapy to the testis
Ovaries	Acute ovarian failure	Alkylating agents
	Premature menopause	Radiotherapy to the ovaries
Adrenals	ACTH deficiency	Direct insult (surgery, tumour expansion)
		Cranial radiotherapy
		Glucocorticoids (transient)
Thyroid	TSH deficiency	Cranial radiotherapy
	Primary hypothyroidism	Radiotherapy (local or scatter)
		TBI
		^{131}I-MIBG and ^{131}I-labeled monoclonal antibody
		Cranial radiotherapy
	Primary hyperthyroidism	Radiotherapy (local or scatter)
	Neoplasms	
	Autoimmune disease	HSCT
Bone	Osteoporosis	Methotrexate
		Glucocorticoids
Metabolism	Obesity	Cranial radiotherapy
		Glucocorticoids
		Surgery
	Diabetes Mellitus	Alkylating agents
		TBI & abdominal irradiation
	Metabolic syndrome	Cranial radiotherapy
		Abdominal irradiation

GH growth hormone; **ACTH** adrenocorticotropic hormone; **TSH** thyroid stimulating hormone; **TBI** total body irradiation; **HSCT** hematopoietic stem cell transplantation

Table 1.1 Endocrine complications and therapy-related risk factors.
Modified from Chemaitilly W and Sklar CA. Endocr Relat Cancer 2010;17(3):R141-59

1.3 Body composition in childhood cancer survivors

In childhood cancer survivors, body composition is affected by treatment, directly, or indirectly, as a consequence of other treatment-related late effects. Lifestyle factors, including dietary habits and physical activity, are important determinants for body composition, particularly in childhood cancer survivors, since lifestyle is known to change after childhood cancer treatment due to psychological or physiological factors. Figure 1.3 shows a simplified overview of determinants influencing alterations in body composition among childhood cancer survivors.

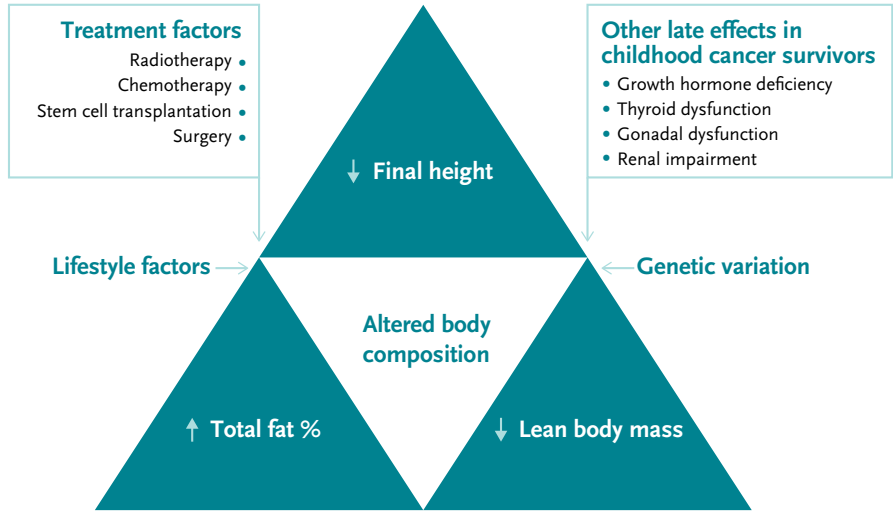

Figure 1.3 Altered body composition in childhood cancer survivors.

Treatment factors

Body composition is affected by high dose cranial radiotherapy (CRT) (>20 Gy)[11, 15(p135)], since it is strongly associated with growth hormone deficiency (GHD) and a subsequent lower insulin-like growth factor I (IGF-I), raised insulin levels, altered fat distribution (particularly abdominal fat accumulation) and dyslipidemia[16(p135)]. During childhood, IGF-I is responsible for linear growth. Consequently, children with GHD caused by childhood cancer treatment, fail to achieve target height. Additionally, GHD is associated with substantial changes of the metabolic system and thereby it is an important denominator for the development of the metabolic syndrome[17(p135)], which is a major treatment-related complication in childhood cancer survivors[18(p135)]. The metabolic syndrome consists of the combination of

abdominal adiposity, insulin resistance, dyslipidemia and hypertension and its presence doubles the risk of cardiovascular disease. Besides CRT, treatment with total body irradiation, as conditioning regimen for stem cell transplantation, is associated with shorter stature, partly explained by loss of pituitary function (GHD), in addition to general damage to organ systems and muscle mass. Apart from GHD-related metabolic changes, more direct irradiation effects are found after abdominal irradiation, which is an important risk factor for the development of the metabolic syndrome and diabetes mellitus, due to radiation-induced damage to liver and pancreas affecting lipid and glucose metabolism[19, 20(p136)]. Local radiation of brain tumours with dosages up to 54 Gy and radical resection of tumours in the hypothalamic region have an extremely high risk for obesity[21, 22(p136)]. The physiopathology of hypothalamic obesity is a complexity of loss of sensitivity to afferent signals (i.e. leptin resistance) and dysfunctional efferent peripheral signals (i.e. insulin hypersecretion), finally leading to compromised energy expenditure[23(p136)].

Long-term influence of chemotherapy on body composition has not been elucidated thoroughly. Survivors treated with both chemotherapy and craniospinal irradiation had shorter stature than those treated with only craniospinal irradiation, suggesting a detrimental effect of chemotherapy[24(p136)]. Since chemotherapy is given as combination treatment, it is hard to determine whether the combination of agents or a particular component caused the effects. Further studies are needed to investigate this. Corticosteroids are known to be associated with temporary height loss and increased body mass index, during and shortly after treatment[25(p136)], whereas several years after treatment cessation these effects had disappeared[17(p135), 26, 27(p136)].

Lifestyle factors

A positive energy balance, caused by high fat, high-caloric diets and minimal physical activity, contributes to the development and maintenance of obesity. This general health concern also plays a role in childhood cancer survivors. Compared to their healthy peers, childhood cancer survivors are less likely to meet physical activity recommendations, particularly individuals treated with high dose CRT[15(p135)]. Risk groups for compromised dietary behaviour are brain tumour survivors (especially craniopharyngioma) and those treated with CRT. However, well-controlled studies describing dietary patterns in childhood cancer survivors are lacking.

Other late effects

Besides growth hormone deficiency, other treatment-related late effects might affect body composition. Primary hypogonadism in individuals exposed to abdominal irradiation and high dosages of alkylating agents, plays a role in obesity development. Untreated hypogonadism among childhood cancer survivors is related to hyperinsulinemia, impaired glucose tolerance and abdominal adiposity in both male and female survivors[28(p136)]. Secondary hypogonadism due to irradiation-induced pituitary damage occurs less often than GHD and particularly with irradiation dosages higher than 30 Gray. Besides the effect on the metabolic system as seen in primary hypogonadism, central hypogonadism causes a delayed puberty, thereby suppressing growth spurt, leading to growth retardation. Apart from pituitary damage, which plays a substantial role in height loss in particularly cranial irradiated patients, chronic kidney disease (CKD) in children, caused by nephrotoxic treatment, is associated with changes in the GH and IGF-I axis, leading to growth retardation. The GH/IGF-I axis is activated at the onset of puberty, which is delayed in CKD patients. Furthermore, GH resistance plays an important role, due to reduced density of GH receptors in target organs[29(p136)]. Additional factors contributing to growth delay are metabolic deficits associated with renal failure, such as water and electrolyte losses, anaemia and metabolic acidosis. Hypertension is present in approximately 80 to 85% of patients with CKD[30(p137)] and can be a causative or contributory factor in the development of kidney disease, due to a combination of factors including sodium retention, increased activity of the renin-angiotensin system, and enhanced activity of the sympathetic nervous system. The metabolic syndrome itself is associated with kidney damage, due to obesity-related glomerular changes, microvascular diseases related to dyslipidemia and hyperglycaemic conditions[31(p137)].

Genetic variation

As in the general population, genetic variation plays a role in the development of obesity in childhood cancer survivors, since it might influence the susceptibility of obesity in response to high-risk treatment. For example, polymorphism of the leptin receptor is associated with a 6-fold higher chance of becoming overweight after CRT treatment than survivors without this polymorphism[32(p137)], while on the other hand polymorphism of the FTO gene has a protective effect on becoming overweight[33(p137)]. However, to date no clear relation has been described between susceptibility for childhood cancer and obesity.

1.4 Aims and Outline of the thesis

In the general population, obesity is associated with higher morbidity and mortality rates. Adverse health implications of obesity may be greater among childhood cancer survivors, due to former diagnosis and treatment exposure, which places them at greater risk for severe chronic health conditions. Understanding the factors that contribute to obesity and detrimental body composition changes in this group, either directly or indirectly, may open possibilities for prevention and management strategies. Therefore, the aim of the thesis was to evaluate the value of current obesity measures in this group (**Chapter 2**) and to define determinants of alterations in

body composition among childhood cancer survivors (**Chapter 3 & 4**). In **Chapter 5** insight is given in ghrelin metabolism in normal weight and obese survivors of childhood cancer. **Chapter 6** describes a study on final height and insulin-like growth factor-I in Wilms tumour survivors treated with nephrectomy. Additionally, risk factors for long-term nephrotoxicity and prevalence of hypertension in a large cohort of adult survivors of childhood cancer is described in **Chapter 7**. This thesis concludes with two studies describing the association between detrimental body composition changes and gonadal reserve in both male and female survivors (**Chapter 8 & 9**).

Obesity is underestimated using body mass index and waist-hip ratio in long-term adult survivors of childhood cancer

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2.1 Abstract

Objective

Obesity, represented by high body mass index (BMI), is a major complication after treatment for childhood cancer. However, it has been shown that high total fat percentage and low lean body mass are more reliable predictors of cardiovascular morbidity. In this study, longitudinal changes of BMI and body composition, as well as the value of BMI and waist-hip ratio representing obesity, were evaluated in adult childhood cancer survivors.

Methods

Data from 410 survivors who had visited the late effects clinic twice, were analysed. Median follow-up time was 16 years (interquartile range 11-21) and time between visits was 3.2 years (2.9-3.6). BMI was measured and body composition was assessed by dual X-ray absorptiometry (DXA, Lunar Prodigy; available twice in 182 survivors). Data were compared with healthy Dutch references and calculated as standard deviation scores (SDS). BMI, waist-hip ratio and total fat percentage were evaluated cross-sectionally in 422 survivors, in whom at least one DXA scan was assessed.

Results

BMI was significantly higher in women, without significant change over time. In men BMI changed significantly with time (ΔSDS =0.19, P<0.001). Percentage fat was significantly higher than references in all survivors, with the highest SDS after cranial radiotherapy (CRT) (mean SDS 1.73 in men, 1.48 in women, P<0.001). Only in men, increase in total fat percentage was significantly higher than references (ΔSDS=0.22, P<0.001). Using total fat percentage as the gold standard, 65% of female and 42% of male survivors were misclassified as non-obese using BMI. Misclassification of obesity using waist-hip ratio was 40% in women and 24% in men.

Conclusions

Sixteen years after treatment for childhood cancer, the increase in BMI and total fat percentage was significantly greater than expected, especially after CRT. This is important as we could show that obesity was grossly underestimated using BMI and waist-hip ratio.

2.2 Introduction

Childhood cancer survival rates have increased enormously over the last few decades[34, 35](p137). As a consequence, the incidence of treatment-related complications is increasing. One of the major sequelae is obesity, with a prevalence of 9 to 30% depending on former treatment modalities[36-38](p137). Body mass index (BMI) is the most widely used measure for obesity. However, it has been shown that a high amount of total body fat, high intra-abdominal fat percentage and low lean body mass are more reliable determinants than high BMI in predicting the development of cardiovascular disease or diabetes mellitus[39-43](p138). In a recent study, Shah and Braverman showed that BMI misclassified 48% of the female and 25% of the male population using total fat percentage measured by dual X-ray absorptiometry (DXA) as the gold standard. This leads to an underestimation of the prevalence of obesity[43](p138). Waist circumference, which approximates the amount of intra-abdominal fat, is a more accurate marker than BMI and is one of the criteria used to define metabolic syndrome[17, 18](p135).

In childhood cancer survivors, abnormal body composition has been thoroughly described and is caused by several factors including damage to the hypothalamus and/or pituitary due to cranial radiotherapy and use of corticosteroids[17, 18](p135), [38](p137), [44-46](p138). Most studies that investigated changes in body composition, in addition to BMI, were cross-sectional and included small cohorts and selected subgroups, such as acute lymphoblastic leukaemia (ALL) survivors[17](p135), [26, 27](p136), [47](p138). Currently, long-term survivor studies measuring body composition longitudinally are not available. The Childhood Cancer Survivor Study has reported changes in BMI with a very long-term follow-up of 25 years[48](p138). The aim of our study was to investigate longitudinal changes in BMI and body composition, and to evaluate the value of BMI and waist-hip ratio as compared with total fat percentage measured by DXA in long-term adult childhood cancer survivors in a single centre in the Netherlands.

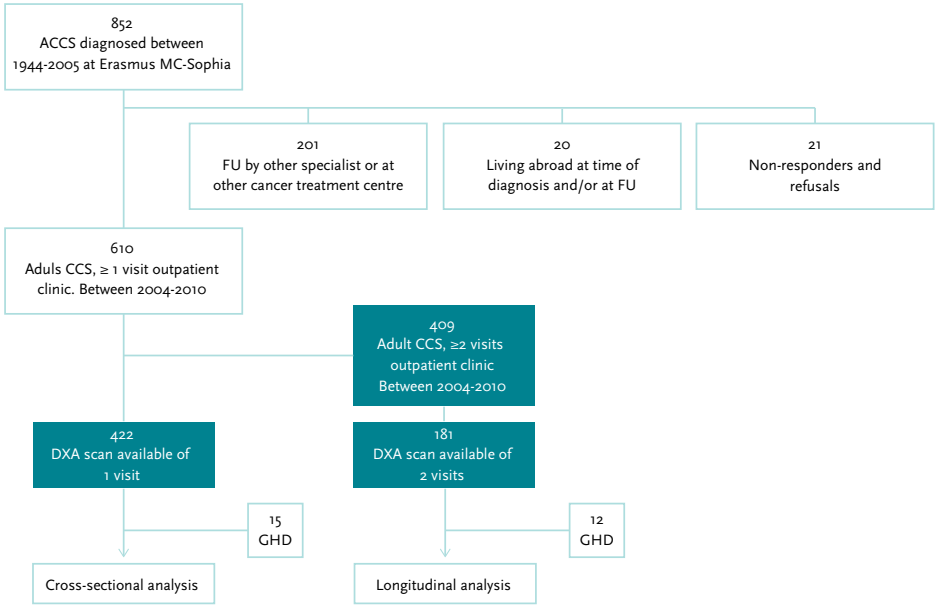
2.3 Subjects and Methods

Ethics Statement

The data described in the current retrospective study were obtained during regular visits at the late effects clinic, and clinical investigations were assessed using the standard guidelines for screening late effects after childhood cancer following Good Clinical Practice (GCP). An official written informed consent from every patient that visited the outpatient clinic was obtained according to standards of the Institutional Review Board (IRB).

Subjects

We performed a retrospective single centre study. Follow-up of subjects at our late effects outpatient clinic for long-term childhood cancer survivors starts 5 years after cessation of therapy and is individualized based on cancer diagnosis and treatment protocol. Out of 852 adult childhood cancer survivors diagnosed and treated between 1964 and 2005, 610 had visited the outpatient clinic and 409 had visited the outpatient clinic twice (Figure 2.1). Longitudinal changes in BMI were evaluated in survivors with 2 visits. Cross-sectional evaluation of BMI, waist-hip ratio and total fat percentage was assessed in 422 survivors. One survivor was diagnosed with Down syndrome and was therefore excluded.



FU follow-up; **CCS** childhood cancer survivors; **GHD** Growth hormone deficiency.

Figure 2.1 Flowchart of included survivors.

Methods

Treatment details, as well as disease and patient characteristics, were retrieved from our local database (Table 2.1). Information regarding pituitary dysfunction and hormone supplementation were evaluated. Body mass index (BMI) was calculated from height and weight[49](p139). Waist-hip ratio was calculated from waist and hip circumference[50](p139). Lean body mass and total fat percentages were measured by DXA using a single machine (DXA, Lunar Prodigy, GE Healthcare, Madison, WI). Data from sequential DXA scans were available in 182 survivors. Obesity was defined as BMI ≥ 30[51](p139), total fat percentage ≥ 25% (M) and ≥ 30% (W)[52](p139) and waist-hip ratio ≥0.85 (W) and ≥0.90 (M)[50](p139).

Final height data were compared with data of Dutch adult references described by Fredriks et al.[53](p139). BMI data were compared with data from self-reported questionnaires, which are sent out yearly to 10 000 subjects by the central office for statistics (CBS) in the Netherlands[54](p139). Lean body mass and total fat percentage data were compared with data of healthy Dutch references[55, 56](p139). To adjust for age and gender, standard deviation scores were calculated for final height, BMI and body composition measures.

Growth hormone deficiency (GHD) was defined by an insufficient growth hormone stimulation test (growth hormone peak <3 µg/L during an insulin tolerance test or <9 µg/L during a GHRH-Arginine test[57](p139)). Hypothyroidism was defined as a fT4 level <11 pmol/L in combination with normal or high TSH levels (>4.3 mU/L; primary hypothyroidism) or a TSH level <0.4 mU/L (secondary hypothyroidism). Hypogonadism was defined as a testosterone level <9 nmol/L in men or oligo-/ amenorrhea in women.

Laboratory measurements

Serum insulin-like growth factor-I (IGF-I; nmol/L) was measured using a chemi-luminescence-based immunoassay (Immulite 2000, Siemens DPC, Los Angeles, CA). Intra- and interassay coefficients of variation (CV) were <5 and <7%. IGF-I levels were compared with reference values by using standard deviation scores (SDS)[58](p139). In cases where GHD was suspected (i.e. IGF-I below -2 SDS) a growth hormone stimulation test was assessed. Testosterone (nmol/L) was measured by coated tube radioimmuno-assay (Siemens DPC). Intra- and interassay CVs were <6 and <9% respectively. Thyroid stimulating hormone (TSH) (mU/L) and free thyroxine (fT4) (pmol/L) were measured using chemoluminescence assays (Vitros ECi Immunodiagnostic System; Ortho Clinical Diagnostics, Rochester, NY). Interassay CV was 4.7–5.4% for fT4 and 2.5–4.1% for TSH.

Statistics

To evaluate longitudinal changes in BMI and body composition, treatment modalities were categorised into five groups: cranial radiotherapy as defined by central nervous system irradiation in leukaemia/lymphoma survivors (CRT), brain tumour irradiation directed to the indicated tumour field (BRT), total body irradiation (TBI), corticosteroids (without irradiation), and other treatment (neither cranial irradiation nor corticosteroids). Standard deviation scores were calculated for BMI, total fat percentage and lean body mass and were compared with healthy references using

the one sample t-test. When analysing the rough data, differences between 1st and 2nd assessments were tested using the paired t-test (Figure 2.2). Growth hormone deficient subjects were excluded from the analysis because growth hormone treatment causes a significant decrease of total body fat[59(p140)]. Multivariate regression analysis was performed to determine the influence of age at diagnosis, hypothyroidism, hypogonadism and use of oral contraceptives on BMI and measures of body composition.
To evaluate the value of BMI and waist-hip ratio, total fat percentage, retrieved from DXA scans, was used as the gold standard. Total fat percentage was compared with BMI and waist-hip ratio, to determine percent agreement and disagreement, separately in men and women. A Receiver Operating Characteristic (ROC) analysis stratified by sex was used to evaluate sensitivity and specificity for BMI and waist-hip ratio and to determine more accurate cut-off points relative to total fat percentage. Survivors who had been treated with abdominal radiotherapy were excluded from the waist-hip ratio analysis. P-values were considered statistically significant if $p<0.05$. Statistical analysis was performed using SPSS 17.0 software (SPSS, Chicago, IL).

2.4 Results

Survivors

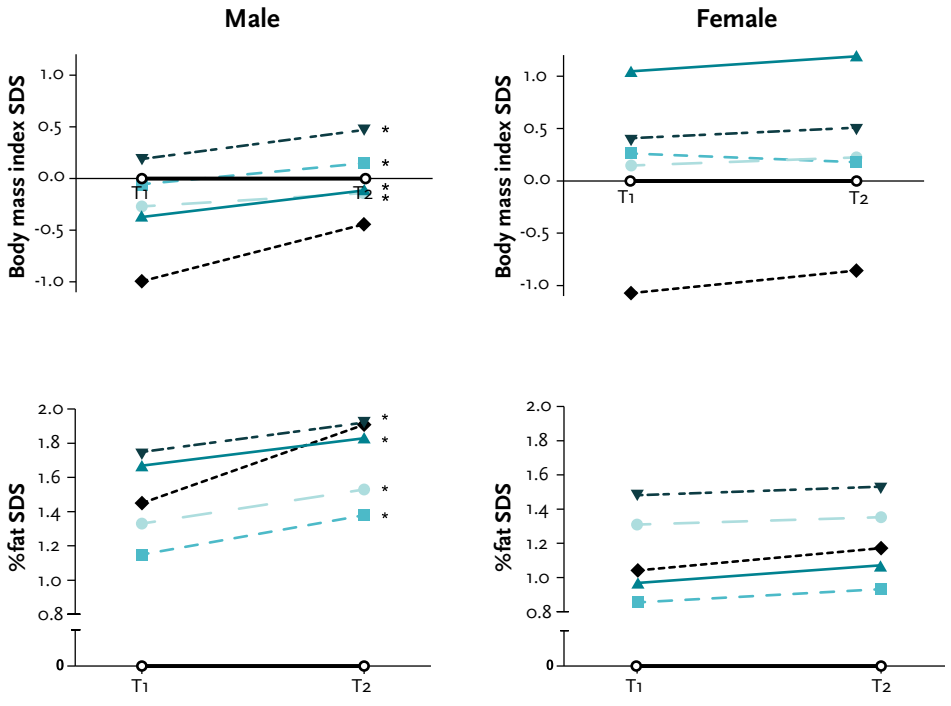
Baseline and treatment characteristics are shown in Table 2.1. Median follow-up time between cessation of therapy and the first visit at the outpatient late effects clinic was 16 years (interquartile range (IQR) 11-21). Median time between the 1st assessment (defined as T1) and 2nd assessment (defined as T2) was 3.2 years (IQR 2.9-3.6). Forty-five, mainly leukaemia survivors, had been treated with CRT (25 Gy (24-25)), including 3 survivors that had received craniospinal irradiation; thirty-nine, mainly brain tumour survivors, had been treated with BRT (40 Gy (35-44)). Two leukaemia survivors had been treated with CRT and TBI and were analysed in the CRT group. One brain tumour survivor had received BRT and TBI and was analysed in the BRT group. One hundred and forty-three survivors had been treated with corticosteroids but not cranial irradiation, and 170 had only been treated with chemotherapy, not with corticosteroids or cranial irradiation.

Fifteen survivors had been diagnosed with GHD earlier and were treated with growth hormone (GH) therapy at time of follow-up, and were excluded from further analysis. Hypothyroidism was present in 14 survivors at T1 and 18 survivors at T2. Hypogonadism was present in 21 survivors at T1 and 25 survivors at T2. There were no subjects with untreated hypogonadism and hypothyroidism. Oral contraceptives were used by 94 women at T1 and 86 at T2. Multivariate analysis showed that total fat percentage was not associated with hypogonadism or hypothyroidism during adequate replacement therapy, nor with oral contraceptive use. After correction for height SDS, lean body mass was significantly associated with hypothyroidism in female survivors, which were therefore excluded from the analysis (β -1.20, p=0.039). BMI was significantly associated with hypogonadism in male survivors, which were therefore excluded from the analysis (β 0.92, p=0.015). Age at diagnosis was not significantly associated with BMI and body composition.

	Survivors with DXA scan (cross-sectional data)		Survivors with two visits (longitudinal data)	
	Male N=243	Female N=179	Male N=229	Female N=180
Age at diagnosis	6.3 (3.4-11.8)	6.5 (3.2-11.7)	6.7 (3.3-11.8)	6.2 (2.9-11.7)
Age at follow-up	25.8 (21.9-31.4)	26.6 (22.3-32.7)	23.8 (20.2-28.0)	25.4 (21.0-30.1)
Time between DXA	NA	NA	3.2 (2.9-3.6)	3.1 (2.9-3.4)
Follow-up time	17.5 (12.1-23.0)	17.9 (13.2-23.7)	15.9 (11.2-20.3)	17.0 (11.9-22.4)
Diagnosis				
ALL, T-NHL	107 (44)	82 (46)	79 (35)	58 (33)
AML	10 (4)	7 (4)	8 (4)	8 (4)
B-NHL	28 (12)	14 (8)	24 (11)	14 (8)
Hodgkin lymphoma	32 (13)	14 (8)	25 (11)	16 (9)
Bone tumour	4 (2)	6 (3)	11 (5)	8 (4)
Wilms tumour	26 (11)	21 (12)	26 (11)	24 (13)
Neuroblastoma	5 (2)	10 (6)	11 (5)	18 (10)
Germ cell tumour	1 (1)	1 (1)	4 (2)	5 (3)
MMT	6 (3)	5 (3)	17 (7)	13 (7)
Brain tumour	15 (6)	9 (5)	15 (7)	7 (4)
Other	9 (4)	10 (6)	9 (4)	9 (5)
Therapy				
CRT	43 (18)	28 (16)	26 (11)	19 (11)
BRT	9 (4)	3 (2)	31 (14)	8 (4)
Corticosteroids	124 (51)	88 (49)	75 (33)	68 (38)
No CRT / corticosteroids	54 (22)	50 (28)	91 (40)	79 (44)
TBI	13 (5)	10 (6)	6 (3)	6 (3)
Abdominal RT	15 (6)	14 (8)	NA	NA

Data are expressed as median (interquartile range) or as frequencies (N %). **NA** not applicable; **ALL** Acute lymphoblastic leukaemia; **AML** Acute myeloid leukaemia; **B-NHL** B-cell non hodgkin lymphoma; **T-NHL** T-cell non hodgkin lymphoma; **MMT** Malignant mesenchymal tumour; **CRT** Cranial radiotherapy; **BRT** Local radiotherapy on cranium; **TBI** Total body irradiation.

Table 2.1 Baseline characteristics of survivors included in this study.



* Significantly higher increase as compared to references (one-paired T-test, p<0.05)

Figure 2.2 Standard deviation scores (SDS) of body mass index, total fat percentage, lean body mass and height in different therapy groups expressed as mean at T1 and T2.

Body mass index

Male survivors had a significantly lower BMI at T1 (SDS= -0.17, p=0.022) and showed a significant change over time (ΔSDS =0.19, P<0.001). When analysing treatment groups separately BMI at T1 was not significantly different from Dutch references, but changed significantly over time in all groups except for TBI (Figure 2.2). In female survivors BMI was significantly higher compared with references, without significant change over time (SDS T1=0.22, p=0.024, T2=0.25, p=0.006). When analysing treatment groups separately only cranial irradiated female leukaemia/lymphoma survivors had significantly higher BMI without significant change over time (SDS T1=0.41, p=0.021, T2 0.51, p=0.031). Both male and female TBI survivors had a significantly lower BMI compared with references (Figure 2.2).

Total fat percentage

Fat percentage was significantly higher compared with Dutch references in both men (SDS T1 1.37, *P*<0.001) and women (SDS T1 1.05, *P*<0.001). The highest fat percentage was found after CRT (mean SDS T1 1.73 in men, 1.48 in women, *P*<0.001) (Figure 2.2). In men, fat percentage increased significantly (ΔSDS=0.22, *P*<0.001). Evaluating treatment groups separately, fat percentage increased significantly in all groups except for the TBI group (Figure 2.2). In female survivors no significant increase of fat percentage was found (ΔSDS=0.07, p=0.22).

	Body Mass Index			Waist-hip Ratio[a]		
	Men N=235	Women N=172	Total N=407	Men N=174	Women N=125	Total N=299
Concordant						
BMI/WHR non-obese, total fat % non-obese	120 (51)	39 (23)	159 (39)	80 (46)	29 (23)	109 (37)
BMI/WHR obese, total fat % obese	17 (7)	21 (12)	38 (9)	40 (23)	41 (33)	81 (27)
Discordant						
BMI/WHR non-obese, total fat % obese	98 (42)	112 (65)	210 (52)	42 (24)	50 (40)	92 (31)
BMI/WHR obese, total fat % non-obese	0	0	0	12 (7)	5 (4)	17 (6)

[a]Survivors treated with abdominal radiotherapy were excluded.

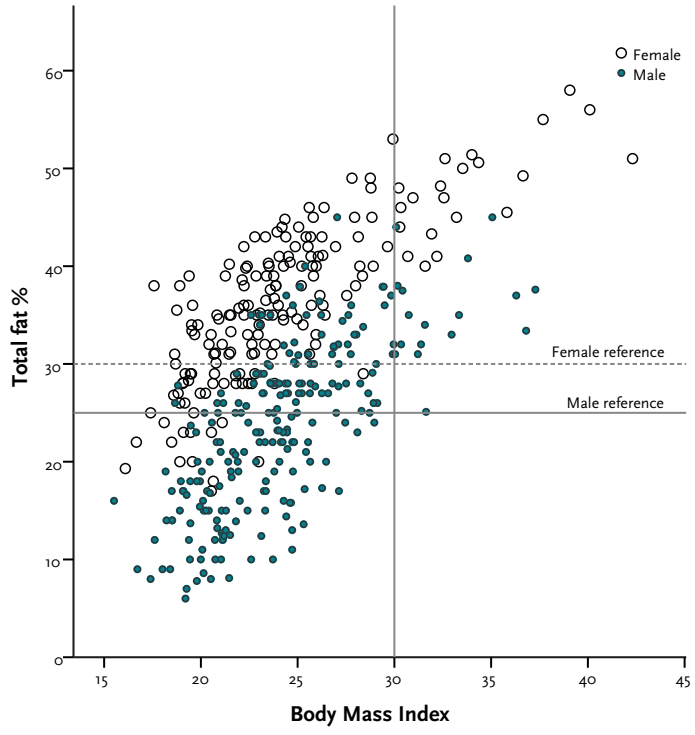
Table 2.2 Obesity as defined by BMI and waist-hip ratio compared to total fat percentage in adult childhood cancer survivors.

Height and lean body mass

Lean body mass, as well as height, were significantly lower in all treatment groups, compared with Dutch references, except for female BRT survivors (Figure 2.2). Adjusted for height SDS, lean body mass SDS was significantly lower in male TBI survivors only (T1, p=0.01; T2, p=0.004). Lean body mass did not change over time in either men or women in any of the treatment groups (Figure 2.2).

BMI and waist-hip ratio as compared with total fat percentage

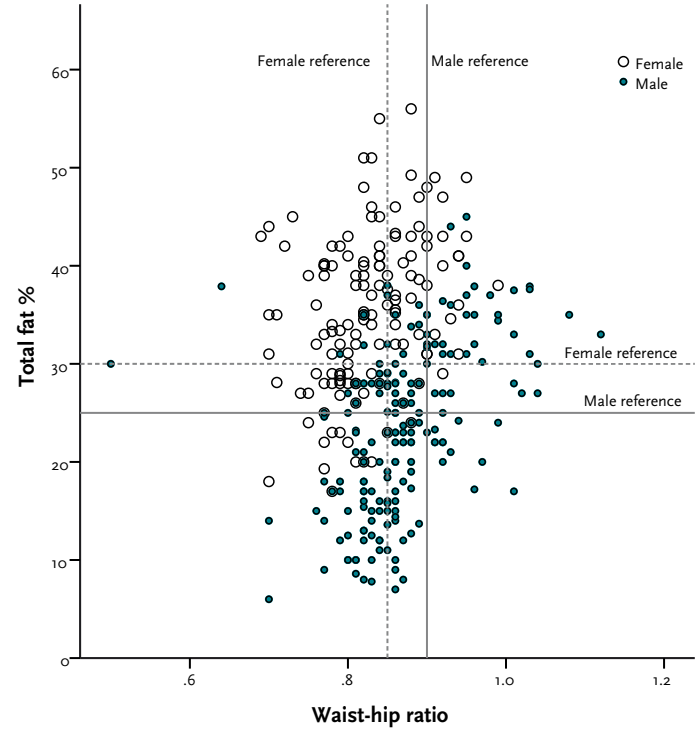
Out of 407 survivors, 210 (52%) were misclassified as non-obese based on BMI, while meeting the obesity criteria based on total fat percentage. In female survivors this percentage of misclassification was even higher (65%) than in men (42%) (Table 2.2, Figure 2.3). Waist-hip ratio was classified as non-obese and total fat percentage as obese in 92 out of 299 survivors (31%). This percentage of misclassification was 40% in female and 24% in male survivors (Figure 2.4).



Female and male survivors in the upper quadrant corner, marked by the dotted respectively the straight horizontal line and vertical line, are classified as non-obese by BMI, but as obese by total fat percentage.

Figure 2.3 Body Mass Index versus total fat percentage.

We attempted to define new cut-off points for BMI and waist-hip ratio to better identify childhood cancer survivors as obese, using total fat percentage as the gold standard (Table 2.3). Sensitivity improved by 58% (from 14% to 72%) in males, with a loss in specificity of only 24% (100% to 76%) when using a BMI cut-off of 24 instead of 30. In females a cut-off of 22 would give an increase in sensitivity of 64 % (16 to 80%) with a loss in specificity of only 18% (100% to 82%). Cut-off points for waist-hip ratio, as defined by the WHO, were more accurate than BMI in childhood cancer survivors (Table 2.3), although a cut-off limit of 0.82 instead of 0.85 in women would give an increase in sensitivity of 26% (45% to 71%), with a loss in specificity of 14% (85% to 71%).



Female and male survivors in the upper quadrant corner, marked by the dotted respectively the straight horizontal and vertical lines, are classified as non-obese by waist-hip ratio, but as obese by total fat percentage.

Figure 2.4 Waist-hip ratio versus total fat percentage.

2.5 Discussion

Male survivors showed an increase of BMI and total fat percentage during a median follow-up time of 3.2 years, more than 15 years after treatment for childhood cancer. Female survivors, especially the cranial irradiated leukaemia / lymphoma subgroup, showed higher BMI and total fat percentage at both time points but without a significant change over time. To our knowledge, the childhood cancer survivor study (CCSS) is the only study to date that describes longitudinal changes in childhood cancer survivors at very long-term follow-up[48(p138)]. In this study, self-reported BMI was evaluated in ALL survivors 25 years after diagnosis, and CRT was defined as a risk factor for BMI change, particularly in women[48(p138)]. Brouwer et al. showed that BMI increased in Dutch childhood cancer survivors, from the end of treatment until attainment of final height and observed that high dose CRT was the most important risk factor for this BMI increase[60(p140)]. Long after attainment of final height, all of our male survivors showed a higher BMI increase compared with the reference population, suggesting that cancer treatment effects persist over time. This BMI increase did not result in a significantly higher BMI 3 years later. However, taking into account the short time-interval, this significant increase in BMI at a relatively young age might indicate an emerging problem in the future. In female survivors BMI was significantly higher after CRT treatment; however, there was no significant change over time, suggesting that changes in BMI had already occurred at a younger age. Furthermore, the interval between measurements was only 3 years and survivors had a younger age at the 1st assessment (median 24 years) as compared with the CCSS (median 32 years) which might explain the different findings. Additionally, since annual reference data from the CBS are self-reported, BMI could be underestimated, possibly affecting the comparison between BMI in survivors and controls in this study.

Previous studies have been mainly based on self-reported or measured BMI values. Recently it was shown that BMI is an inaccurate marker for total body fat. Obesity was underestimated in 39% of subjects, using BMI[43(p138)]. Therefore, we used DXA to assess total fat percentage and lean body mass, which are considered to be more relevant predictors for cardiovascular disease or diabetes mellitus[39-42(p138)]. In the current study, total fat percentage was persistently and significantly higher than the reference population and was more pronounced in CRT survivors. Furthermore, while mean BMI standard deviation scores varied around zero, increases in total fat percentage SDS and decreases in lean body mass SDS were more pronounced. This implies that although BMI may seem normal, the distribution of total fat and lean body mass might be dramatically changed. Therefore, in order to test the hypothesis that BMI underestimates the adiposity risk in childhood cancer survivors, BMI data were compared with total fat percentage of all the survivors in whom one DXA scan was performed. This revealed that indeed 42% of male survivors and 65% of female survivors were classified as non-obese by BMI, but as obese by total fat percentage. These percentages of misclassification are even greater than compared with earlier

	Male		Female	
	Sensitivity	Specificity	Sensitivity	Specificity
BMI cut-off point				
18	100	4	99	10
20	98	25	92	54
22	91	58	80	82
24	72	76	53	97
26	47	95	32	97
28	28	98	23	97
30[a]	14	100	16	100
32	6	100	11	100
WHR cut-off point				
0.77	98	3	93	9
0.78	98	8	89	21
0.79	98	9	84	32
0.80	96	12	80	50
0.81	94	15	75	59
0.82	91	22	71	71
0.83	88	34	60	76
0.84	86	42	54	82
0.85[a]	81	48	45	85
0.86	73	56	42	88
0.87	63	70	33	88
0.88	59	76	31	91
0.89	54	85	24	94
0.90[a]	49	87	20	97
0.91	42	88	13	97
0.92	40	91	12	97
0.93	33	95	9	100
0.94	28	96	NA	NA
0.95	28	97	NA	NA
0.96	22	97	NA	NA
0.97	19	98	NA	NA
0.98	17	99	NA	NA

NA not applicable. [a]Cut-off limits of BMI respectively waist-hip ratio generally used for the classification of obesity.

Table 2.3 Sensitivity and specificity for different cut-off points of BMI and waist-hip ratio as defined by total fat percentage of ≥ 25% for men and ≥30% for women.

findings in the general population by Shah and Braverman, although our subjects were much younger[43 (p138)]. This finding emphasizes the need for more precise measurements of total fat percentage in order to define obesity. Since DXA is costly and not easily accessible for less developed countries, we attempted to define more accurate cut-off limits for BMI in childhood cancer survivors. It was shown that a cut-off limit of 24 for males and 22 for females resulted in greater sensitivity, with only small reductions in specificity. Although the group was too small to draw definite conclusions, these findings indicate that the current cut-off of 30 is not useful in defining obesity in childhood cancer survivors. A more reliable determinant than BMI that predicts the development of cardiovascular disease is the amount of intra-abdominal fat, which is approximated by the waist-hip ratio[17, 18 (p135)]. Above all, waist-hip ratio is an easy to use clinical tool. However, when we compared waist-hip ratio with total fat percentage in childhood cancer survivors, percentage misclassification was lower than with BMI, but still 31%.

Mechanisms that contribute to abnormal body composition in childhood cancer survivors include decrease in physical activity, hypothalamic, pituitary and/or gonadal damage, due to cranial and abdominal radiotherapy and leading to endocrinopathies and use of corticosteroids, followed by metabolic changes[17, 18 (p135), 38 (p137), 44-46 (p138)].

In the current retrospective study, no data concerning socio-economic status, smoking, diet and physical activity for the total cohort of survivors were available, which could be important predictors for BMI change. Reduced energy expenditure and excess energy intake may play a role in the development of overweight in childhood cancer survivors, and has been described during and shortly after treatment[61, 62 (p140)]. Furthermore, since obesity is not an independent risk factor for cardiovascular morbidity, prospective studies are needed, investigating multiple risk factors such as hypertension, dyslipidemia, insulin resistance and lifestyle changes in very long-term survivors of childhood cancer.

Conclusion

In this large and unique longitudinal study among childhood cancer survivors, a greater increase in BMI and total fat percentage compared with the reference population was found, especially in adult male survivors. Furthermore, we found that BMI underestimated obesity by 52% of adult survivors while waist-hip ratio underestimated 31% of survivors when compared with measurements of total fat percentage. Therefore, we have suggested new cut-off limits, which need to be confirmed in future prospective studies, in order to define obesity more precisely in childhood cancer survivors.

The limited screening value of insulin-like growth factor-I as a marker for alterations in body composition in very long-term adult survivors of childhood cancer

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3.1 Abstract

Background

The clinical relevance of low IGF-I levels, caused by cranial radiotherapy, in adult childhood cancer survivors has not been studied extensively. We evaluated whether IGF-I is a useful marker for altered body composition and growth hormone deficiency in this group.

Procedure

We analysed retrospective data from 610 adult childhood cancer survivors, retrieved from the late effects clinic. Median age at diagnosis was 6 years (interquartile range 3-11) and follow-up time was 18 years (13-24). We assessed IGF-I standard deviation scores (SDS), anthropometrical measures, growth hormone stimulation tests in patients with clinical signs of GHD and measures of body composition (assessed by dual X-ray absorptiometry, Lunar Prodigy).

Results

In 58 cranially irradiated acute leukaemia survivors (25 Gy (24-25)) and 56 locally irradiated brain tumour survivors (42 Gy (35-54)) we found significantly lower IGF-I SDS ($p<0.001$), lower height SDS ($p<0.001$), higher body mass index ($p=0.01$), higher waist-hip ratio ($p=0.001$), higher total fat percentage SDS ($p<0.001$) and lower lean body mass SDS ($p<0.001$), as compared to 452 non-cranially irradiated survivors. IGF-I showed a weak inverse correlation with BMI ($r=-0.12$, $p=0.04$), waist-hip ratio ($r=-0.15$, $p=0.01$), total fat percentage ($r=-0.14$, $p=0.02$) and a positive correlation with lean body mass ($r=0.15$, $p=0.01$). In patients with low IGF-I levels, IGF-I did not significantly differ between subjects with and without GHD as determined by GH stimulation testing ($p = 0.39$).

Conclusion

This study shows that IGF-I has limited value as a marker for alterations in body composition in adult childhood cancer survivors.

3.2 Introduction

Childhood cancer survival rates have improved significantly, with 70-80% of patients becoming long-term survivors[35(p137)]. It has been estimated that 1 out of 640 young adults in the U.S. is a survivor of childhood cancer[63(p140)]. Consequently, the incidence of late, treatment-related complications is increasing. Endocrine sequelae, such as the metabolic syndrome, osteopenia, subfertility, thyroid dysfunction and growth hormone deficiency, represent an important category of such late effects[5, 16, 18(p135), 44(p138), 64, 65(p140)].

Growth hormone deficiency (GHD), which has an incidence of 29-39% after CRT treatment[66(p140)], has many effects on the metabolic system, such as decreased lean body mass and impaired bone mineral density, increased abdominal fat mass, dyslipidemia, insulin resistance and subsequent raised cardiovascular morbidity later in life[64(p140)]. GHD, due to damage of the radiosensitive hypothalamic-pituitary region by CRT, has been associated with low IGF-I levels in adult childhood cancer survivors[16(p135), 67(p141)]. However, it has been shown that IGF-I is a poor marker for GHD in patients treated with CRT[68(p141)] and that only very low levels of IGF-I are predictive of GHD[69(p141)].

In a previous study in 114 childhood acute lymphoblastic leukaemia (ALL) survivors that were treated with CNS irradiation, it was shown that low IGF-I levels were associated with altered body composition, represented by decreased lean body mass, increased total fat percentage and 65% more visceral fat in combination with a worsened metabolic risk profile, represented by insulin resistance and dyslipidemia[46(p138)]. This study confirmed results of earlier studies of CRT treated ALL survivors that showed low IGF-I levels associated with total and visceral fat percentage, but not with BMI[16, 17(p135)]. However, most studies focused on relatively small groups of ALL survivors and after CRT treatment. The value of IGF-I as a marker for body composition, and its association with GHD, is uncertain. Therefore, in this study we analysed IGF-I levels in a single centre cohort of 610 adult childhood cancer survivors.

3.3 Patients and Methods

Patients

We performed a retrospective single centre study. Follow-up at our adult late effects outpatient clinic for long-term childhood cancer survivors starts 5 years after cessation of therapy and consultation is individualized, based on cancer diagnosis and treatment protocol. Out of 885 adult survivors, diagnosed and treated between 1964 and 2005, we identified and included 610 survivors, in whom IGF-I had been measured between 2004 and 2009, at least 5 years after cessation of cancer treatment. Twenty survivors were living abroad at time of follow-up, 21 refused to participate and 201

survivors did not show up at the outpatient clinic, due to the fact that the late effects clinic was a new setting and survivors could be under treatment by another specialist. Up until 2009, IGF-I levels were assessed based on former diagnosis and treatment as routine follow-up screening for GHD. Thereafter, according to the national guidelines of screening for late effects as implemented in 2010 by the Dutch Childhood Oncology Group (DCOG), routine measurement of IGF-I levels was omitted.

Clinical parameters

Data concerning treatment protocols, as well as disease and patient characteristics, were retrieved from our local database and completed from the medical records where necessary (Table 3.1). Follow-up time was defined as time between cessation of therapy and the most recent visit. Follow-up data of the most recent visit were evaluated including the following variables: use of medication, weight, height, target height (TH) calculated from the height of the survivors' parents[53(p139)], body mass index (BMI) calculated from height and weight[49(p139)], and waist-hip ratio (WHR) as measured by waist circumference divided by hip circumference[70(p141)]. Final height standard deviation score (SDS) was calculated using reference values for Dutch adults: a mean (SD) of 184.0 (7.1) cm for males and 170.6 (6.5) cm for females[53(p139)].

Body composition

Data on total body fat mass (kg), lean body mass (kg) and percentage of body fat were retrieved from dual energy X-ray absorptiometry (DXA, GE Lunar Prodigy, GE Healthcare, Madison, WI) (measured in 422 survivors) and assessed as previously described, on the same day as IGF-I measurement. In addition, visceral fat percentage was calculated from intra-abdominal fat (kg) and total fat (kg) using the DXA scan[71(p141)]. Values for lean body mass and total fat percentage were compared with normal Dutch reference values and calculated as standard deviation scores (SDS)[55(p139)]. Waist-hip ratio and visceral fat percentage were not analysed in the subset of survivors treated with abdominal radiotherapy because of detrimental local effects on body fat and the frequent occurrence of scoliosis.

Laboratory assessments

IGF-I was assessed by Immulite 2000 (DPC Biermann GmbH/Siemens, Fernwald, Germany), a solid-phase, enzyme-labelled chemiluminescent immunometric assay, with an intra assay variability of 2-5% and an inter assay variability of 3-7%. IGF-I levels were compared with reference values by using standard deviation scores (SDS)[58(p139)]. In cases where GHD was suspected (i.e. IGF-I below -2 SDS) either insulin tolerance tests or, if contra-indications were present (diabetes mellitus and heart failure), GHRH-Arginine tests were performed[69(p141)]. However, only 28 out of the 85 survivors with an IGF-I below -2 SDS were tested for GHD. Eight out of these 28 survivors were tested before 18 years of age because of growth impairment during childhood. One possible reason that only a proportion of the survivors with IGF-I below -2 SDS were tested for growth hormone deficiency is that some endocrinologists are reluctant to treat childhood cancer survivors with growth hormone because of the resultant high recurrence rate[72(p141)].

Treatment groups

Baseline characteristics of the included survivors are shown in Table 3.1. Three hundred and eighty one survivors had been treated with chemotherapy only. Seventy three subjects, mainly brain tumour survivors, had been treated with cranial irradiation focused on the tumour field (tumour field CRT). Fifty seven subjects, mainly leukaemia survivors, had been treated with irradiation of the whole cranium (CRT). Twenty survivors had received additional irradiation of the spinal cord. In three leukaemia survivors both cranial irradiation and total body irradiation (TBI) were administered. The following treatment groups were analysed separately after excluding survivors that were treated with growth hormone at the time of the study (n=23): CRT treated leukaemia survivors (25 Gy (24-25)), including 57 CRT survivors, plus 3 survivors that had been treated with CRT and TBI, minus 2 subjects that received GH therapy at follow-up (n=58); survivors treated with local irradiation of the cranium (mainly brain tumours) (42 Gy (35-54)) (n=56); and TBI survivors (8 Gy (8-12)) (n=21). These 3 groups were compared separately with all other survivors (n=452), hereafter referred to as the non-cranially irradiated group.

Confounding factors Oral contraceptive use can result in lower IGF-I levels, and impaired thyroid function or impaired gonadal function can alter body composition. No subjects were identified with untreated hypothyroidism. Therefore, in our analysis we only adjusted for hypogonadism, represented by oligo- or amenorrhea in women or low testosterone levels in men (<9 nmol/L) and the use of oral contraceptives in women. As previous therapy with corticosteroids may influence body composition, we also adjusted for corticosteroid treatment.

Statistics

All data were expressed as median (interquartile range, IQR) unless specified otherwise. Differences between treatment groups were tested using the Mann Whitney-U test for scaled data and the chi-squared test or the Fisher's exact test for nominal data. Correlations were tested using partial correlations. Regression analysis was used to correct for possible confounding factors. If no SD scores were available, body composition parameters were adjusted for sex and age. P-values are all measured in the two-way classification with $p<0.05$ considered statistically significant. Statistical analysis was performed using SPSS 17.0 software (SPSS, Chicago, IL).

	Complete group N=610	No radiotherapy N=381	Radiotherapy					
			Tumour field CRT 42 Gy (35-54) N=73		CRT 25 Gy (24-25) N=57	TBI 8 Gy (8-12) N=22	CRT & TBI 22 Gy (18-25) N=3	Abdominal RT 20 Gy (20-30) N=44	Other RT 40 Gy (20-45) N=30
Sex (male)	344 (56)	211 (55)	51 (70)		30 (53)	14 (64)	2 (67)	19 (43)	16 (53)
Age at diagnosis	6 (3-11)	6 (2-12)	10 (7-12)		3 (2-6)	10 (7-12)	4 (3-11)	4 (0-6)	7 (5-11)
Follow-up time	18 (13-24)	17 (12-21)	16 (11-25)		25 (21-28)	14 (11-21)	15 (13-29)	29 (24-34)	14 (12-23)
Diagnosis									
ALL	183 (30)	116 (30)	0		51 (89), 5 (9)[a]	8 (36)	2 (67)	0	1 (3)
AML	20 (3)	10 (3)	0		1 (2)	8 (36)	1 (33)	0	0
T-NHL	6 (1)	5 (1)	1 (2)		0	0	0	0	0
B-NHL	51 (8)	44 (12)	2 (3)		0	3 (14)	0	0	2 (7)
Hodgkin	49 (8)	34 (9)	8 (11)		0	1 (5)	0	0	6 (20)
Bone tumour	29 (5)	19 (5)	1 (1)		0	0	0	0	9 (30)
Wilms tumour	70 (12)	37 (10)	0		0	0	0	33 (75)	0
Neuroblastoma	41 (7)	31 (8)	0		0	0	0	8 (18)	2 (7)
Germ cell	12 (2)	12 (3)	0		0	0	0	0	0
MMT	52 (9)	31 (8)	10		0	0	0	2 (5)	9 (30)
LCH	13 (2)	11 (3)	2		0	0	0	0	0
Brain tumour	63 (10)	17 (5)	46 (63), 15 (33)[a]		0	0	0	0	0
Other	21 (3)	14 (4)	3 (4)		0	2 (9)	0	1 (2)	1 (3)
Recurrence (≥1)	62 (11)	30 (8)	19 (26)		14 (25)	13 (59)	3 (100)	8 (18)	7 (23)
Corticosteroids [b]	299 (49)	192 (50)	21 (29)		57 (100)	18 (82)	3 (100)	2 (5)	6 (20)
GH therapy [c]	23	3	17		2	1	-	-	-

Data are expressed as median (interquartile range) or as totals n (%). **ALL** acute lymphoblastic leukaemia; **AML** acute myeloid leukaemia; **T-NHL** T-cell non-Hodgkin lymphoma; **B-NHL** B-cell non-Hodgkin lymphoma, Burkitt lymphoma; **MMT** malignant mesenchymal tumour; **LCH** Langerhans Cell Histiocytosis; **CRT** cranial radiotherapy; **TBI** Total body irradiation; **RT** radiotherapy; **other RT** radiotherapy not including the cranium, abdomen and total body irradiation, i.e. radiotherapy on the thorax, mediastinum, pelvis, testes and extremities. [a]craniospinal radiotherapy; [b]Former treatment with corticosteroids as part of the childhood cancer protocol. [c]Twenty three survivors received growth hormone therapy at the time of the study and therefore were excluded from further analyses.

Table 3.1 Details of diagnosis and therapy in the cohort of studied adult childhood cancer survivors.

3.4 Results

IGF-I values

IGF-I SDS of the total group was -0.35 (range -5.55 – 2.55), which indicates that IGF-I in this group of adult childhood cancer survivors was lower compared to normal subjects. After correction for possible confounders, both cranially irradiated leukaemia survivors and brain tumour irradiated survivors had significantly lower IGF-I SDS, as compared to those treated without cranial irradiation (p<0.001) (Table 3.3). No significant difference in IGF-I SDS was found when comparing the TBI / BMT group with the non-cranially irradiated group (p=0.06). Compared with non-irradiated survivors, the risk of having an IGF-I SDS below minus 1 is 4.3 times higher in CRT survivors and 3.2 times higher in survivors treated with localized irradiation therapy for brain tumours (OR 4.3 p<0.001, OR 3.2, p<0.001).

	Non-cranially irradiated CCS N=452	Tumour field CRT 42 Gy (35-45) N=56	P[a]	CRT 25 Gy (24-25) N=58	P[a]	TBI 8 Gy (8-12) N=21	P[a]
Height SDS	-0.4 (-1.2-0.2)	-1.3 (-2.1; -0.3)	<0.001	-1.6 (-2; -0.4)	<0.001	-1.1 (-2; -0.1)	0.02
Target Height SDS	0.1 (-0.4-0.7)	0.3 (-0.3-0.5)	0.84	-0.3 (-0.6-0.3)	0.06	-0.1 (-0.6- 0.1)	0.18
ΔTH – height SDS	0.6 (0.2-1.2)	1.5 (0.9-2.6)	<0.001	1.5 (0.8-2.2)	<0.001	1.6 (0.3-3.5)	0.03
Body Mass Index	23 (21-25)	24 (21-28)	0.02	26 (23-29)	<0.001	21 (19-22)	0.004
Waist-hip ratio	0.84 (0.8-0.88)	0.88 (0.84-0.92)	<0.001	0.89 (0.86-0.93)	<0.001	0.86 (0-84-0.9)	0.03
Visceral fat %	7.4 (6.4-8.6)	7.9 (7-9.3)	0.008	8.6 (7.8-9.6)	<0.001	8.3 (7.2-8.8)	0.04
Total fat % SDS	1.2 (0.7-1.8)	1.9 (1.2-2.5)	<0.001	2 (1.4-2.2)	<0.001	1.8 (1.3-2.1)	0.02
LBM SDS	-0.7 (-1.3-0.2)	-1.2 (-2.1 -0.7)	0.005	-1 (-2; -0.1)	0.04	-2.3 (-3; -1.9)	<0.001
IGF-I SDS (range)	-0.28 (-4.9-2.5)	-0.6 (-5.3-2.6)	<0.001	-1.3 (-5.6-0.5)	<0.001	-0.75 (2.4-1.2)	0.06
IGF-I SDS <-1	85 (23)	24 (43)	<0.001	35 (60)	<0.001	8 (38)	0.06

Data are expressed as median (interquartile range). **SDS** standard deviation score; **TH** target height; **ΔTH - height SDS** difference between target height SDS and height SDS; **LBM** lean body mass. [a]P-value is calculated using Mann-Whitney test for scaled variables and chi-square test or Fisher's exact test for nominal variables.

Table 3.2 IGF-I levels and body composition in the different radiotherapy groups compared to the non-irradiated group.

IGF-I and growth hormone deficiency

Twenty-eight out of the 85 subjects with an IGF-I below -2 SDS were tested with a growth hormone stimulation test, as described earlier (a GHRH-Arginine test was performed in 11 survivors). Seventeen patients were diagnosed with severe GHD by growth hormone stimulation testing, defined as a GH peak <3 µg/L (insulin tolerance test) or <9 µg/L (GHRH-Arginine test)[73(p147)], whereas 11 subjects did not have GHD. The 17 GHD subjects had a median IGF-I SDS of -4.6 (-5.7; -2.3), the 11 non-GHD subjects had a median IGF-I SDS of -3.8 (-5.6; -2.7), (p=0.39).

	Tumour field CRT 42 Gy (35-54) N=56	CRT 25 Gy (24-25) N=58	TBI 8 Gy (8-12) N=21
Height SDS	-0.8 (-1.2; -0.5) **	-0.7 (-1.1; -0.4) **	-0.7 (-1.3; -0.2) *
ΔTH SDS – height SDS	1.2 (0.8-1.7) **	0.8 (0.4-1.2) **	1.1 (0.5-1.8) **
Body Mass Index (kg/m²)	3.7 (-12.2- 8.9)	11.9 (6.8-17.2) **	-10.2 (-16.7; -3) *
Waist-hip ratio	0.02 (0-0.04)	0.04 (0.02-0.07) *	0.02 (-0.01-0.06)
Visceral fat percentage (%)	-0.2 (-0.4-0.8)	1.1 (0.6-1.7) **	0.8 (0-1.6)
Total fat percentage (%) SDS	0.4 (0.1-0.7) *	0.6 (0.3-0.9) **	0.4 (-0.02-0.9)
Lean body mass (kg) SDS	-0.7 (-1.2; -0.3) *	-0.1 (-0.5-0.3)	-1.6 (-2.2; -0.9) **
IGF-I SDS	-0.9 (-1.2; -0.6) **	-1.2 (-1.5; -0.8) **	-0.2 (-0.7-0.4)
IGF-I SDS <-1 (OR, 95% CI)	3.2 (1.7-6) **	4.3 (2.3-8.2) **	1.4 (-0.5-4.1)

Regression coefficients are adjusted for sex, age at diagnosis, follow-up time, recurrence, use of oral contraceptives and hypogonadism (expressed as testosterone <9 nmol/L (m) or oligo- / amenorrhea without OC use (f)). SD scores are adjusted for all these variables minus sex and follow-up time. Height, body mass index, waist-hip ratio, visceral fat percentage, total fat percentage and lean body mass are also adjusted for treatment with corticosteroids. **SDS** standard deviation score; **TH** target height; **ΔTH SDS - height SDS** difference between target height SDS and height SDS; **OR** odds ratio; **CI** confidence interval. *p<0.05; **p<0.001

Table 3.3 Adjusted regression coefficients (95% CI) of IGF-I levels and components of body composition of the different radiotherapy groups compared to the non cranially irradiated group (n=452), adjusted for possible confounders.

Final height and body composition

Raw data on height and body composition data of the total cohort are depicted in Table 3.2. Adjusted regression coefficients of the different radiotherapy groups as compared to the not cranially irradiated group are depicted in Table 3.3, which is referred to in the following section. Survivors treated with cranial irradiation and irradiated brain tumour patients had significantly lower height SDS (p<0.001) and higher total fat percentage SDS (p<0.001). Leukaemia survivors treated with cranial irradiation had higher body mass index (p<0.001), higher waist-hip ratio (p<0.05) and higher visceral fat percentage (p<0.001) than those treated without radiotherapy. Irradiated brain tumour survivors had a higher total fat percentage and lower lean body mass, than those treated without radiotherapy (p<0.05 respectively p<0.001). Survivors

treated with total body irradiation had significantly lower height SDS ($p<0.05$), lower BMI ($p<0.05$) and lower lean body mass ($p<0.001$). To correct for target height, the difference between target height SDS and final height SDS was calculated. This was significantly greater in all cranially irradiated groups compared to the non cranially irradiated group ($p<0.001$).

IGF-I and body composition

After correction for possible confounders, in the whole cohort, IGF-I SDS was positively correlated with height SDS ($r=0.23$, $p<0.001$) and lean body mass SDS ($r=0.15$, $p=0.01$); IGF-I SDS was inversely correlated with BMI ($r=-0.12$, $p=0.04$), waist-hip ratio ($r=-0.15$, $p=0.01$) and total fat percentage SDS ($r=-0.14$, $p=0.02$) . Visceral fat percentage, measured by DXA, was not correlated with IGF-I ($r=-0.1$, $p=0.1$). When analysing only the subgroup that had been treated with cranial irradiation ($n=135$), IGF-I SDS was positively correlated with height SDS ($r=0.24$, $p=0.02$) and lean body mass ($r=0.23$, $p=0.03$. IGF-I SDS was not significantly correlated with BMI ($r=-0.07$, $p=0.58$), waist-hip ratio ($r=-0.1$,$p=0.37$) or total fat percentage ($r=-0.07$, $p=0.51$)

3.5 Discussion

The value of IGF-I as a marker for body composition in long-term childhood cancer survivors has not been studied extensively. Here, we show significantly lower IGF-I SDS and altered body composition in subgroups of survivors, i.e. ALL survivors treated with CRT and brain tumour survivors treated with local irradiation. However, IGF-I and all measures of body composition are so weakly correlated that the value of IGF-I as a marker for clinically relevant alterations in body composition has to be considered insufficient.

In this study we assessed the value of IGF-I as a marker for body composition and growth hormone deficiency. Several studies have shown that IGF-I is the best marker for GH secretory status[74-76](p141). For this reason, it was initially assumed that IGF-I would also be predictive in the diagnosis of GHD, and therefore IGF-I was used as screening instrument in adult childhood cancer survivors. Our data showed that more than 60% of the 28 survivors that were tested for GHD turned out to be severely GH deficient, leading us to conclude that low IGF-I is a useful screening instrument. Indeed, pathologically low IGF-I levels (below -2 SDS) have been reported to be a sensitive screening marker, especially in young adults with severe GHD[77](p141). However, low IGF-I levels do not always represent GHD, because IGF-I levels are dependent upon many other states, such as thyroid function, insulin action, nutritional status or chronic disease and therefore do not fully represent GH status[78](p142). Furthermore, it is known that IGF-I levels can be artificially normal after CRT treatment because of neurosecretory dysfunction[78](p142) and therefore, the general consensus guideline for adult GHD screening prescribes a mandatory GH stimulation test in cases of clinically suspected GHD[69](p141).

Previous studies in childhood ALL survivors have shown that low IGF-I levels associate with high BMI, high total and visceral fat percentage and low lean body mass[16, 17](p135), [46](p138). BMI is often used to quantify the level of obesity; however it is known to be only a crude indicator of body fat mass[39-42](p138). High amounts of total body fat, high visceral fat percentage and low lean body mass are more accurate markers for true body composition and subsequently are more reliable as risk factors for the development of cardiovascular disease or diabetes mellitus[39-42](p138). Our study in a large heterogeneous group of adult childhood cancer survivors, confirms that there is a significant negative correlation between IGF-I levels and BMI, waist-hip ratios and total fat percentage, and a positive correlation with lean body mass. However, if we focus on the subgroups that had been treated with cranial irradiation, IGF-I SDS showed a stronger positive correlation with lean body mass, whereas the other measures of body composition were not correlated with IGF-I SDS. However, the correlation coefficients were very weak, meaning that only 2-5% of the change in body composition is explained by low IGF-I levels.

According to the national guidelines of screening for late effects as implemented by the Dutch Childhood Oncology Group (DCOG), routine measurement of IGF-I levels was omitted, except in groups that are at high risk for GHD, i.e. CRT treated ALL survivors and locally irradiated brain tumour survivors[79](p142). Whereas low IGF-I levels can guide us to test for GHD, normal IGF-I levels, especially after CRT treatment, do not exclude GHD. Therefore, we recommend that routine IGF-I measurement in these groups be replaced with regular assessment of an insulin tolerance test, which is the gold standard test for GHD. Furthermore, because IGF-I is not a useful marker for clinically relevant alterations in body composition, DXA scans are recommended in risk groups, as defined in the current study, to assess fat mass and lean body mass.

In this large study we evaluated BMI and body composition in different treatment groups and concluded that after CRT treatment BMI, total fat percentage, waist-hip ratio and visceral fat percentage, which is a major risk factor for cardiovascular morbidity, were significantly raised in comparison with non cranially irradiated survivors. Lean body mass was not significantly different. This finding is in agreement with earlier smaller studies in ALL survivors which showed higher BMI, higher total and visceral fat percentage in CRT survivors, in combination with lower lean body mass, which was not found in our CRT survivors[17](p135), [46](p138). This is the first study to measure body composition in a large group of irradiated brain tumour survivors, whereas most of the previous studies on body composition are in ALL survivors. Alterations in body composition were represented by higher total fat percentage and lower lean body mass, without significantly higher body mass index. Gurney et al. showed that brain tumour survivors treated with high dose radiotherapy of the hypothalamus had increased BMI[16](p135). We did not find raised BMI in irradiated brain

tumour survivors, which might be explained by the fact that we did not make a distinction between hypothalamic and non-hypothalamic irradiation.

Conclusion

This study shows that IGF-I is not of additional value for identifying subjects at risk for alterations in body composition. Patients treated with cranial irradiation had higher total fat percentage and lower lean body mass than patients not treated with cranial irradiation.

Endocrine sequelae and metabolic syndrome in adult long-term survivors of childhood acute myeloid leukaemia

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4.1 Abstract

This study focuses on the effect of chemotherapy on endocrinopathies and the metabolic syndrome in adult survivors of childhood acute myeloid leukaemia (AML). Endocrine function and metabolic syndrome were evaluated in 12 AML survivors, treated with chemotherapy, and in 9 survivors of myeloid leukaemias treated with stem cell transplantation (SCT), after a median follow-up time of 20 years (range 9-31). In survivors treated with chemotherapy, no endocrinopathies or metabolic syndrome were present, although AMH and inhibin B levels tended to be lower than in controls. In SCT survivors, pituitary deficiencies and metabolic syndrome were more frequent.

4.2 Introduction

Five-year survival of childhood acute myeloid leukaemia (AML) has increased significantly over the past few decades and is now approximately 65%. Numerous studies have investigated late effects in survivors of childhood acute lymphoblastic leukaemia (ALL), however -due to lower patient numbers and lower survival rates- only a small number of studies have been published on late effects in survivors of acute myeloid leukaemia (AML). Endocrine late effects, including decreased bone mineral density, infertility, growth retardation, growth hormone deficiency and hypothyroidism and components of the metabolic syndrome (MetS) such as insulin resistance[28(p136), 80(p142)] and dyslipidemia[81(p142)] have been extensively studied in stem cell transplanted (SCT) survivors, especially in those treated with total body irradiation (TBI)[82-90(p142)].

Currently, intensive chemotherapy has become the standard treatment in childhood AML and SCT is only advised in less than 20% in most treatment protocols[91(p143)]. Consequently, data on long-term outcome after such intensive chemotherapy schedules are scarce. The Nordic AML Late effect Study among 102 AML survivors treated with chemotherapy only between 1984 and 2003, concluded that self-reported health was good and use of health care service was limited [92(p143)]. However, Mulrooney et al. reported recurrent cancer, second malignancies and cardiac events exceeding the rates in the normal population in 272 survivors of childhood and adolescent AML, who where treated with chemotherapy only between 1970 and 1986[93(p143)]. Limited data exist on very late endocrine effects and cardiovascular risk factors after intensive treatment for AML with chemotherapy only[81(p142), 84, 94(p143)]. In this case-controlled study we therefore evaluated the effect of chemotherapy only on endocrine sequelae and the components of the MetS in adult long-term survivors of myeloid leukaemias.

4.3 Materials and Methods

Subjects

Fifteen long-term (≥ 5 years after cessation of treatment) adult survivors of childhood acute myeloid leukaemia (AML), treated with chemotherapy only, between 1961-2004 in the Erasmus MC-Sophia Children's Hospital, participated in this prospective study. In addition, nine survivors (7 AML, 1 CML, 1 MDS) treated with SCT including TBI in the conditioning regimen, were recruited. Informed consent was obtained according to the Helsinki declaration[95(p143)] and the study was approved by the local medical ethical committee.

In addition, a healthy control group, consisting of 60 siblings, friends or neighbours, of the same sex and within an age range of five years of the related survivor,

was cross-sectionally recruited. In each individual, all measurements were performed on one and the same day.

Methods

Disease and treatment data were obtained from our local database. Information regarding smoking status, socio-economic status (SES), statines and antihypertensive medication, hormone supplementation and diabetes was collected using a self-designed questionnaire. Smoking status was defined as non-smoker, former smoker or current smoker. Socio-economic status was defined by the highest level of educational attainment and was obtained in seven categories based on the Dutch educational system. Information about physical activity was collected by a Dutch translation of the Short Questionnaire to Assess Health-enhancing physical activity (SQUASH), a questionnaire to assess physical activity[96](p144). Height was measured to the nearest millimetre using a Harpenden Stadiometer and weight was assessed with underwear only to the nearest 0.1 kg with a standard clinical balance. Body mass index (BMI) was calculated (weight(kg)/(height(m)^2)[97](p144). Waist and hip circumference were measured to the nearest 1 cm, midway between last rib and the iliac crest and at the maximum circumference of the buttocks, respectively[98](p144). Waist-hip ratio was calculated. Final height standard deviation score (SDS) was calculated using reference values for Dutch adults[53](p139).

Blood pressure was measured with the subject in sitting position after an hour of rest on the right arm with the Dinamap® Procare and was defined as the mean of three measurements. Standard deviation scores (SDS) were calculated using the reference value from the Dutch epidemiologic MORGEN-study[99](p144). Using the National Education Program Adult Treatment Panel Guideline III, participants with at least three of the following components were diagnosed with MetS: waist circumference >102 cm in males or >88 cm in females; triglycerides ≥1.7 mmol/L or treatment for dyslipidemia; high density lipoprotein-cholesterol (HDL-C) <1.03 in males or <1.30 in females); fasting plasma glucose ≥5.6 mmol/L or treatment for type 2 diabetes; blood pressure ≥130/85 mmHg or treatment for hypertension[100, 101](p144). ALT levels were evaluated as a marker for hepatic steatosis[102](p144).
Data on bone mineral density, lean body mass (kg) and percentage of body fat were retrieved from dual energy X-ray absorptiometry (DXA, GE Lunar Prodigy, Madison, WI) and were only available in survivors and values for bone mineral density, lean body mass and total fat percentage were compared with normal Dutch reference values and calculated as standard deviation scores (SDS) as previously reported from our institute [55](p139).

Laboratory measurements

Fasting blood samples were taken from an intravenous cannula before 10:00 AM. Serum insulin-like growth factor I (IGF-I) (nmol/L), cortisol (nmol/L), ACTH (pmol/L), luteinizing hormone (LH) (U/L), follicle stimulating hormone (FSH) (U/L) and insulin (pmol/L) were measured using a chemi-luminescence-based immunoassay (Immulite 2000, Siemens DPC, Los Angeles, CA). IGF-I levels were compared with reference values by using standard deviation scores (SDS)[58](p139). Testosterone (nmol/L) was measured by coated tube radioimmuno-assay (Siemens DPC, New York, NY). Serum AMH levels were determined using an in-house double-antibody enzyme-linked immunosorbent assay (ELISA) (commercially available through Beckman-Coulter, Brea, CA)[103, 104](p144). Inhibin B levels were measured using kits purchased from Serotec Ltd (Oxford, UK). Thyroid stimulating hormone (TSH) (mU/L) and free thyroxine (fT_4) (pmol/L) were measured using chemoluminescence assays (Vitros ECi Immunodiagnostic System; Ortho Clinical Diagnostics, Rochester, NY). Triglycerides (mmol/L), high-density lipoprotein-cholesterol (HDL-C) (mmol/L), low-density lipoprotein-cholesterol (LDL-C) (mmol/l), glucose levels (mmol/L) and alanine transaminase (ALT) (U/L) as a marker for liver steatosis were measured using an enzymatic in vitro assay (Roche Diagnostics, Mannheim, Germany). Homeostatic Model Assessment (HOMA) was calculated as a measure of insulin resistance[105](p145).

Statistics

Statistical analyses were performed with the Statistical Package for Social Sciences (SPSS 17.0, Chicago, IL). Mann Whitney U-tests were used to compare results between survivors and controls. P-values <0.05 (two-tailed) were considered statistically significant. Chisquare test were used to evaluate the frequency of MetS and its separate components. Multiple ordinal and linear regression analyses were performed, to correct for relevant confounders. Linear and ordinal regression analyses were all performed using the following strategy: first, all models were corrected for age and sex. Then the effects of the possible confounders socio-economic status, smoking, physical activity, BMI and use of oral contraceptives were evaluated using backward regression modelling. When P<0.200, the confounders were kept in the subsequent model. Subsequently, the effect of the subgroups treated with SCT and chemotherapy only were evaluated.

4.4 Results

Survivors and controls

Of 47 adult survivors of childhood myeloid leukaemias in our centre, 12 AML patients, treated with chemotherapy only (chemo-only group), and nine survivors treated with SCT for myeloid malignancies (SCT group) participated in this study.

Sixteen survivors moved to another outpatient clinic for late effects, two refused to participate and four survivors were lost to follow-up. Two AML survivors were treated with chemotherapy in combination with cranial radiotherapy respectively abdominal radiotherapy and were therefore excluded. One AML survivor with Down syndrome was excluded. One AML survivor was immobilized due to paralyses[106](p145). Baseline and treatment characteristics of controls and survivors, as well as representativeness as compared to excluded survivors are shown in Table 4.1.

Treatment protocols included BFM derived AML protocols[107-109](p145). In addition to chemotherapy, seven AML survivors, one CML survivor and one MDS survivor had been treated with SCT including TBI as conditioning regimen (SCT group) (Table 4.1). Data of 12 chemo-only survivors were compared with normal healthy controls and with data of SCT survivors. Age at diagnosis, age at follow-up and follow-up time of AML-chemo-only survivors were not significantly different from SCT survivors (p=0.310, p=0.193 and p=0.602 respectively). Numbers of patients receiving specific chemotherapeutic drugs and cumulative doses did not significantly differ between groups, except for cyclofosfamide which total cumulative dose was significantly higher in the SCT group (median dose of 3600 mg/m² vs 500 mg/m² in the chemo-only group).

Growth and growth hormone deficiency

Height SDS and IGF-I levels of chemo-only survivors were not significantly different from controls (Table 4.1). None of the chemo-only survivors was GH deficient and one survivor in the SCT group received growth hormone substitution for growth hormone deficiency diagnosed by an insulin tolerance test[110](p145).

Adrenal and Thyroid function

In the chemo-only group, none of the AML survivors had abnormal TSH levels or fT4 levels, whereas five SCT survivors received thyroxin because of primary hypothyroidism. Cortisol levels were significantly higher in the chemo-only group, but after correction for use of oral oestrogen supplementation in women, this effect disappeared (β= -20, p=0.900).

Fertility and gonadal dysfunction

Median testosterone level was 18.3 nmol/L (p=0.952) in chemo-only survivors and 11.3 nmol/L in SCT survivors (p=0.039) as compared to 18.7 nmol/L in controls.

An inhibin B level below 100 ng/L was found in 2/11 male chemo-only survivors, in all four SCT survivors and in 2/33 controls (Figure 4.1). Median inhibin B level was 167 ng/L in chemo-only survivors (p=0.086) and 21 ng/L in SCT survivors (P<0.001) as compared to 201 ng/L in controls. Inhibin B levels were significantly associated with cumulative dose of cyclofosfamide, but not with cytosine arabinoside dose.

Mean testicular volume was significantly lower in survivors after SCT than in chemo-only survivors (12.25 versus 20 mL, p=0.004), but after correction for age, BMI, physical activity and cumulative dose of cyclofosfamide, this difference was not significant (β= -3.4, p=0.387).

An AMH level <1 µg/L was found in 1/3 female chemo-only survivors and in 6/27 controls (Figure 4.2). Median AMH level was 1.2 µg/L in chemo-only survivors and 2.3 in controls (p=0.799), whereas in all 5 SCT survivors AML level was <0.1 (Table 4.2). The number of the patients was too small to study the effect of alkylating agents on AMH levels.

Components of the metabolic syndrome

Three out of 48 controls had the MetS, as compared to 1/12 subjects in the chemo-only group (p=1.000) and 1/8 subjects in the SCT group (p=0.507). After correcting for age, sex, smoking and BMI, chemo-only survivors did not have more MetS components than controls (OR=1.31, p=0.687), however SCT survivors did have more MetS components (OR=24.1, p<0.001).

Adiposity

High waist circumference was present in 15% of chemo-only survivors (p=0.569), none of the SCT survivors (p=0.346) and 13% of controls. BMI, waist and waist-hip ratio were not significantly different from controls in chemo-only survivors, whereas in SCT survivors waist-hip ratio was significantly higher than in controls (0.95 versus 0.87, p=0.001).

Total fat percentage was significantly higher compared with healthy Dutch references in both chemo-only survivors and SCT survivors (SDS 1.09, p=0.010 and SDS 1.19, p=0.005 respectively). Lean body mass was significantly lower than in healthy Dutch references in SCT survivors (SDS -1.31, p=0.024) but not in chemo-only survivors (SDS -0.46, p=0.248).

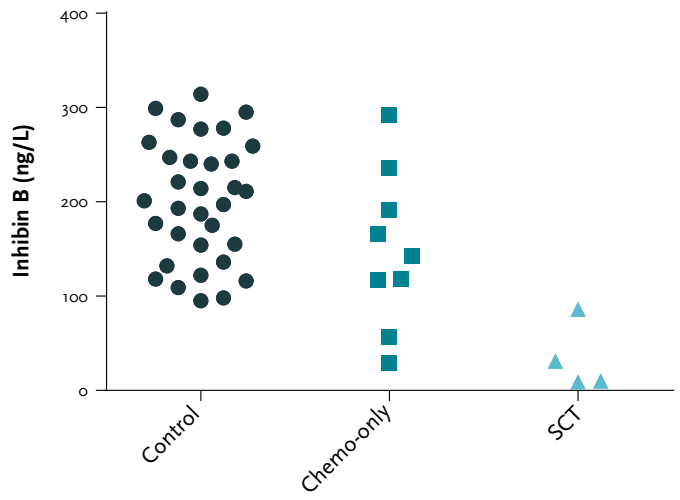
Insulin resistance

Elevated fasting glucose levels or treatment for diabetes were present in 7% of chemo-only survivors (p=0.437) and 13% of SCT survivors (p=0.697) as compared to 14% in controls. Fasting glucose, fasting insulin and HOMA were not significantly different between chemo-only survivors and controls (Table 4.2), while SCT survivors had significantly higher fasting glucose, fasting insulin and HOMA than controls (median 5.2 versus 4.9, p=0.021, 123 versus 25, p<0.001 and 2.3 versus 0.5, p<0.001, respectively).

	Chemo-only N=12			SCT N=9		Controls N=60	Survivors not included N=22	
Male/female	9/3			4/5		33/27	13/9	
Age at follow-up (years)	27.4 (22.0-39.2)			32.4 (23.4-44.5)		32.1 (18.0-61.7)	NA	
Age at diagnosis (years)	5.1 (0.1-15.8)			11.5 (1.1-15.0)		NA	9.6 (0.2-16.8)	
Follow-up time (years) [a]	21.6 (9.1-30.7)			19.0 (11.6-30.0)		NA	NA	
Diagnosis								
AML	12			7		NA	13	
MDS	0			1			5	
CML	0			1			4	
Recurrence or 2nd malignancy	1			2		NA	4	
Allogeneic / Autologous SCT	-			6/3		NA	9/0	
Radiotherapy (n)	0			9		NA	11[b]	
Cumulative dose (Gy)	-			TBI: 8 (4-12)		NA	CRT: 25 (24-26) / TBI: 8 (4-12)	
Chemotherapy (n)	12			9		NA	16	
	N	Cumulative dose (mg/m²)		N	Cumulative dose		N	Cumulative dose
Prednison i.v.	9	1235 (560-4000)		5	1230 (1225-4000)	NA	12	1225 (1225-3540)
Prednison i.th.	2	36		0	-	NA	0	NA
Vincristin	9	6 (3-10)		5	6 (5-8)	NA	10	10 (3-156)
Anthracyclines	11	320 (80-520)		7	176 (80-356)	NA	12	300 (146-376)
Cyclophosphamide	9	500 (400-8800)		9	3600 (1000-6000)	NA	13	1400 (120-7200)
Iphosphamide	1	32,100		1	21,400	NA	1	4000
Cytosine arabinoside	11	20,300 (10,690-62,000)		8	28,730 (3500-71,160)	NA	12	24,000 (520 – 39,200)
Busulfan	0	NA		1	300	NA	2	510 (80-940)
Melfalan	0	NA		0	NA	NA	1	ND
Etoposide	11	1200 (400-3600)		4	1925 (1350-2450)	NA	10	1200 (450-1500)
Methotrexate i.v.	4	150 (150 -225)		1	150	NA	3	30
Methotrexate i.th.	3	36 (36-66)		2	33 (30-36)	NA	8	36 (12-72)
Thioguanine [c]	5	ND		6	ND	NA	7	ND
Mercaptopurine [c]	3	ND		2	ND	NA	7	ND

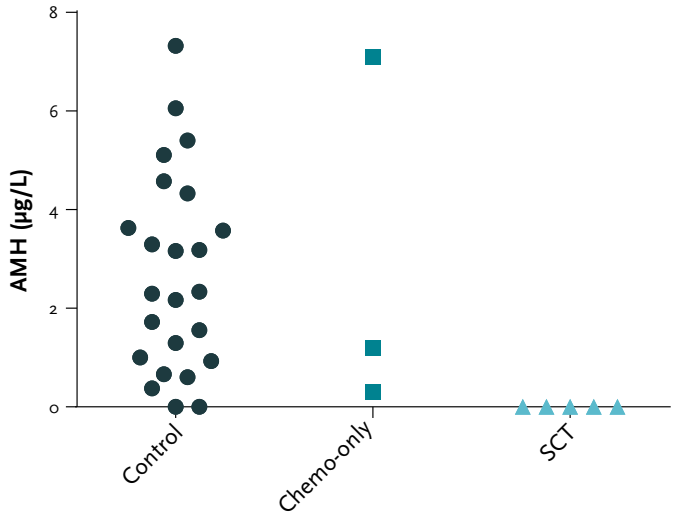
Data expressed as median (range) or as frequencies (N). [a] Time after cessation of treatment; [b] Three survivors were treated with CRT, six with TBI, one with CRT and TBI and one with abdominal RT; [c] Cumulative doses are unknown. **SCT** stem cell transplantation; **Gy** gray; **i.v.** intravenous; **p.o**. per os; **TBI** total body irradiation; **NA** not applicable; **ND** not determined.

Table 4.1 Baseline characteristics of study participants.



SCT stem cell transplantation

Figure 4.1 Levels of inhibin B (ng/L) in controls, chemo-only survivors and SCT survivors.



SCT stem cell transplantation

Figure 4.2 Levels of AMH (µg/L) in controls, chemo-only survivors and SCT survivors.

Dyslipidemia

In chemo-only survivors, the frequency of dyslipidemia was not significantly different from controls (7% versus 6%, p=0.953), whereas in SCT survivors, frequency of dyslipidemia was higher than controls (63% vs. 6%, p<0.001). In chemo-only survivors, triglyceride levels were not different from controls, whereas triglyceride levels were significantly higher in SCT survivors as compared to controls (median 2.5 vs. 0.8, p<0.001). Moreover, ALT levels were higher in SCT survivors than in controls (median 35 versus median 19, p=0.004) (β=103 (%), p=0.001)[102(p144)]. ALT levels were not different between the chemo-only group (median 20 (U/L), p=0.392) and controls.

	Chemo-only N=12	SCT N=9	Controls N=60
IGF-I SDS	-0.3 (-1.9-1.1)	-0.2 (-4.1-1.5)	-0.7 (-3.6-1.9)
TSH (mU/L)	1.3 (0.0-5.3)	3.3 (0.5-4.0)*	1.3 (0.4-3.9)
fT4 (pmol/L)	17.8 (11.8-21.3)	11.2 (10.0-17.0)**	15.9 (11.8-21.3)
Cortisol (nmol/L)	505 (301-748)*	386 (320-679)	348 (125-1054)
LH (U/L)	4.6 (3.1-10.1)*	6.8 (1.6-11.6)*	3.3 (0.2-34.8)
FSH (U/L)	4.6 (1.4-11.1)	10.5 (2.5-28.4)*	3.8 (0.2-48.4)
Testosterone (nmol/L)	18.3 (12.1-24.7)	11.3 (9.2-13.2)*	18.7 (6.0-32.2)
BMI (kg/m²)	27.2 (18.6-32.2)	28.2 (18.6-32.8)	24.2 (20.2-41.8)
Waist circumference (cm)	84 (68-106)	87 (68-114)	81 (68-132)
Waist-hip ratio	0.88 (0.81-1.05)	0.95 (0.91-1.13)*	0.87 (0.72-1.06)
Fasting glucose (mmol/L)	5.1 (4.3-5.6)	5.2 (4.9-6.5)*	4.9 (4.3-8.4)
Fasting insulin (pmol/l)	14 (13-118)	123 (77-159)**	25 (13-34)
HOMA	0.3 (0.2-2.2)	2.3 (1.4-3.1)**	0.5 (0.2-0.7)
Triglycerides (mmol/L)	1.1 (0.6-3.1)	2.5 (1.6-6.7)*	0.8 (0.4-3.4)
HDL-C (mmol/L)	1.3 (0.9-2.2)	1.2 (0.8-1.5)	1.3 (0.7-2.5)
Systolic pressure SDS	0.5 (-1.0-1.6)	0 (-1.5-2.8)	0.1 (-1.5-2.1)
Diastolic pressure SDS	-0.3 (-1.4-1.0)	0 (-2.2-2.8)	-0.2 (-2.0-2.3)

Data expressed as median (range). **IGF-I** Insulin-like growth factor-I; **SDS** standard deviation score; **TSH** thyroid stimulating hormone; **fT4** free thyroxine; **LH** luteinizing hormone; **FSH** follicle stimulating hormone; **BMI** body mass index; **HOMA** homeostatic model assessment; **HDL-C** high density lipoprotein-cholesterol. * p<0.05, ** p<0.001 (compared with controls).

Table 4.2 Univariate analysis of endocrine parameters and components of the metabolic syndrome.

Blood pressure

The frequency of hypertension was not different in the chemo-only group (21%,

p=0.415) nor in the SCT group (25%, p=0.391) as compared with controls (15%). Systolic and diastolic blood pressure in both therapy groups were not different from controls.

Bone mineral density

Bone mineral density of the total body (BMD-TB) was not significantly different from Dutch reference values in both chemo-only survivors and SCT survivors (SDS -0.21, p=0.567, and SDS -1.0, p =0.122 respectively). One adult chemo-only survivor had a BMD-TB SDS of -2 or lower, in contrast to four SCT survivors. SDS did not significantly differ between groups in univariate analysis (p=0.552) nor after adjustment for height.

4.5 Discussion

After twenty years of follow-up, AML survivors were not found to be at increased risk of endocrinopathies and components of the metabolic syndrome after treatment with chemotherapy only. As stem cell transplantation is replaced by intensive chemotherapy for 80% in current treatment protocols for childhood AML, this study focused on endocrine late effects and cardiovascular risk factors in survivors treated with chemotherapy only. In the current study, chemotherapy included cytosine arabinoside in combination with cyclofosfamide, anthracyclines, vincristin and corticosteroids.

Interestingly, we did not find an adverse effect on thyroid function after chemotherapy only, in contrast to hypothyroidism documented in half of the SCT survivors, treated with TBI. A previous study also found a higher frequency of hypothyroidism among SCT survivors (3/15) as compared with 1/44 of chemo-only survivors at 17 years of follow-up [84(p142)]. Similarly, Leahy et al. did not find any endocrinopathies after chemotherapy only in 26 survivors of childhood AML after seven years of follow-up[94(p143)], suggesting that hypothyroidism after SCT is due to the effect of total body irradiation as conditioning regimen for SCT.

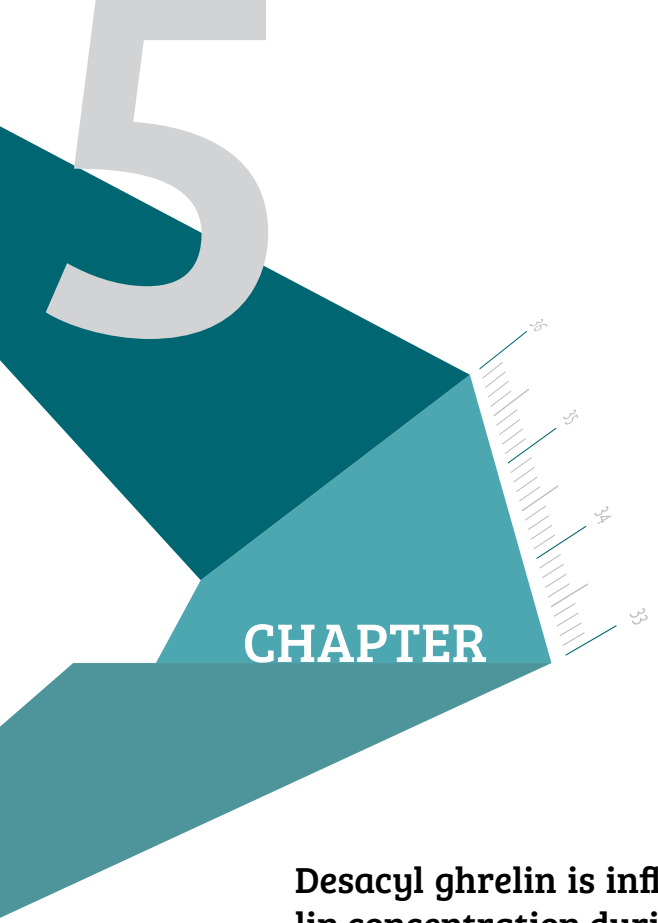
AMH and inhibin B levels tended to be lower in chemo-only survivors as compared to controls and 3/14 chemo-only survivors had gonadal dysfunction, indicating that intensive chemotherapy like cyclofosfamide may affect gonadal function, although gonadal dysfunction was much more pronounced in the SCT group[83(p142)]. Recently, in adult survivors of childhood non-Hodgkin lymphoma (NHL) survivors, we reported a contribution of cytosine arabinoside to gonadal dysfunction, which may be a previously disguised gonadotoxic drug, masked by the SCT regimens in the past[111(p145)]. The present study was underpowered to confirm our previous finding, so larger studies are needed to evaluate this.

Chemotherapy only survivors did not have a higher frequency of components of the Mets than controls. This is in contrast to the higher risk of the MetS after SCT treatment, which is mainly determined by a higher frequency of dyslipidemia. In a study by Oudin et al, describing both ALL (n=150) and AML survivors (n=34) at 15 years of follow-up, frequency of the MetS was higher after SCT treatment compared to chemo-only survivors (18% versus 5%), which was comparable with our findings (13% in SCT survivors versus 8% in chemo-only survivors)[81(p142)].

Although the present study is limited by sample size, our results are strengthened by the prospective study design with a cross-sectionally recruited control group. Furthermore, we described the complete spectrum of endocrine functions and components of the MetS in a representative group of AML survivors at very long-term follow-up. As to date most paediatric AML patients are treated with chemotherapy only, larger nationwide prospective epidemiological studies are needed to focus on the effects of intensive chemotherapy later in life.

Conclusion

Childhood AML survivors treated with chemotherapy only did not suffer from components of the MetS nor from endocrine late effects at very long-term follow-up, which is in contrast to childhood AML survivors treated with SCT. Gonadal function should however be evaluated with care in both therapy subgroups.



Desacyl ghrelin is influenced by changes in insulin concentration during an insulin tolerance test

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5.1 Abstract

Objective

Ghrelin, a gut-brain peptide, regulates energy homeostasis and glucose metabolism and is present in acylated and nonacylated form in the circulation. Although desacyl ghrelin (DAG), the predominant form of ghrelin, is associated with insulin sensitivity and improved metabolic state, not much is known about its direct regulation by insulin. We aimed to assess changes in DAG in response to the rapid increase in insulin concentration during an insulin tolerance test (ITT) in normal weight and obese subjects.

Design

We performed an observational single centre study.

Methods

An ITT was assessed in eight subjects (four males), median age 29.9 years (range 19.6-42.0). DAG concentrations were measured at 20, 40, 60 and 90 minutes after insulin infusion. Homeostatic Model Assessment (HOMA) was calculated from fasting insulin and glucose. Body mass index (BMI) and waist circumference were assessed.

Results

Three subjects were obese (BMI ≥30 kg/m²), one subject was overweight (BMI 25-30 kg/m²) and four subjects had normal weight (BMI 18.5-25 kg/m²). Median DAG decreased after insulin infusion (90 pg/mL, p=0.028), especially in normal weight subjects. Baseline DAG was lower in subjects with higher BMI (ρ -0.76, p=0.028) and higher fasting insulin (ρ -0.76, p=0.030). DAG change correlated with fasting insulin levels (ρ -0.85, p=0.007), HOMA (ρ -0.86, p=0.007), BMI (ρ -0.83, p=0.010) and waist circumference (ρ -0.93, p<0.001).

Conclusion

DAG levels rapidly decreased in response to insulin administration in normal subjects, but not in insulin-resistant obese who are in a state of relative DAG deficiency.

5.2 Introduction

Ghrelin, a gut-brain peptide, regulates energy homeostasis and glucose metabolism and is present in acylated and nonacylated form in the circulation[112](p145). For a long time acylated ghrelin was considered to be the only active form of ghrelin, since it stimulates food intake and displays a strong growth hormone (GH) releasing activity by binding to the GH secretagogue receptor 1a (GHS-R1a)[113, 114](p145). The predominant circulating form of ghrelin, however, is unacylated (desacyl ghrelin; DAG), which might have its own activities as well. While AG is associated with reduced insulin sensitivity, elevated DAG levels correlate with insulin sensitivity and reduced fat mass[115-117](p146). DAG also modulates lipid metabolism and energy balance in normal conditions[116, 118](p146). Subsequently, AG/DAG ratios have been found to be high in insulin resistant conditions and low in fasting, insulin sensitive subjects[117](p146), [119, 120](p146). In addition, it was reported that administration of DAG was found to reduce insulin resistance in healthy subjects, as well as in GH-deficient and in morbid obese subjects[116](p146), [121, 122](p146). This positive correlation between DAG and insulin sensitivity and improved metabolic state could be of great importance for future treatment strategies for insulin resistant diseases. However, there is still much unknown about the direct regulation of circulating DAG by insulin. In this study, we assessed the changes in DAG in response to a rapid change of insulin concentration during an insulin tolerance test (ITT) in normal weight and obese subjects.

5.3 Subjects and Methods

An ITT was performed in eight subjects (four males, median age 29.9 years (range 19.6-42.0) as part of patient care, because of insufficient hormone response during regular screening at the outpatient clinic. Five subjects were survivors of childhood cancer and three subjects had been recruited as healthy controls for survivor studies to the outpatient clinic. Eventually, none of the subjects turned out to have any pituitary insufficiencies or diabetes mellitus. Subjects gave their written informed consent to assess the additional laboratory data.

Subjects had been fasting overnight and were at rest for at least one hour before the start of the test. At baseline and at 20, 40, 60 and 90 minutes after insulin administration (0.15-0.25 IU / kg body weight, dependent on fasting insulin level), cortisol, GH, glucose and DAG were measured. Glucose was also measured at 30 minutes. Fasting insulin (pmol/L), GH (µg/L) and cortisol (nmol/L) were measured using a chemiluminescence-based immunoassay (Immulite 2000, Siemens DPC, Los Angeles, CA). Glucose (mmol/L) was assessed with an automatic hexokinase method (Roche, Almere, the Netherlands). Homeostatic Model Assessment (HOMA) was calculated as a measure of insulin resistance[105](p145). DAG concentrations were measured in 1 mL EDTA-plasma stabilized with 10 µL PMSF (0.1 mg/ml; phenylmethylsulphonyl fluoride, Sigma) and 50 µL acidification (50 mM HCl), using a human DAG enzyme immunoassay (Bertin Pharma, Motigny le Bretonneux, France). Body mass index (BMI) was calculated (weight(kg)/(height(m)²)[97](p144). Waist circumference was measured to the nearest 1 cm, midway between last rib and the iliac crest[98](p144). Obesity was defined as a BMI ≥30 kg/m² or waist circumference >88 cm (women) and >102 cm (men)[50, 51](p139).

Statistics

Statistical analyses were performed with the Statistical Package for Social Sciences (SPSS 17.0, Chicago, IL) and GraphPad Prism 5 (San Diego, CA). Results are expressed as median (range) unless specified otherwise. A Wilcoxon signed rank test was performed to evaluate change in DAG during the ITT. Spearman's correlation was used to evaluate the relationship between BMI, waist circumference and fasting insulin with DAG levels. Statistical analysis to evaluate the differences between subgroups (normal weight subjects versus overweight/obese subjects) were not performed, because of the small numbers in both subgroups (n=4). P-values <0.05 (two-tailed) were considered statistically significant.

	Normal weight subjects BMI 18.5-25 kg/m2 N=4	Overweight / obese subjects BMI ≥25 kg/m2 N=4
Male sex	2	2
Age (years)	29.9 (26.6-34.2)	30.3 (19.6-42.0)
BMI (kg/m²)	21.7 (19.5-23.6)	31.2 (26.4-41.8)
Waist circumference (cm)	66.0 (58.0-76.0)	93.5 (75.0-132.0)
Fasting glucose (mmol/L)	4.4 (4.2-4.7)	5.3 (4.8-5.5)
Fasting insulin (pmol/L)	14 (14-19)	42 (31-118)
HOMA	0.41 (0.38-0.55)	1.36 (1.09-3.393)
Baseline DAG (pg/mL)	319 (235-478)	105 (67-266)
DAG change (pg/mL)	161 (62-285)	17 (8-136)
% DAG decrease	48 (26-60)	18 (9-51)
Growth hormone peak (µg/L)	23.1 (21.3-82.0)	12.5 (7.8-14.9)
Cortisol peak (nmol/L)	629 (535-693)	834 (662-1242)

Data are expressed as median (ranges); **BMI** body mass index; **HOMA** Homeostatic Model Assessment; **DAG** desacyl ghrelin

Table 5.1 Baseline characteristics and outcome parameters of normal weight and overweight/obese subjects.

5.4 Results

According to BMI, three subjects were obese, one overweight and four subjects had normal weight. According to waist circumference, two male subjects were obese (waist circumference 132 cm and 106 cm respectively). Median fasting serum insulin was 25 pmol/L and ranged between 14 pmol/L (normal weight subjects) and 118 pmol/L (obese subject, Figure 5.1). All subjects showed normal GH and cortisol response after insulin injection and subsequent hypoglycaemia of <2.5 mmol/L (GH peak ≥7.8 µg/L and cortisol response ≥535 nmol/L) (Table 5.1, Figure 5.1).

Median serum DAG levels significantly decreased within 60 minutes (90 pg/mL, p=0.028) and this change in DAG was more pronounced in normal weight subjects (Figure 5.2). Median percentage decrease in DAG was 48% (range 26-60) in normal weight subjects and 18% (9-51) in overweight / obese subjects (Table 5.1). Both baseline DAG and change in DAG correlated significantly with fasting insulin (ρ -0.76, p=0.030 and ρ -0.85, p=0.007), baseline HOMA (ρ -0.76, p=0.028 and ρ -0.86, p=0.007), BMI (ρ -0.76, p=0.028 and ρ -0.83, p=0.010), and waist circumference (ρ -0.95, p<0.001 and ρ -0.93, p<0.001). Both baseline DAG and change in DAG significantly correlated with maximum GH response (ρ 0.88, p=0.004), but not with maximum cortisol response (ρ -0.55, p=0.160).

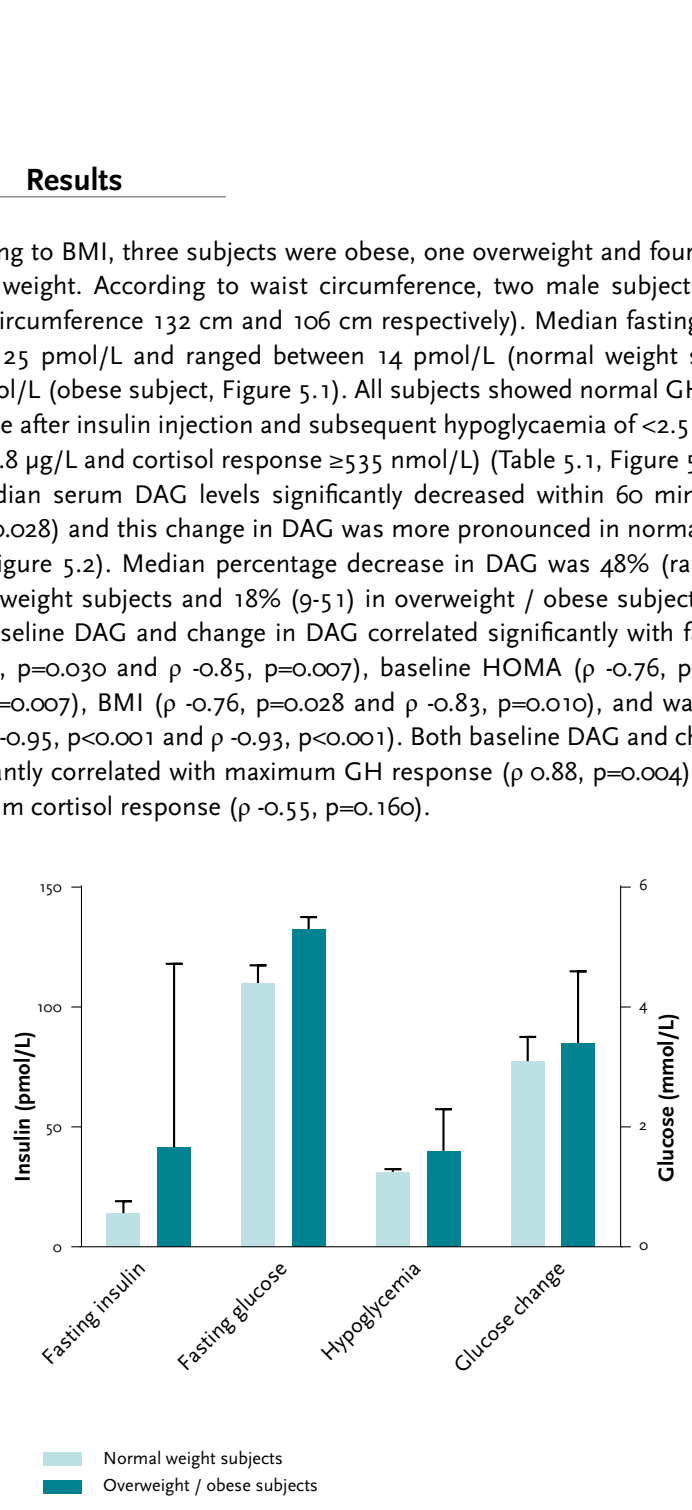


Figure 5.1 Levels of fasting insulin and fasting glucose and level of hypoglycaemia in normal weight and overweight / obese subjects during the insulin tolerance test.

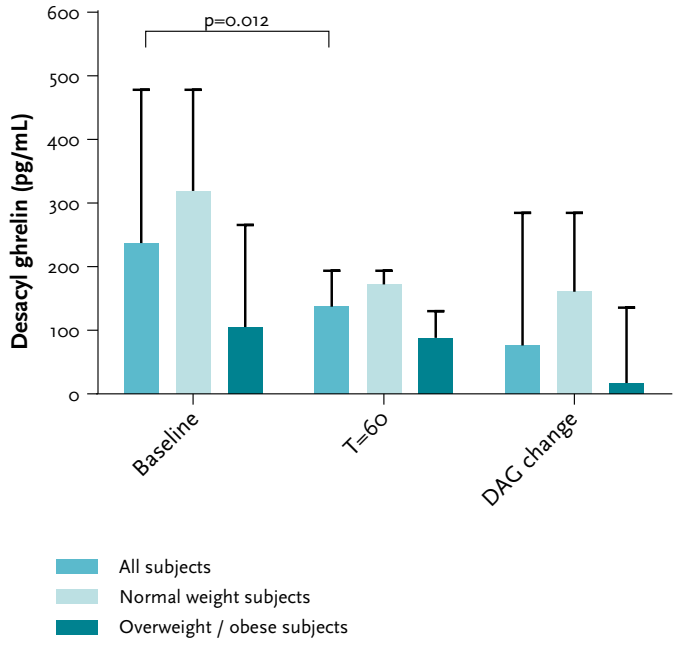


Figure 5.2 Desacyl ghrelin levels at baseline, at 60 minutes after insulin infusion and the DAG decrease in normal weight and overweight / obese subjects.

5.5 Discussion

Significant negative correlations between change in DAG and BMI, waist circumference and HOMA were observed during an ITT, even in this small number of subjects. Our data suggest that a relative DAG deficiency is present in obese insulin resistant subjects, since DAG levels are lower at baseline and hardly change after insulin administration in contrast to normal weight subjects, which show a markedly DAG decrease after insulin infusion.

Our findings confirm earlier studies describing significantly lower DAG levels in obese subjects compared with normal weight subjects, suggesting that DAG is regulated by body weight[119(p146), 123, 124(p147)]. However, it has been described that in insulin resistant obese subjects AG/DAG ratios were higher than in insulin sensitive obese subjects, indicating that insulin rather than body weight seems to play a dominant role in the regulation of DAG levels[117(p146), 119, 125(p147)]. The current study showed that indeed high fasting insulin and HOMA were inversely correlated with DAG levels. Above all, we found that circulating DAG concentrations rapidly decreased after insulin administration, especially in insulin sensitive subjects, suggesting a direct effect of insulin on DAG levels. The fact that insulin and DAG appear

to be associated has already been indicated by previous studies from our group in which insulin sensitivity was improved after co-administration of DAG and AG, while administration of AG alone reduced insulin sensitivity[116, 118(p146)]. Furthermore, in another study, DAG levels increased with improvements in body composition during long-term exercise intervention and were correlated with a higher level of lipolysis[124(p149)].

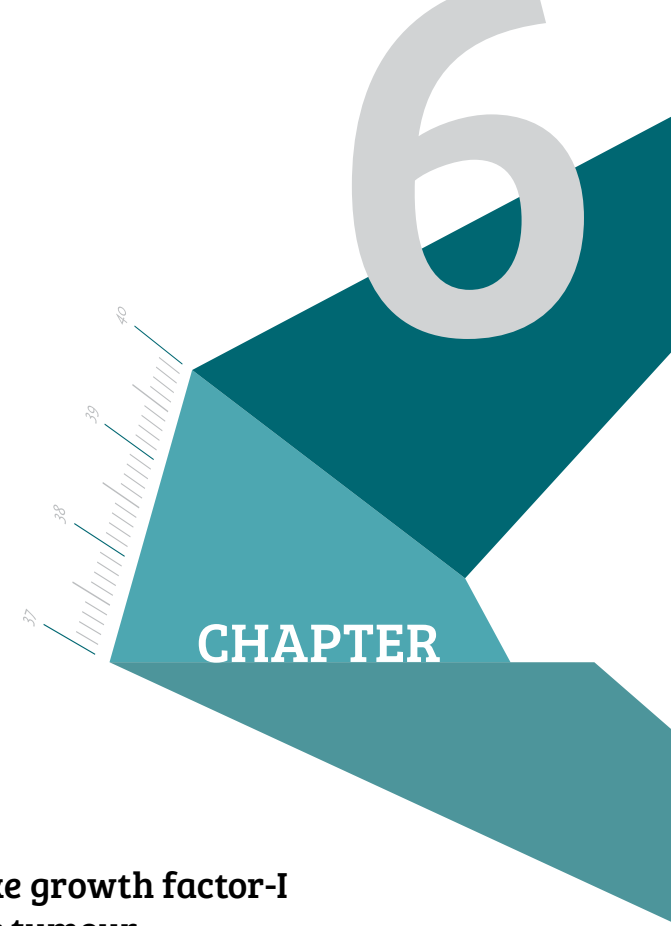
Roux et al. reported that insulin-resistant obese subjects display a significantly lower postprandial suppression of total ghrelin levels[126(p147)]. St-Pierre et al. described a significant reduction in total ghrelin, as well as in DAG and AG, in insulin sensitive subjects compared to insulin resistant individuals during an euglycaemic hyperinsulinemic clamp[117(p146)]. Furthermore, total ghrelin levels decreased in response to high insulin levels and hypoglycaemia during an ITT in six healthy subjects[127(p147)]. Additionally, the current study shows that in the presence of high insulin levels DAG concentrations are suppressed not only in a chronic state of raised insulin levels but also in response to a rapid increase in insulin concentration. Moreover, the capacity to reduce DAG seems to be dependent on the degree of insulin sensitivity.

In this study we did not exclude the possibility that the decrease in DAG was a time effect rather than an insulin effect. However, DAG levels increased after 60 minutes in all subjects (data not shown) suggesting that not time, but another external factor influenced DAG levels. Future intervention studies should focus on comparing DAG concentrations after infusion of insulin versus placebo to distinguish between time effect and insulin effects on DAG concentrations.

To our knowledge, this is the first study that describes the response of DAG during an ITT. Due to the small numbers of subjects, results should be interpreted with care and larger studies are needed to confirm our results. Nevertheless, the positive correlation between DAG levels and insulin sensitivity and improved metabolic state shown is this study in addition to previous reports, is of great importance for the development of future treatment strategies for insulin resistant diseases such as the metabolic syndrome and diabetes mellitus.

Conclusion

DAG levels rapidly decrease in response to insulin administration during an ITT in normal subjects, but not in obese insulin resistant subjects who are in a state of relative DAG deficiency.



Final height and insulin-like growth factor-I in adult survivors of Wilms tumour

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Submitted

6.1 Abstract

Objective

One-sided nephrectomy is followed by increased levels of insulin-like growth factor-I (IGF-I), associated with linear growth during childhood. The aim was to evaluate final height and IGF-I levels in nephrectomized Wilms tumour survivors as compared to healthy Dutch references and survivors of other cancer types.

Patients

Data of 575 adult childhood cancer survivors were analysed retrospectively. Median follow-up time was 17.8 years (range 5.0-48.8). Analysis of (co)variance was performed to evaluate differences between subgroups: nephrectomized Wilms tumour survivors treated with or without abdominal irradiation (n=41 and n=36); survivors of other cancer types treated with or without irradiation involving the cranium, abdomen or total body (n=149 and n=349). IGF-I and height were compared with Dutch references by calculating standard deviation scores (SDS).

Results

After adjustment for age at diagnosis, former corticosteroid treatment and renal impairment, height SDS in non-irradiated nephrectomized Wilms tumour survivors was significantly higher than height SDS in non-irradiated survivors of other cancer types (estimated mean SDS -0.09 versus -0.49, p=0.044), abdominal irradiated survivors (SDS -0.70, p=0.015) and other irradiated survivors (SDS -1.47, p<0.001). In uni- and multivariate analysis, non-irradiated nephrectomized Wilms tumour survivors had significantly higher IGF-I SDS than other irradiated survivors (estimated mean SDS -0.05 versus -1.36, p<0.001 and -0.06 versus -1.37 p<0.001), while there was no significant difference with the other two subgroups.

Conclusions

Adult survivors of Wilms tumour showed better attainment of final height and relatively higher IGF-I levels than survivors of other cancer types who had significantly shorter stature and lower IGF-I levels than Dutch references.

6.2 Introduction

Wilms tumour or nephroblastoma is the most common solid tumour during childhood[128](p147). The survival of Wilms tumour patients has increased over the past decades due to improved treatment regimens and better stratification. In Europe, treatment exists of tumour–nephrectomy after pre-operative chemotherapy and, depending on histology and stage, chemotherapy (e.g. vincristine, dactinomycin, and doxorubicin). Additionally, abdominal irradiation is given in stage III and in some stage IV patients[129, 130](p147).

In general, nephrectomy is followed by compensatory renal growth of the contralateral kidney, possibly as a result of hypervascularization and increased glomerular pressures[131, 132](p148). Simultaneously, serum insulin-like growth factor-I (IGF-I) increases after surgery and is associated with recovery of renal function and regeneration of kidney tissue[132-134](p148). Since IGF-I is responsible for linear growth during childhood, we hypothesize that temporary high IGF-I levels after nephrectomy during childhood contribute to faster growth in survivors of Wilms tumour. This may result in a taller stature than survivors of other childhood cancer types.

We have to take into account that Wilms tumour is associated with predisposing syndromes, including WT1-associated syndromes and overgrowth syndromes, which are present in 9-19% of Wilms tumour patients[135](p148). Previous studies have shown that the pathophysiology of both Wilms tumour and overgrowth syndromes might be associated with growth factor excess[136-141](p148). The fact that Wilms tumour patients often have a significantly higher birth weight than healthy newborns, supports this hypothesis[142](p149). The effect of this suggested mechanism on final height in adulthood is however not elucidated.

The aim of the current study was to compare final height and IGF-I levels of nephrectomized Wilms tumour survivors to data of healthy Dutch references and survivors of other cancer types.

6.3 Methods

Subjects

We performed a single centre study in all adult long-term survivors of childhood cancer, including Wilms tumour, diagnosed between 1952 and 2005. Regular follow-up at the late effects outpatient clinic for long-term childhood cancer survivors starts 5 years after cessation of treatment and is individualized based on cancer diagnosis, treatment protocol and clinical condition. Exclusion criteria for our evaluation were: no availability of height or IGF-I during follow-up, growth hormone treatment during follow-up, former diagnosis of Wilms tumour without one-sided nephrectomy and treatment with nephrectomy for other diagnoses than Wilms tumour.

Data collection

Data concerning disease and treatment protocol were retrieved from our local paediatric oncology database and missing data were retrieved from the medical records. Follow-up time was defined as time since cessation of treatment. Follow-up data for all survivors included height, oral contraceptive (OCP) use, growth hormone replacement and renal function, as defined by estimated glomerular filtration rate (eGFR), calculated with the abbreviated Modification of Diet in Renal Disease (MDRD) equation using serum creatinine[143-145](p149). Final height standard deviation scores (SDS) were calculated using reference values for Dutch adults: a mean (SD) of 184.0 (7.1) cm for males and 170.6 (6.5) cm for females[53](p139). In Wilms tumour survivors, the diameter of the remaining kidney was retrieved from abdominal ultrasounds and compared to a normal value of 10.8 cm[146](p149). IGF-I was assessed by Immulite 2000 (DPC Biermann GmbH/Siemens, Fernwald, Germany), a solid–phase, enzyme labeled chemiluminescent immunometric assay, with an intra assay variability of 2-5%, and an inter assay variability of 3-7%[58](p139). IGF-I levels were compared with reference values by using SDS[58](p139). Serum creatinine, assessed using the Roche enzymatic assay, was analysed in a fully automated computerized laboratory system with a Hitachi 917 chemistry analyser (Roche Diagnostics, Almere, the Netherlands).

Genetic analysis

All Wilms tumour survivors were offered clinical genetic assessment, genetic counselling and molecular analysis of the WT1-gene and the methylation status of the imprinted gene clusters on locus 11p15 after informed consent from the included patients was obtained, as previously described by Segers et al[147](p149).

Statistics

Statistical analyses were performed with the Statistical Package for Social Sciences (SPSS 19.0, Chicago, IL). Results are reported as medians (range) for baseline characteristics and non-normative outcome variables and as means (95% CI) for standard deviation scores. Included survivors were divided into four subgroups: Wilms survivors treated with nephrectomy without abdominal irradiation (n=36); other survivors treated without stem cell transplantation (SCT) and without irradiation of the cranium, abdomen, spine, or total body (TBI) since these treatments affect height and / or IGF-I (n=349); Wilms tumour survivors treated with nephrectomy and abdominal irradiation (n=41); other survivors treated with SCT or one of the above mentioned types of irradiation (n=149). To compare IGF-I and height of these four subgroups of survivors to healthy references, SDS were calculated and analysed with the one-sample t-test. Analysis of variance (ANOVA) was used for univariate comparisons between subgroups and analysis of covariance (ANCOVA) for multivariate comparisons to adjust for potential confounders. Models for height SDS and IGF-I SDS were adjusted for age at diagnosis and renal impairment, defined as eGFR <60 ml/ min/1.73 m². Because of its negative effect on IGF-I levels, OCP use was added to the model with IGF-I SDS as outcome measure. Former corticosteroid treatment has been described to negatively influence final height attainment and therefore was added to the model for height SDS. ANCOVA, adjusted for sex, age at follow-up and age at diagnosis, was used to compare kidney length between Wilms tumour survivors treated with or without abdominal irradiation. P-values of <0.05 (two-tailed) were considered statistically significant.

6.4 Results

Survivors

Out of 885 adult survivors of childhood cancer diagnosed between 1964 and 2005, 600 survivors had visited the outpatient clinic at least once, of which 79 adults survived a Wilms tumour more than 5 years since cessation of treatment. Baseline and treatment characteristics are presented in Table 6.1. Median age at diagnosis was 3.3 years (range 0.2-12.7) for Wilms tumour survivors and 7.1 (0-18.0) for survivors of other cancer types. Median follow-up time was 24.5 years (range 5.1-48.8) for Wilms tumour survivors and 17.3 years (range 5.0-48.8) for other survivors. A second tumour was diagnosed in one Wilms tumour survivor (1.2%) and 8 other survivors (1.5%). Forty-two Wilms tumour survivors had been additionally treated with abdominal radiotherapy.

Clinical genetic assessment was performed in 42 Wilms tumour survivors and a genetic mutation was found in 4 cases. In one survivor a WT1 mutation was found and in one survivor hypomethylation of LIT1. None of these survivors had been clinically diagnosed with overgrowth. Two Wilms tumour survivors had been clinically diagnosed with Stickler syndrome; in one survivor this syndrome was confirmed by mutation of COL2A1.

Excluded from the analysis were 2 Wilms survivors not treated with nephrectomy, 10 non-Wilms survivors treated with nephrectomy and 13 survivors treated with growth hormone replacement during follow-up.

Final height

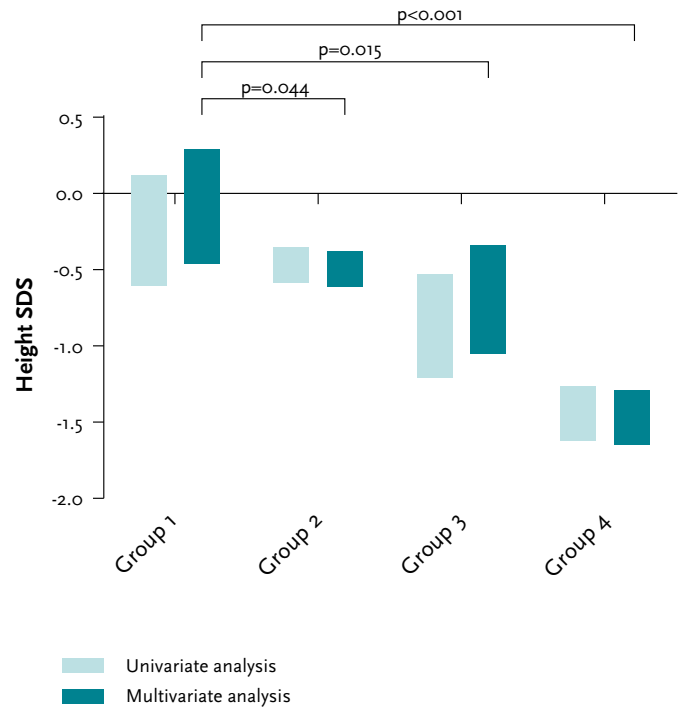
IGF-I SDS was significantly but weakly correlated with height SDS (ρ =0.24, p<0.001). Figure 6.1 shows height SDS in the four different subgroups. Except for non-irradiated Wilms tumour survivors treated with nephrectomy (SDS -0.24, p=0.155), height SDS was significantly lower than Dutch references in all other three groups (others treated without irradiation: SDS -0.47, p<0.001; Wilms treated with abdominal irradiation: SDS -0.87, p<0.001; others treated with irradiation: SDS -1.45, p<0.001). After

	Wilms tumour survivors N=79		Survivors of other cancer types N=521	
Male sex (n)	41 (52)		293 (56)	
Age at diagnosis (years)	3.3 (0.2-12.7)		7.1 (0-18.0)	
Age at follow-up (years)	28.4 (18.1-50.8)		25.8 (18.0-57.4)	
Follow-up time (years)	24.5 (5.1-48.8)		17.3 (5.0-48.8)	
Diagnosis				
Wilms tumour	79 (100)		-	
Non-Wilms renal tumours	-		5 (1)	
ALL & T-NHL	-		182 (35)	
ANLL	-		20 (4)	
B-NHL	-		50 (10)	
Hodgkin	-		46 (9)	
Bone tumour	-		29 (6)	
Neuroblastoma	-		39 (8)	
LCH			13 (3)	
Extracranial GCT	-		12 (2)	
MMT	-		51 (10)	
Brain tumour	-		55 (11)	
Hepatoblastoma	-		3 (0.5)	
Other cancers	-		16 (3)	
Second tumour	1 (1)		8 (2)	
Recurrence	12 in 9 patients		83 in 70 patients	
Therapy				
Nephrectomy	77 (98)		10 (2)*	
left kidney	42 (55)		6 (1)	
right kidney	35 (46)		4 (1)	
No nephrectomy	2 (3)*		511 (98)	
	N (%)	Cumulative RT dose (Gy)	N (%)	Cumulative RT dose (Gy)
Abdominal RT	42 (53)	25 (15-40)	12 (2)	20 (10-50)
CRT	-	NA	59 (11)	25 (15-30)
BRT	-	NA	61 (12)	45 (10-66)
TBI	-	NA	25 (5)	8 (6-12)
SCT autologous /allogenous	1/0		8 / 17	
Spinal irradiation	-	NA	23 (5)	33.6 (12-50.4)
Follow-up data				
GH treatment	-		13 (3)*	
eGFR <60 ml/ min/1.73 m2	2 (3)		6 (1)	

*Excluded from the analyses; **ALL** acute lymphoblastic leukemia; **T-NHL** T-cell non-Hodgkin lymphoma; **ANLL** acute non-lymphoblastic leukemia; **B-NHL** B-cell non Hodgkin lymphoma; **MMT** malignant mesenchymal tumour; **RT** radiotherapy; **CRT** cranial RT; **BRT** brain tumour RT; **TBI** total body irradiation; **GH** growth hormone; **NA** not applicable

Table 6.1(p66) Baseline characteristics of adult Wilms tumour survivors and other survivors

adjustment for age at diagnosis, former treatment with corticosteroids and renal impairment, height SDS in non-irradiated nephrectomized Wilms survivors was significantly higher than height SDS in non-irradiated survivors of other cancer types (estimated mean SDS -0.09 versus -0.49, p=0.044), survivors treated with abdominal irradiation (SDS -0.70, p=0.015) and other irradiated survivors (SDS -1.47, p<0.001).

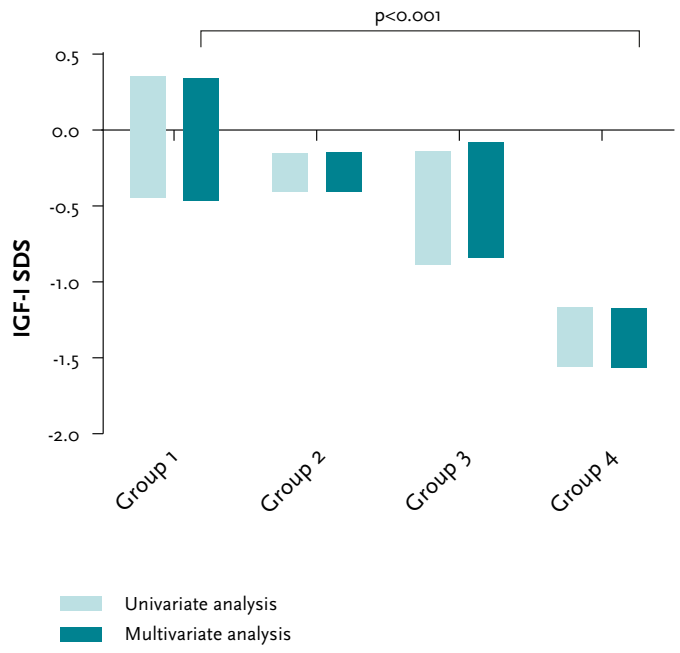


Data are expressed as mean (95% confidence interval). P-values represent the differences between groups after adjusting for age at diagnosis, renal impairment and corticosteroid treatment.
Group 1: wilms tumour survivors treated with nephrectomy, not with abdominal radiotherapy (RT) or stem cell transplantation (SCT); **group 2**: survivors of other cancer types treated without SCT or RT involving the cranium, abdomen, spine and total body; **group 3**: wilms tumour survivors treated with nephrectomy and abdominal RT; **group 4**: survivors of other cancer types treated with RT and/or SCT.

Figure 6.1 Uni- and multivariate analysis of height SDS in the four subgroups.

IGF-I levels

IGF-I standard deviation scores per subgroup are outlined in Figure 6.2. Except for non-irradiated Wilms tumour survivors treated with nephrectomy (SDS -0.05, p=0.817), IGF-I SDS was significantly lower than Dutch references in all other three groups (others treated without irradiation: SDS -0.28, p<0.001; Wilms treated with abdominal irradiation: SDS -0.51, p=0.011; others treated with irradiation: SDS -1.36, p<0.001). In uni- and multivariate analysis, non-irradiated Wilms tumour survivors treated with nephrectomy had significantly higher IGF-I than others treated with irradiation (estimated mean SDS -0.05 versus -1.36, p<0.001 and -0.06 versus -1.37 p<0.001), while there was no significant difference with the other two subgroups (Figure 6.2).



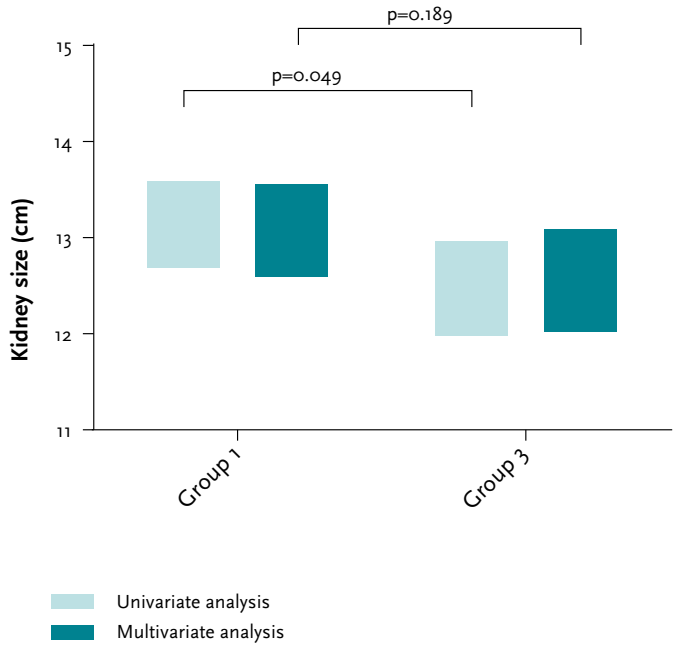
Data are expressed as mean (95% confidence interval). P-value represents the difference between groups after adjusting for age at diagnosis, renal impairment and oral contraceptive use.

Figure 6.2 Uni- and multivariate analysis of IGF-I SDS in the four subgroups.

Kidney size

Kidney size of the contralateral kidney in survivors treated with nephrectomy was significantly larger than Dutch references (12.8 cm versus 10.8 cm, P<0.001, Figure 6.3).

Survivors treated with nephrectomy without abdominal irradiation had larger kidney size than abdominal irradiated Wilms survivors (13.1 versus 12.5, p=0.049), but this difference was not significant after adjustment for sex, age at follow-up and age at diagnosis (Figure 6.3).



Data are expressed as mean (95% confidence interval). P-value represents the difference between groups after adjusting for sex, age at follow-up and age at diagnosis.

Figure 6.3 Kidney size as expressed by maximal diameter of the contralateral kidney in survivors of Wilms tumour treated with nephrectomy with and without abdominal irradiation.

6.5 Discussion

Adult survivors of Wilms tumour treated with nephrectomy showed better attainment of final height than survivors of other cancer types, in whom final height was significantly lower than Dutch references. We hypothesize that temporary high IGF-I levels after nephrectomy in paediatric Wilms tumour patients contribute to faster catch up growth post-therapy than in survivors of other cancer types, leading to a better attainment of final height.

The mechanism of increasing serum IGF-I levels after nephrectomy has been described in both adults[133](p148) and children[148](p149). In adults, IGF-I levels showed

a significant increment post-surgery, with a peak at 6 months and thereafter normalized to baseline within one year. In nephrectomized children, higher IGF-I levels than controls were found more than three years after surgery. IGF-I is associated with hypervascularization, compensatory growth of the contralateral kidney and recovery of renal function[133, 134,(p148), 149, 150(p149)]. The fact that we found larger kidney size than references in all our Wilms tumour survivors support this mechanism. The effect of high IGF-I levels on growth in paediatric patients undergoing nephrectomy has not been elucidated yet, but a positive relationship is plausible since IGF-I is an important determinant of linear growth during childhood. Our data supports this hypothesis since nephrectomized Wilms tumour survivors had significantly higher final height than a comparable group of non-irradiated survivors of other cancer types. The only way to confirm this hypothesis would be to longitudinally evaluate and compare IGF-I levels in relation to growth data shortly after treatment in both Wilms tumour survivors and survivors of other cancer types.

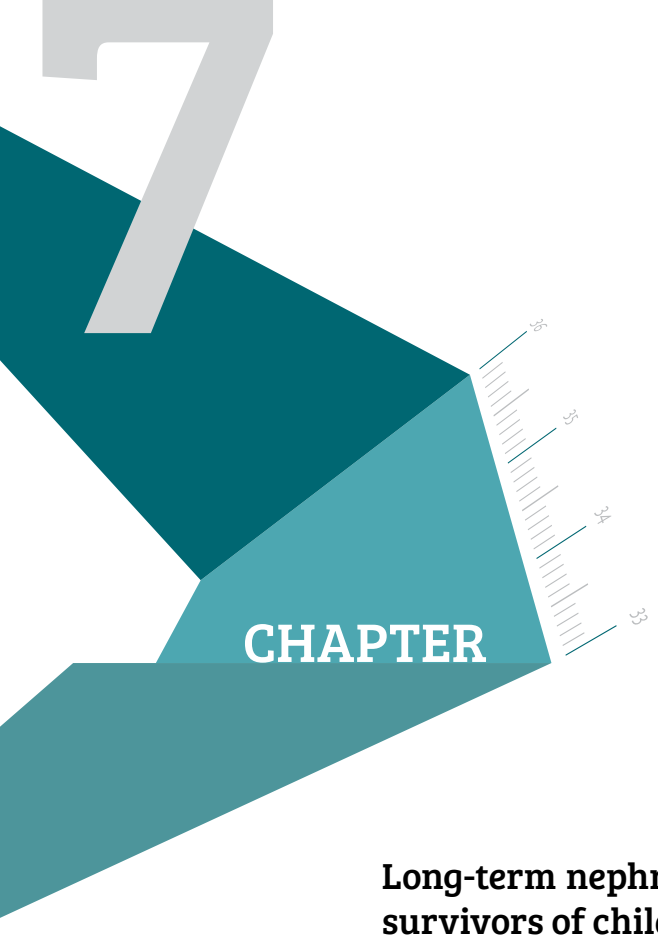
Since IGF-I levels are temporarily high after nephrectomy, we do not expect IGF-I SDS to be higher in adult long-term nephrectomized Wilms tumour survivors, which indeed was not the case. However, comparable to height SDS data, we found lower IGF-I levels than Dutch references in all childhood cancer survivors, except for non-irradiated survivors of Wilms tumour. Reduced IGF-I levels and shorter stature in childhood cancer survivors might partly be explained by treatment factors, since it is well known that irradiation at young age involving the whole brain, or any part of it, and TBI affect growth and IGF-I levels[64(p140), 151, 152(p149)]. However, even in the group of non-irradiated survivors, IGF-I levels were significantly lower than in the normal population in contrast to non-irradiated survivors of Wilms tumour, who had IGF-I levels comparable to the normal population, which is a remarkable finding.

Although in one survivor a hypomethylation of LIT1 was found, no clinically diagnosed overgrowth syndrome was present in this patient. However, several studies suggested that growth factor excess may not be limited to patients diagnosed with known germ line mutations, which could be illustrated by the fact that Wilms tumour patients without associated anomalies had higher birth weights than controls[142(p149), 153(p150)]. Another study in 200 patients however could not confirm this[154(p150)]. IGF2 binds to the IGF-I receptor (IGFIR) and is over expressed in Wilms tumour patients. In vitro studies showed that inhibition of IGFIR suppressed growth of Wilms tumour cells[155(p150)], suggesting that indeed growth factor excess may be an important determinant of clinical overgrowth in survivors of Wilms tumour. It is important to realize that IGF2 is a potent fetal growth factor and plays a significant role in particularly overgrowth syndromes, but is not responsible for linear growth, like IGF-I.

It would have been appropriate to include data on target height, since it indicates individual height attainment. Unfortunately, due to the retrospective character of this study, these data were not available. Secondly, data on clinical genetic analysis and related clinical overgrowth were lacking in half of the Wilms tumour patients. However, final height and birth weight did not significantly differ between survivors with and without available genetic data.

Conclusion

Non-irradiated nephrectomized adult survivors of Wilms tumour showed better attainment of final height and relatively higher IGF-I levels than survivors of other cancer types who had significantly shorter stature and IGF-I levels than Dutch references.



Long-term nephrotoxicity in adult survivors of childhood cancer

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7.1 Abstract

Background & objectives

Since little is known about long-term treatment-related nephrotoxicity, the aim was to determine risk factors for renal impairment long after childhood cancer treatment.

Design, setting, participants, and measurements

Data of 763 adult childhood cancer survivors (414 males) were obtained during regular visits at the late effects clinic between 2003 and 2009. Median follow-up time was 18.3 years (range 5.0-58.2). Glomerular function was assessed by estimated glomerular filtration rate (eGFR, using the MDRD formula) and urinary albumin creatinine ratio (U-ACR) and tubular function by urinary β2-microglobulin creatinine ratio (U-β2MCR). The association with treatment factors was analysed with covariance analysis for eGFR and with logistic regression for U-ACR and U-β2MCR.

Results

Survivors treated with nephrectomy and abdominal irradiation had significantly lower eGFR than survivors not treated with nephrectomy / abdominal irradiation (estimated mean 90 ml/ min/1.73 m² versus 106, p<0.001). eGFR was significantly lower after treatment with high dose ifosfamide (88 versus 98, p=0.02) and high dose cisplatin (83 versus 101, p=0.004) compared to survivors not treated with these regimens. Nephrectomy combined with abdominal radiotherapy (OR 3.14, 95% confidence interval (CI) 1.02; 9.69) and high dose cisplatin (OR 5.19, 95% CI 1.21; 22.21) were associated with albuminuria. Former treatment with high dose ifosfamide (OR 6.19, 95% CI 2.45; 15.67) was associated with increased U-β2MCR. Hypertension was present in 23.4% of survivors and 31.4% of renal tumour survivors.

Conclusion

Treatment with unilateral nephrectomy, abdominal radiotherapy, cisplatin, and ifosfamide was associated with lower eGFR. Persisting tubular damage was related to ifosfamide treatment.

7.2 Introduction

Childhood cancer survival rates have improved substantially over the last decades, leading to a five-year survival of 80%[156](p150). To date, 1 out of 570 young adults is a childhood cancer survivor[157](p150). However, even long time after cessation of treatment, mortality rates appear to be significantly higher than in the general population[158](p150). Not only excess of mortality is an important issue, but also excess of morbidity including second malignancies, endocrinopathies and cardiovascular disease[159](p150). Impaired renal function is one of the known potential late effects after childhood cancer treatment, either at the glomerular or at the tubular level. Nephrotoxic chemotherapy, abdominal irradiation, and nephrectomy contribute to renal injury[160](p150). Nephrotoxicity induced by chemotherapeutic drugs including ifosfamide, cisplatin and carboplatin can manifest as acute reversible renal failure at the glomeruli or the proximal tubules[161-165](p151). Cyclophosphamide, an isomer of ifosfamide, is thought not to cause nephrotoxicity, possibly due to different pharmacology, but clinical studies to confirm this, especially at very long-term follow-up, are lacking. In general, acute kidney injury during childhood cancer treatment remains subclinical and reversible, but may lead to chronic kidney disease later in life. To date, studies on nephrotoxicity in large cohorts of childhood cancer survivors at long-term (>5 years) and very long-term (>20 years) follow-up are limited. Studies so far mainly focus on single therapies and short term follow-up[161, 162](p150), [164, 165](p151). Since reduced glomerular filtration rate and albuminuria are independent predictors of cardiovascular disease and all-cause mortality, studies focusing on long-term renal function to define risk groups, to implement early interventions and to limit potentially nephrotoxic treatments are needed[166-171](p151). In the present study, we determined risk factors for renal impairment many years after childhood cancer treatment.

7.3 Patients and Methods

Ethics statement

The data described in the current retrospective study were obtained during regular visits at the late effects clinic, and clinical investigations were assessed using the standard guidelines for screening late effects after childhood cancer following Good Clinical Practice (GCP). An official written informed consent from every patient that visited the outpatient clinic was obtained according to standards of the Institutional Review Board (IRB).

Subjects

We performed a retrospective cross-sectional single centre study. Follow-up at the late effects outpatient clinic for long-term childhood cancer survivors starts 5 years after cessation of treatment and is individualized based on cancer diagnosis, treatment protocol and current clinical condition. Patients younger than 18 years of age and of whom no serum creatinine was available were excluded.

Data collection

Data concerning disease and treatment protocol were retrieved from our local database and completed from the medical records. Follow-up time was defined as time since cessation of treatment until most recent renal function measurement. Follow-up data included height, weight and blood pressure, glomerular function defined as eGFR, glomerular damage defined as albuminuria and tubular injury defined as elevated β2-microglobulinuria. Blood pressure was electronically measured and defined as hypertensive if either systolic blood pressure was ≥140 mmHg or diastolic blood pressure was ≥90 mmHg or any use of antihypertensive medication [172](p152). Body mass index (BMI) was calculated as weight in kilograms divided by the squared height in meters.

Laboratory measurements

Serum creatinine, assessed using the Roche enzymatic assay, and urinary creatinine, urinary β2-microglobulin and urinary albumin were analysed in a fully automated computerized laboratory system with a Hitachi 917 chemistry analyzer (Roche Diagnostics, Almere, the Netherlands).

Evaluation of renal function

Serum creatinine (Cr) concentration was used to calculate the eGFR by using the abbreviated Modification of Diet in Renal Disease (MDRD) equation[145](p149), [173, 174](p152). Kidney disease was categorized according to the K/DOQI guidelines: stage 3 for eGFR 30-59 ml/ min/1.73 m², stage 4 for 15-29 and stage 5 for <15, with or without renal replacement therapy[173](p152). Age-specific standard deviation scores were calculated to compare eGFR data with those of healthy Dutch references, retrieved from the Nijmegen Biomedical study (n=3732, aged 18-85 yrs)[175](p152). Urinary albumin creatinine Ratio (U-ACR) was calculated to determine the presence of microalbuminuria, which was defined as U-ACR ≥3.5 mg/mmol Cr (female) and ≥2.5 mg/mmol Cr (male). Macroalbuminuria was defined as U-ACR >35 mg/mmol Cr (females) and >25 mg/mmol Cr (males)[173, 176](p152). Urinary β2-microglobulin creatinine ratio (U-β2MCR) was measured at the same time point as U-ACR and was expressed in mg/mmol Cr (normal value <0.04 mg/mmol Cr). If urinary pH was <6, measurements of U-β2MCR were unreliable and were excluded from the analysis.

	Total Cohort N (%) / Median (range) N=763		Renal tumour survivors N (%) / Median (range) N=85	
Sex (male)	414 (54.3)		45 (52.9)	
Age at diagnosis (years)	7.3 (0.0-18.0)		2.8 (0.0-15.0)	
Age at follow-up (years)	26.9 (17.8-65.8)		27.9 (17.9-49.0)	
Follow-up time (years)	18.3 (5.0-58.2)		24.4 (12.2–41.1)	
Long-term survivors (FU time >5 yrs)	438 (57.4)		-	
Very long-term survivors (FU time >20 yrs)	325 (42.6)		-	
Diagnosis				
Acute lymphoblastic leukaemia / T-NHL	216 (28.3)		-	
Acute myeloid leukaemia	26 (3.4)		-	
B-cell non Hodgkin lymphoma	68 (8.9)		-	
Hodgkin lymphoma	80 (10.5)		-	
Bone tumour	35 (4.6)		-	
Renal tumour	85 (11.1)		-	
Neuroblastoma	50 (6.6)		-	
Langerhans Cell Histiocytosis	14 (1.8)		-	
Germ cell tumour	18 (2.4)		-	
Malignant Mesenchymal Tumour	67 (8.8)		-	
Brain tumour	76 (9.9)		-	
Other tumours	28 (3.7)		-	
Recurrence (≥1)	91 (11.9)		8 (9)	
Treatment	**N (%)**	**Median (range)**	**N (%)**	**Median (range)**
Chemotherapy		**TCD (mg/m²)**		**TCD (mg/m²)**
Cisplatin	51 (6.7)	450 (18-900)	1 (1.2)	450
Carboplatin	16 (2.1)	2 050 (500-7 150)	0	Na
Ifosfamide	75 (9.8)	18 000 (4-96 000)	4 (4.7)	36 000 (30 000-36 000)
Cyclophosphamide	305 (40.0)	3 500 (45-45 990)	5 (5.9)	5 250 (250-7 400)
Methotrexate	319 (41.8)		1 (1.2)	Unknown
Intrathecal	277 (36.3)	108 (1-420)	-	
Intravenous	236 (30.9)	10 000 (45-198 000)	-	
Oral	250 (32.7)	unknown	-	
Radiotherapy		**TCD (Gray)**		**TCD (Gray)**
Abdominal RT	47 (6.2)	23 (10-40)	29 (34)	21 (15-30)
Total body irradiation	26 (3.4)	10 (6-20)	-	
Spinal irradiation	23 (3.0)	40 (21-44)	-	
Nephrectomy	85 (11.3)			
Renal replacement therapy[a]	3 (0.5)			

TCD total cumulative dose; **T-NHL** T-cell non Hodgkin lymphoma. [a]Renal replacement therapy includes dialysis and renal transplantation.

Table 7.1 Baseline and treatment characteristics of adult childhood cancer survivors.

Statistics

Statistical analyses were performed with the Statistical Package for Social Sciences (SPSS 18.0, Chicago, IL). Results are reported as median (range) for baseline characteristics and non-normative outcome variables and as mean (95% CI) for standard deviation scores. For univariate analysis, the one-sample t-test was used for SDS and the Mann-Whitney-U test for group comparisons. The Chi-square (X^2) test was used for nominal variables. The associations between eGFR, and baseline and treatment characteristics were analysed using covariance analysis and are expressed as adjusted means. The association between albuminuria and high U-β2MCR (≥0.04 mg/mmol Cr) and baseline and treatment characteristics were analysed with logistic regression and are expressed as odds ratios (ORs). Models were corrected for age, sex (except for eGFR), age at diagnosis and BMI. Dummy variables for subjects treated with nephrectomy only, nephrectomy with abdominal irradiation and abdominal irradiation only were added to the models. Total cumulative dosages (TCD) of chemotherapeutics were divided into two groups using the median as cut-off limit. Since only 16 survivors had been treated with carboplatin, this group was analysed as a whole. For all chemotherapeutics, the group of survivors not been treated with the subsequent chemotherapy was used as reference category. Since the TCD's of methotrexate (MTX) were missing for 235 survivors, we added being treated with MTX as dichotomous variable. To assess the influence of hypertension, this variable was added to all models. P-values of <0.05 (two-tailed) were considered statistically significant.

7.4 Results

Survivors

Out of 885 adult survivors of childhood cancer diagnosed and treated between 1964 and 2005, 763 survivors met the inclusion criteria, of which 85 survived a renal tumour. One patient was excluded because of bilateral nephrectomy and one patient because of nephrophtosis before cancer diagnosis. Baseline and treatment characteristics are presented in Table 7.1 & 7.2. Median follow-up time was 18.3 years (range 5.0-58.2), median age was 26.9 years (17.8-65.8). Cisplatin, carboplatin and/or ifosfamide had been administered in respectively 51 (7%), 16 (2%) and 75 (10%) survivors. Cyclophosphamide and methotrexate had been administered in 305 (39.9%) and 319 (41.8%) survivors respectively. The group of survivors who had received abdominal radiotherapy (n=47) consisted mainly of renal tumour survivors (n=29) and neuroblastoma survivors (n=8). None of the survivors had received renal shielding during abdominal or total body irradiation. Unilateral nephrectomy was performed in all 85 renal tumour survivors (11.1%, Table 7.2). Data of urinary albumin and β_2-microglobulin were not available in survivors that had visited the outpatient clinic before 2006

(n=266, 35%). Survivors of whom data were available were significantly younger than survivors of whom data were missing (25.9 versus 29.3, p<0.001). Also, a higher percentage of them had been treated with nephrotoxic chemotherapy (19% versus 9%. p<0.001). However, the cohort was comparable with regard to sex, diagnosis, and treatment with nephrectomy, abdominal radiotherapy, and total body irradiation.

Glomerular function

Estimated GFR of the total group was not significantly different from healthy controls (mean SDS 0.03, 95% CI -0.05; 0.11, p=0.47). An eGFR between 60-90 ml/min/1.73m² was found in 241 (31.5%) cases, between 30-60 ml/min/1.73m² in 16 (2.1%), between 15 and 30 in 2 (0.3 %) and below 15 in 3 (0.4%) (Table 7.2).

Of the latter 3 (all renal tumour survivors), 2 had a functioning renal transplant, 1 since childhood (due to Denys Drash syndrome and nephroblastomatosis of the contralateral kidney), and one was treated with dialysis since 2009. Prevalence of End Stage Renal Disease was ten times higher than in the normal Dutch population (<0.04%)[177 (p152)]. Survivors treated with unilateral nephrectomy (all 85 renal tumour survivors, SDS= -0.59, 95% CI -0.85; -0.33, p<0.001), abdominal radiotherapy (n=47, SDS= -0.42, 95% CI -0.83; -0.01, p=0.04), or the combination of both (n=29, SDS= -0.49, 95% CI -0.98; -0.01, p=0.05) had significantly lower eGFR compared to healthy references (Supplementary Table). After adjustment for age at diagnosis and BMI, survivors treated with nephrectomy alone (91 ml/ min/1.73 m², 95% CI 76; 106, p<0.001) or in combination with abdominal irradiation (90 ml/ min/1.73 m², 95% CI 74; 106, p<0.001) had significantly lower eGFR than survivors not treated with nephrectomy or abdominal radiotherapy (106 ml/ min/1.73 m², 95% CI 95; 119) (Table 7.3). Additionally, eGFR was significantly lower after treatment with high dose ifosfamide (88, 95% CI 73; 103, versus 98 ml/ min/1.73 m², 95% CI 85; 112, p=0.02) and high dose cisplatin (83, 95% CI 66; 100, versus 101, 95% CI 89; 113, p=0.004) compared to survivors who had not been treated with these regimen. Treatment with carboplatin, cyclophosphamide and methotrexate was not significant associated with eGFR (Table 7.3).

Albuminuria

Albuminuria was significantly more often present in survivors after nephrectomy (25% versus 12% if no nephrectomy, p=0.01) and after abdominal radiotherapy (39% versus 11% if no abdominal radiotherapy, p<0.001) (Supplementary Table). Macroalbuminuria was rare (n=10, 2.0%) and was present in 4 renal tumour survivors (Table 7.2). After adjustment for age, sex, age at diagnosis, and BMI, this risk of albuminuria was significantly higher after combination treatment with nephrectomy and abdominal radiotherapy (OR 3.14, 95% CI 1.02; 9.69, p=0.05) compared to survivors not treated with this combination treatment. Furthermore, being treated with high dose cisplatin (OR 5.19, 95% CI 1.21; 22.21, p=0.03) was significantly and independently associated with albuminuria, whereas treatment with ifosfamide and/or carboplatin was not (Table 7.3).

Tubular function

Median U-β_2MCR was 0.02 mg/mmol Cr and was significantly higher in patients treated with ifosfamide (0.03 versus 0.02 if no ifosfamide treatment, p=0.01) and spinal irradiation (0.07 versus 0.02 if no spinal irradiation, p=0.02) (Supplementary Table). After adjustment for age, sex, age at diagnosis and BMI, survivors treated with high dose ifosfamide (OR 6.19, 95% CI 2.45; 15.67 p<0.001) and spinal irradiation (OR 12.4, 95% CI 2.06; 78.99, p=0.006) had a higher risk of having tubular dysfunction, represented by U-β_2MCR ≥0.04 mg/mmol Cr.

Blood pressure

Arterial hypertension was present in 142 (23.4%) survivors. In renal tumour survivors treated with nephrectomy 22 subjects were hypertensive (31.4%). In survivors treated with abdominal irradiation 18 subjects were hypertensive (43%) versus 22% in survivors not treated with abdominal irradiation (p=0.003) (Supplementary Table).
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	Complete group of adult CCS	Adult renal tumour survivors
Glomerular function (eGFR in ml/ min/1.73 m²)	**N (%)**	**N (%)**
> 90	501 (65.7)	34 (40.0)
60-90	241 (31.6)	44 (51.8)
30-59	16 (2.1)	4 (4.7)
15-29	2 (0.3)	0
<15	3 (0.4)	3 (3.5)
Albumin to creatinine ratio (mg/mmol Cr)		
Measurements / total group	496/763	61/85
< 2.5 (if male) or <3.5 (if female)	430 (86.7)	46 (75.4)
≥ 2.5-25 (if male) or ≥ 3.5-35 (if female)	56 (11.3)	11 (18.0)
>25 (if male) or >35 (if female)	10 (2.0)	4 (6.6)
U-β_2MCR (mg/mmol Cr)		
Measurements / total group	478/763	59/85
<0.04	348 (73)	41 (69)
≥0.04	130 (27)	18 (31)

CCS childhood cancer survivors; **eGFR** estimated glomerular filtration rate; **U-β_2MCR** Urinary β2-microglobulin creatinine ratio.

Table 7.2 Frequency of eGFR categories, albumin-to-creatinine ratio and urinary β_2-microglobulin creatinine ratio.

	eGFR (ml/ min/1.73 m²)				albuminuria			U-β_2MCR ≥0.04 mg/mmol Cr		
	Adjusted Mean	95% CI	P		OR	95% CI	p	OR	95% CI	p
Hypertension no	96	83; 110			1.0			1.0		
Hypertension yes	96	82; 109	0.82		1.71	0.86; 3.40	0.13	2.05	1.17; 3.61	0.01
No cisplatin	101	89; 113			1.0			1.0		
Cisplatin ≤450	104	88; 120	0.54		1.73	0.44; 6.85	0.44	0.58	0.15; 2.26	0.43
Cisplatin >450	83	66; 100	0.004		5.19	1.21; 22.21	0.03	0.52	0.08; 3.29	0.49
No ifosfamide	98	85; 112			1.0			1.0		
Ifosfamide ≤16 000	102	86; 117	0.42		1.35	0.34; 5.33	0.67	1.34	0.48; 3.76	0.58
Ifosfamide >16 000	88	73; 103	0.02		1.49	0.49; 4.54	0.48	6.19	2.45; 15.67	<0.001
No carboplatin	94	81; 106			1.0			1.0		
Carboplatin treatment	98	81; 115	0.50		2.18	0.45; 10.54	0.33	2.93	0.68; 12.64	0.15
No cyclophosphamide	96	82; 110			1.0			1.0		
Cyclophosphamide ≤3 500	96	83; 110	0.98		0.54	0.21; 1.39	0.20	1.09	0.56; 2.15	0.80
Cyclophosphamide >3 500	95	81; 109	0.74		0.84	0.35; 2.00	0.69	1.61	0.81; 3.20	0.18
No methotrexate	97	84; 110			1.0			1.0		
Methotrexate treatment	95	81; 109	0.36		0.94	0.49; 2.16	0.94	1.07	0.59; 1.92	0.83
No total body irradiation	93	81; 106			1.0			1.0		
Total body irradiation	99	83; 115	0.29		3.28	0.88; 12.22	0.08	0,48	0.12; 1.96	0.30
No Spinal irradiation	90	80; 101			1.0			1.0		
Spinal irradiation	102	82, 120	0.14		2.12	0.21; 21.21	0.52	12.4	2.06; 78.99	0.006
No nephrectomy / abd RT	106	95; 119			1.0			1.0		
Nephrectomy only	91	76; 106	<0.001		1.83	0.66; 5.17	0.25	1.69	0.67; 4.31	0.27
Abdominal irradiation only	96	78; 113	0.09		3.29	0.69; 15;67	0.14	1.12	0.23; 5.55	0.89
Nephrectomy & abd RT	90	74; 106	<0.001		3.14	1.02; 9.69	0.05	1.31	0.43; 3.99	0.63

Models were adjusted for age and sex (except for eGFR), age at diagnosis and body mass index. **eGFR** estimated glomerular filtration rate; **CI** confidence interval; **U-ACR** Urinary Albumin Creatinine Ratio; **U-β_2MCR** Urinary β2-microglobulin creatinine ratio.

Table 7.3 Multivariate analysis illustrating the association of hypertension and treatment factors with eGFR, albuminuria and U-β**2**MCR.

7.5 Discussion

In the current study among nearly 800 survivors of childhood cancer we investigated a broad spectrum of parameters of chronic kidney impairment after a median follow-up of 18 years (range 5-58 years). Nephrectomy, abdominal irradiation, high dose cisplatin and high dose ifosfamide were found to be independent risk factors for renal impairment, while former treatment with cyclophosphamide or methotrexate was not.

High dosed abdominal radiotherapy, total body irradiation, ifosfamide, cisplatin, and carboplatin have been described as risk factors for renal impairment in several studies[178-181](p152). However, in these studies follow-up time was relatively short and patient numbers were small. A recent study in a large cohort of childhood cancer survivors 12 years after diagnosis, described nephrectomy and the combination of nephrectomy with abdominal radiotherapy as risk factors for renal damage, represented by hypertension, proteinuria and reduced glomerular function[182](p153). In the present study, with a median follow-up of 18 years, we showed that nephrectomy and the combination of nephrectomy with abdominal radiotherapy, was associated with a decreased glomerular function and albuminuria, but not with tubular dysfunction.

Albuminuria however, has been described to be associated with both glomerular and tubular dysfunction, since it is the result of the balance between glomerular filtration and tubular reabsorption[183, 184](p153). In case of tubular dysfunction, reabsorption of filtered albumin is decreased causing albuminuria. Based on in vitro studies, Birn and Christensen hypothesized that excess albumin in the tubular lumen, caused by glomerular dysfunction, may lead to interstitial inflammation and fibrosis, causing tubular damage[183, 185, 186](p153). These findings illustrate that both glomerular and tubular dysfunction play a role in the existence of albuminuria.

Short-term glomerular and/or tubular dysfunction after treatment with cisplatin, carboplatin or ifosfamide have been thoroughly described before, while studies investigating multiple treatment effects after very long-term follow-up are not available [178](p152), [180](p152), [187](p153). Our data show that tubular and glomerular impairment due to former treatments with high dose ifosfamide and high dose cisplatin are still present at very long-term follow-up.

Some studies suggested that cyclophosphamide is not nephrotoxic in children with cancer but up until now, long-term follow-up studies in large cohorts that confirmed this are not available. We show for the first time that cyclophosphamide on the long-term is not nephrotoxic. The difference between the nephrotoxic effect of cyclophosphamide and its isomer ifosfamide may be explained by the differences in pharmacokinetics and pharmacodynamics[188](p153). A recent study using murine and human proximal tubule cells, showed that specific renal uptake of the metabolites of ifosfamide and not of cyclophosphamide is the basis for the differential effect in nephrotoxicity between ifosfamide and cyclophosphamide[189](p153). Our finding that ifosfamide and not cyclophosphamide is persistently nephrotoxic even after almost 20 years of follow-up, may be useful for the ongoing discussion on the role of ifosfamide in current treatment protocols for paediatric sarcomas[190](p153). However, in the design of upfront protocols, not only nephrotoxicity, but also other late sequelae such as gonadal damage should be taken into account[191, 192](p154).

Carboplatin is a cisplatin analogue, but less nephrotoxic than cisplatin. Carboplatin treatment has been reported to be associated with tubular, and to a lesser extent with glomerular dysfunction. In the current study former treatment with carboplatin was not associated with tubular nor with glomerular dysfunction. However, since the number of survivors treated with carboplatin was relatively small in this study, results should be interpreted with caution.

The contribution of methotrexate to nephrotoxicity was reported during and shortly after treatment[160](p150), [193, 194](p154). For the first time we show that methotrexate related acute nephrotoxicity appears to be completely reversible, as after long-term follow-up methotrexate treated cancer survivors did neither manifest glomerular nor tubular dysfunction.

Renal tumour survivors treated with nephrectomy had a significantly lower glomerular function than survivors who kept both kidneys. Nephrectomy is known to result in compensatory hypertrophy and hyperfiltration of the remaining kidney due to loss of 50% of nephrons, in the long run leading to glomerulosclerosis, albuminuria and high blood pressure[195](p154). A meta-analysis reported a decrease in eGFR of 10-20 ml/min/1,73m² after 5-10 years in healthy adult renal graft donors. Moreover, 12% of healthy donors reached an eGFR below 60 ml/min/1,73m² [196, 197](p154). Furthermore, unilateral nephrectomy for several renal diseases in childhood (including obstructive uropathy and reflux nephropathy) resulted in a reduced eGFR (median 85 ml/min.1,73m2) after very long-term follow-up [198](p154). It is remarkable that in our group of renal tumour survivors treated with nephrectomy and additional nephrotoxic treatment, renal function was well maintained with a mean eGFR of 87.3 ml/min/1,73m², although eGFR was significantly lower than in the normal population (SDS -0.6). Thus, renal function was relatively well preserved in our cohort of childhood cancer survivors, 18 years after treatment with nephrectomy whether or not combined with abdominal irradiation or nephrotoxic agents.

Hypertension can be both a cause as well as a complication of renal disease and it is therefore of great importance to be monitored in childhood cancer survivors, since it can be a disguised symptom. Hypertension was present in 23%, which is relatively high compared to previous findings of 14% and 19% among childhood survivors, which could be explained by the fact that survivors were older and follow-up time was longer in the current study[28](p136), [182](p153), [199](p154). This percentage was even higher (31%) in survivors treated with unilateral nephrectomy. Since renal impairment is highly associated with an increased risk of cardiovascular morbidity and death, renal tumour survivors should therefore be monitored with extra care[167](p151). Based on our results we recommend intensive renal screening (once per 3 years) in high risk groups (being treated with high dose cisplatin (>450 mg/

m²) or high dose ifosfamide (>16 000 mg/m²), nephrectomy with or without abdominal irradiation) by measurement of creatinine, eGFR and albuminuria. Furthermore, treatment with ACE-inhibition is indicated not only for hypertension but also in case of isolated microalbuminuria, since it is described to reduce cardiovascular risk in non-diabetic patients[200(p154)].

Our study comprises nearly 90% of all adult survivors treated and diagnosed at the Erasmus Medical Centre between 1964 and 2005 with a long and nearly complete follow-up. A limitation of this study is the fact that urine measurements were not part of the follow-up scheme of the late effects clinic before 2006. Because the reason for selection was based on timing of follow-up, rather than based on patients or treatment characteristics, the chance of selection bias is small, although not completely excluded.

Conclusion

Lower eGFR in the long run is associated with former treatment with unilateral nephrectomy whether or not combined with abdominal irradiation, cisplatin and ifosfamide. Albuminuria is related to combination treatment of nephrectomy and abdominal radiotherapy. Persisting tubular damage particularly is associated with treatment with ifosfamide. Treatment with cyclophosphamide and methotrexate is not related to very long-term nephrotoxic sequelae in childhood cancer survivors.

		eGFR SDS N=762		eGFR (ml/min/1.73 m²) N=762	Albuminuria N=496		U-ACR (mg/mmol Cr) N=496		U-β_2MCR ≥0.04 mg/mmol Cr N=478	U-β_2MCR (mg/mmol Cr) N=481		Hypertension N=604
		Mean	95% CI	Mean ± SD	N positive (%)		Median	IQR	N positive (%)	Median	IQR	N positive (%)
Total cohort		0.03	-0.05; 0.11	100 ± 24.6	66 (13)		0.7	0.3 – 1.6	130 (27)	0.022	0.015 – 0.042	142 (23)
Nephrectomy	No	0.11	0.02; 0.19*	102 ± 24.4	51 (12)		0.6	0.3 – 1.4	112 (27)	0.022	0.015 – 0.041	120 (23)
	Yes	-0.59	-0.85; -0.33**	87 ± 21.9**	25 (33)*		1.3*	0.4 – 3.0	18 (31)	0.021	0.014 – 0.046	22 (31)
Cisplatin	No	0.05	-0.04; 0.13	100 ± 24.3	59 (13)		0.7	0.3 – 1.6	124 (28)	0.023	0.015 – 0.043	134 (24)
	Yes	-0.30	-0.68; 0.08	97 ± 28.6	7 (18)		0.5	0.3 – 1.8	6 (17)	0.018	0.012 – 0.030	8 (20)
Ifosfamide	No	0.05	-0.03; 0.14	100 ± 24.7	56 (13)		0.7	0.3 – 1.5	106 (25)	0.021	0.015 – 0.040	130 (25)
	Yes	-0.23	-0.50; 0.03	100 ± 23.8	10 (16)		0.6	0.4 – 1.8	24 (39)	0.029*	0.016 – 0.088	12 (17)
Carboplatin	No	0.03	-0.05; 0.11	100 ± 24.7	63 (13)		0.7	0.3 – 1.6	123 (26)	0.022	0.015 – 0.041	139 (24)
	Yes	-0.29	-0.73; 0.15	98 ± 16.3	3 (25)		1.0	0.3 – 4.8	7 (64)*	0.052	0.015 – 0.162	3 (20)
Cyclophosphamide	No	-0.01	-0.12; 0.10	99 ± 25.2	42 (15)		0.7	0.4 – 1.7	80 (31)	0.024	0.015 – 0.045	88 (26)
	Yes	0.07	-0.05; 0.20	102 ± 23.5	24 (11)		0.6	0.3 – 1.4	50 (23)*	0.021*	0.014 – 0.037	54 (21)
Methotrexate	No	0.05	-0.17; 0.07	98 ± 26.6	42 (17)		0.7	0.4 – 1.8	73 (30)	0.022	0.015 – 0.045	89 (27)
	Yes	0.12	0.02; 0.23*	103 ± 21.2*	24 (10)*		0.6*	0.3 – 1.4	57 (24)	0.022	0.015 – 0.038	53 (19)*
Abdominal RT	No	0.06	-0.02; 0.14	101 ± 24.3	53 (11)		0.6	0.3 – 1.4	127 (28)	0.022	0.015 – 0.041	124 (22)
	Yes	-0.42	-0.83; -0.01*	86 ± 25.1**	13 (39)**		1.8**	0.8 – 4.9	3 (16)	0.025	0.017 – 0.061	18 (43)*
Total body irradiation	No	0.01	-0.07; 0.10	100 ± 24.6	61 (13)		0.6	0.3 - 1.5	119 (27)	0.022	0.015 – 0.043	139 (24)
	Yes	0.30	-0.20; 0.79	107 ± 24.8	5 (25)		1.3*	0.6 – 2.8	11 (36)	0.024	0.015 – 0.038	3 (13)
Spinal irradiation	No	0.01	-0.08; 0.09	100 ± 24.5	65 (13)		0.7	0.3 – 1.6	125 (27)	0.022	0.015 – 0.041	137 (23)
	Yes	0.61	0.05; 1.17*	105 ± 25.9	1 (14)		0.9	0.4 – 1.4	5 (71)*	0.069*	0.026 – 0.091	5 (33)

eGFR estimated glomerular filtration rate; **SDS** standard deviation score; **U-β_2MCR** Urinary β2-microglobulin creatinine ratio; **IQR** interquartile range. *p<0.05, **p<0.001, p-value was calculated using One-sample T-test for standard deviation scores, Independent samples T-test for eGFR (ml/min/1.73 m²), Chi-Square test for albuminuria, U-β_2MCR ≥0.04 mg/mmol Cr, hypertension and the Mann-Whitney U-test for U-ACR and U-β_2MCR.

Supplementary Table Univariate analysis of eGFR SDS, eGFR, albuminuria, U-β_2MCR and hypertension in different treatment groups.

CHAPTER 8

Decreased ovarian function is associated with obesity in adult female survivors of childhood cancer

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8.1 Abstract

Objective

Obesity and gonadal dysfunction are known major side effects of treatment in adult childhood cancer survivors. In the general population, obesity has a negative influence on female fertility. We aimed to evaluate whether obesity and serum insulin are associated with decreased ovarian reserve markers in childhood cancer survivors.

Design

Retrospective single centre cohort study.

Methods

Data of 191 female survivors of childhood cancer were analysed. Median follow-up time was 18.8 years (2.3-48.8). Outcome measures were serum anti-Müllerian hormone (AMH) and total follicle count (FC). Potential risk factors were body mass index (BMI), body composition measures, determined by dual energy X-ray absorptiometry (total fat percentage, lean body mass and visceral fat percentage) and fasting insulin.

Results

Lower serum AMH was found in obese subjects (β(%) -49, p=0.007), and in subjects with fasting insulin in the highest tertile (β(%) -43, p=0.039). Total fat percentage tended to be associated with serum AMH (β(%) -2.1, p=0.06). Survivors in the highest tertile of insulin had significantly lower FC than survivors in the lowest tertile (β -6.3, p=0.013). BMI and other measures of body composition were not associated with FC. Correlation between serum AMH and antral follicle count (AFC) was ρ=0.32 (p=0.08).

Conclusions

Obesity and insulin resistance are associated with gonadal damage, as reflected by decreased AMH and reduced FC in adult survivors of childhood cancer. In contrast to its highly predictive value for AFC in the healthy female population, serum AMH does not seem to correlate as well with AFC in childhood cancer survivors.

8.2 Introduction

The prevalence of obesity has increased dramatically since the 1970s[201(p155)]. It is a well-described risk factor for diabetes, hypertension, heart disease, stroke, and cancer[202(p155)]. In healthy women, high body mass index (BMI) also affects reproductive health[203, 204(p155)] as reflected by impaired ovulatory function and a lower pregnancy rate[205(p155)]. In childhood cancer survivors, obesity is a major side effect, occurring in 9-30%, mainly depending on previous treatment[11(p135), 38(p137)]. In adult female childhood cancer survivors, the risk of obesity is increased by 50% when compared to the general population[48(p138)].

Gonadal dysfunction is an important side effect of cancer treatment in childhood cancer survivors. Alkylating agents and abdominal radiotherapy, in particular, can have a deleterious effect on ovarian function[191(p154)]. To determine ovarian reserve, anti-Müllerian hormone (AMH) was identified as a reliable serum marker[103(p144)]. It is produced by the granulosa cells of small growing follicles, reflects the size of the primordial follicle pool in the ovaries and is an indicator of a woman's reproductive capacity[206]. It is stable during and between menstrual cycles, in contrast to FSH[207-209(p155)], and is considered to be a valued marker for ovarian reserve, since it corresponds well with antral follicle count (AFC), which reflects reproductive status[103(p144)].

In their late reproductive years the gonadal function of women seems to be affected by obesity[210, 211(p156)]. Insulin resistance, which occurs at a higher frequency in adult childhood cancer survivors, may affect granulosa cell function[212, 213(p156)]. The association between obesity, insulin resistance and gonadal function, which are all more prevalent in childhood cancer survivors than in the normal population, has never been explored. Therefore, the aim of this study was to determine the association between BMI, body composition, insulin levels and ovarian reserve as reflected by AMH and AFC, in a substantial single centre cohort of female adult survivors of childhood cancer.

8.3 Subjects and Methods

Subjects

A retrospective single centre cohort study was performed among female survivors that visited our late effects outpatient clinic for long-term childhood cancer survivors. Inclusion criteria were: age ≥18 years and < 50 years, female childhood cancer survivors diagnosed and treated between 1964 and 2005, who had both serum AMH levels and BMI determined at the same moment at least 5 years after cessation of cancer treatment. Exclusion criteria were: ovariectomy, PCOS and AMH > 5 µg/L. One hundred and five survivors visited the gynaecological outpatient clinic for screening. In the remaining 180 CCS, we were not able to distinguish between PCOS and non-PCOS subjects, because data on hyperandrogenism (clinical and biochemical) and total follicle counts (transvaginal ultrasound) were not available. Since we were not able to use the Rotterdam criteria in these remaining cases, and Dewailly et al. suggested a cut-off limit of AMH > 5 µg/L to define PCOM[214(p156)], we used this marker and cut-off limit for the presence of PCOM as one of the criteria of PCOS and excluded these survivors.

Outcome measures (AMH/FC)

Serum samples were taken randomly during the menstrual cycle since AMH has been shown not to fluctuate during the menstrual cycle and during OCP use[207(p155)]. AMH was measured using an in-house double-antibody enzyme-linked immunosorbent assay (ELISA) (commercially available through Beckman-Coulter)[103, 104(p144)]. Intra- and inter-assay coefficients of variation were <10 and <5%, respectively[103, 104(p144)]. A subset of patients (n=91) underwent a standardized gynaecological examination. Clinical examination was performed after an overnight fasting period on a random day and included menstrual history, current cycle length, cycle regularity, height and weight. Transvaginal ultrasonography was performed to assess ovarian volume and total follicle count (FC) for both ovaries and to exclude other genital abnormalities. Follicle count was called antral follicle count (AFC) if the transvaginal ultrasound was performed during the follicular phase (day 2-5) or during amenorrhoea. Polycystic ovary syndrome (PCOS) was diagnosed according to the revised Rotterdam 2003 criteria[215(p156)]. The presence of polycystic ovaries was defined as ≥ 12 follicles in one or both ovaries and/or increased ovarian volume (>10 ml), without the presence of a cyst (>10mm)[216(p156)].

Obesity variables

Follow-up data of the most recent visit included the following variables: weight, height, body mass index (BMI) calculated from height and weight[49(p139)], and waist-hip ratio (WHR), as measured by waist circumference divided by hip circumference[70(p141)]. Serum insulin (pmol/l) was measured using a chemi-luminescence-based immunoassay (Immulite 2000, Siemens DPC, Los Angeles, CA, USA) after an overnight fasting period. Glucose levels were measured using a Hitachi 917 analyzer (Roche Diagnostics). Insulin resistance was determined by calculation of the homeostatis model assessment (HOMA) score as plasma glucose (mmol/L) x plasma insulin (mU/L) / 22.5[217(p156)]. Lean body mass (kg) and percentage of body fat were measured by dual energy X-ray absorptiometry (DXA, Lunar Prodigy, GE Healthcare, Madison, WI, USA). Visceral fat percentage was calculated from intra-abdominal fat (kg) and total fat (kg), measured by DXA[71(p141)]. Waist, waist-hip ratio and visceral fat percentage were not analysed in the subset of survivors treated with abdominal radiotherapy, because of impairment of local body fat and the frequent occurrence of scoliosis.

Potential confounders

Data concerning treatment protocols, disease and patient characteristics were retrieved from our local database and were completed using the medical records where necessary. Follow-up time was defined as time since cessation of treatment. Among patients exposed to alkylating agents, the alkylating agent dose (AAD) score was calculated by determining the drug dose tertile distribution in our entire cohort of survivors and adding the tertile scores (1, 2 and 3) for each of the alkylating agents given to a particular patient as previously described by Tucker et al. and Green et al. [218, 219(p156)]. An AAD score of zero was assigned to patients not exposed to any alkylating agent.

Statistics

To examine the associations between obesity variables and AMH or FC, we used univariate and multiple linear regression analyses. In all multiple linear regression models, age, age at diagnosis, total body irradiation, abdominal radiotherapy and AAD score were included as potential confounders. The analyses were performed in several steps. First, BMI, body composition and insulin were entered as continuous variables. Secondly, to evaluate if there was an exponential association with AMH, squared variables of BMI, body composition measures and insulin were added to the relevant model. Subsequently, all variables were divided into quintiles or tertiles (depending on sample size) and added to the relevant model as dummy variables, with the lowest quintile/tertile as reference category. Additionally, BMI was divided in 4 categories: BMI > 30 kg/m² (obese), BMI 25-30 kg/m² (overweight), BMI 18-25 kg/m² (normal weight) and BMI < 18 kg/m² (underweight) and added to the model as dummy variables with normal weight as reference category. Associations are expressed as standardised regression coefficients because this measure allows direct comparison of the strengths of associations between different determinants. The distribution of AMH was normalised by ^{10}log transformation to improve the plots of the residual analyses and expressed as percentage. P-values < 0.05 (two-tailed) are considered statistically significant. SPSS 17.0 software (SPSS, Chicago, IL, USA) was used for statistical analysis.

8.4 Results

Gonadal function

The cohort consisted of 425 female survivors of childhood cancer, of whom 292 visited the late effects outpatient clinic. Seven survivors were excluded because of previous one-sided or two-sided ovariectomy (n=6 and n=1, respectively) and two because they were > 50 years old. Sixteen survivors were clinically diagnosed with polycystic ovary syndrome (PCOS) by a gynaecologist and were therefore excluded. Another 76 survivors were excluded because their AMH levels were > 5 µg/L. Finally, 191 female survivors were included in the analysis.

Clinical characteristics and treatment details of the total cohort of female CCS of our centre and the survivors included in the study are shown in Table 8.1. The included sample is representative for the total cohort of female CCS of our centre.

	Total group of adult female CCS N=425		Female CCS with AMH measurement N=292		Included survivors in this study[a] N=191	
Age at diagnosis (yrs)	7.3 (0-17.9)		6.2 (0-16.8)		6.3 (0-16.2)	
Age at follow up (yrs)	NA		26.5 (17.7-57.4)		27.1 (17.7-50.0)	
Follow up time (yrs)	NA		17.8 (2.3-48.8)		18.8 (2.3-48.8)	
Diagnosis n (%)						
ALL & T-NHL	128 (30)		91 (31)		52 (27)	
Acute myeloid leukaemia	9 (2)		9 (3)		7 (4)	
B-cell non Hodgkin lymphoma	25 (6)		18 (6)		12 (6)	
Hodgkin lymphoma	29 (7)		21 (7)		18 (9)	
Bone tumour	22 (5)		14 (5)		14 (7)	
Wilms tumour	47 (11)		41 (14)		31 (16)	
Neuroblastoma	32 (8)		29 (10)		18 (9)	
Germ cell tumour	12 (3)		8 (3)		2 (1)	
Malignant mesenchymal tumour	35 (8)		27 (9)		17 (9)	
Brain tumour	52 (12)		18 (6)		13 (7)	
Other	34 (8)		16 (5)		7 (4)	
Therapy n (%)	n (%)	TCD (Gy)	n (%)	TCD (Gy)	n (%)	TCD (Gy)
Abdominal radiotherapy	27 (7)	25 (10-71)	24 (8)	25 (10-62)	20 (11)	25 (10-60)
Total body irradiation	13 (3)	8 (7-15)	16 (5)	8 (6-12)	11 (6)	12 (7-12)
AAD score						
0	237 (56)		150 (51)		93 (49)	
1	47 (11)		35 (12)		19 (10)	
2	47 (11)		39 (13)		26 (14)	
3	70 (17)		55 (19)		42 (22)	
4	9 (2)		5 (2)		5 (3)	
≥5	15 (4)		8 (2)		6 (3)	

Data are expressed as median (range) or frequencies (%). **NA** not applicable; **CCS** childhood cancer survivors; **AMH** anti-Müllerian hormone; **ALL** Acute Lymphoblastic Leukaemia, **T-NHL** T-cell non Hodgkin lymphoma; **TCD** total cumulative dose; **AAD** alkylating agent dose. [a]After exclusion of ovariectomized subjects (n=7), PCOS subjects as classified according to the revised PCOS Rotterdam criteria (n=16), AMH > 5 µg/L N=76) if subjects were not classified according to the revised PCOS Rotterdam criteria since information about follicle count and hyperandrogenism was not available, and women >50 years (n=2).

Table 8.1 Representativeness of included survivors compared to the total group of female adult CCS and survivors in who AMH was measured.

At the time of inclusion, 42 of 191 included survivors (22.0 %) had regular menstrual cycles, whereas 8 survivors (4.2 %) had an oligo- or amenorrhoea. One survivor had shortening of mean menstrual cycle length (1.3 %). Of one survivor (1.3 %), no data on menstrual cycles were available since she delivered recently, and one survivor was pregnant (1.3 %). In 23 of 191 survivors (30.3 %), data on menstrual cycle at time of screening were not available. All other survivors used oral contraceptive pills (99/191, 51.8 %) or hormonal replacement therapy (16/191: 8.4 %) at the time of follow-up.

Median AMH level of the total group was 1.7 µg/L (range 0.1-4.9). Median total follicle count (FC) was 10 (0-25). The Spearman correlation coefficient (ρ) between AMH and antral follicle count (AFC) was 0.32 (p=0.08) in survivors who were screened during the follicular phase or during amenorrhoea. FSH was significantly inversely correlated with AMH (ρ= -0.30, P<0.001) and was significantly higher in survivors with AMH <0.1 µg/L compared to survivors with AMH >0.1 µg/L (20.9 U/l versus 4.9 U/l).

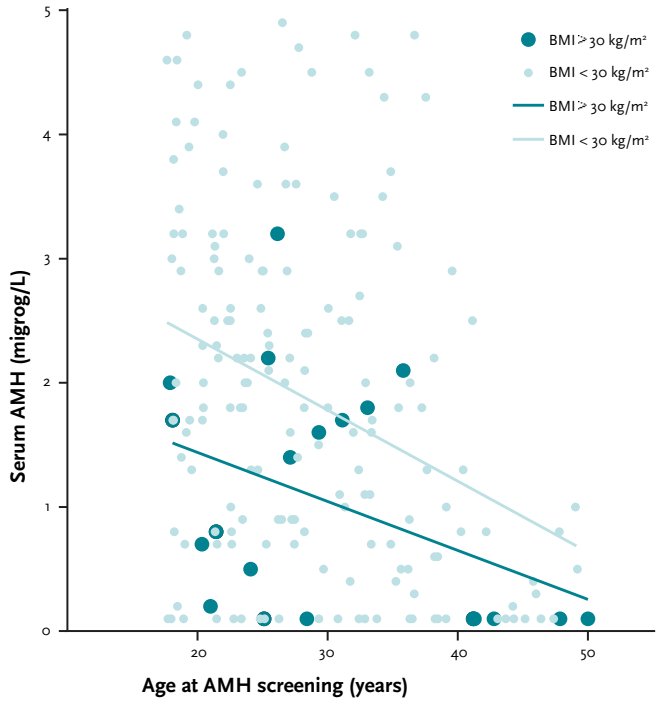

Figure 8.1 Anti-Müllerian hormone (AMH) in obese (BMI ≥30 kg/m²) survivors as compared to non-obese survivors.

Influence of obesity, body composition and insulin on AMH

Twenty subjects (10%) were defined as obese (BMI ≥ 30 kg/m²), 25 (13%) as overweight (BMI 25-30 kg/m²), seven (4%) as underweight (BMI 18 < kg/m²) and 121 (63%) had normal weight. AMH levels were significantly inversely associated with obesity (BMI ≥ 30 kg/m²), high fasting insulin (>54 pmol/L), total fat percentage, waist circumference, WHR and visceral fat percentage, but not with HOMA and lean body mass (Table 8.2). After adjustment for confounders (age, age at diagnosis, treatment with abdominal or total body irradiation and AAD score), obesity (BMI ≥30 kg/m²) and high fasting insulin (>54 pmol/L) remained significantly associated with AMH (β(%) -48, p=0.008 and β(%) -43, p=0.039 respectively) (Table 8.2, Figure 8.1, 8.2.1 and 8.2.2).

	AMH (%) N=191 (122) [a]					
	Univariate model			Multivariate model		
	β (%)	95% CI	P-value	β (%)	95% CI	P-value
Obesity (BMI ≥ 30 kg/m²)	-55*	-75; -18	0.009	-49*	-68; -17	0.007
Overweight (BMI 25-30 kg/m²)	1	-35; 56	0.974	15	-20; 64	0.450
Underweight (BMI < 18 kg/m²)	-17	-68; 116	0.698	-21	-64; 76	0.568
Body mass index	-3.3	-6.8; 0.4	0.081	-2.0	-5.0; 1.0	0.191
Total fat percentage	-3.7*	-6.4; -1.0	0.009	-2.1	-4.2, 0.1	0.06
Lean body mass (kg)	2.0	-11.3; 5.2	0.22	0.4	-2.2, 3.0	0.76
Waist circumference (cm) **	-2.0*	-3.7; -0.2	0.025	-0.5	-1.8, 0.9	0.511
Waist – hip ratio **	-2.5*	-4.3; -0.2	0.037	0.1	-1.8, 2.6	0.894
Visceral fat percentage **	-13*	-22; -2	0.02	-1	-10, 8	0.79
Insulin	-0.4	-0.9; 0.1	0.132	-0.2	-0.6; 0.3	0.48
Insulin 2nd tertile (24-54 pmol/L)	2	-47; 96	0.952	-8	-45; 55	0.761
Insulin 3rd tertile (>54 pmol/L)	-54*	-76; -12	0.019	-43*	-67; -3	0.039
HOMA	-13	-28; 5	0.135	-6	-18; 9	0.387
HOMA 2nd tertile (0.58-1.40)	17	-40; 127	0.636	-8	-46; 9	0.753
HOMA 3rd tertile (>1.40)	-45	-71; 8	0.080	-41	-65; 0.3	0.051

AMH anti-Müllerian hormone; **CI** confidence interval; **HOMA** homeostatic model assessment. [a]Number of available DXA scans; *P < 0.05; **For the dependent variables waist, waist-hip ratio and visceral fat percentage, survivors treated with abdominal radiotherapy are excluded from the analysis (n=19). Corrected for age, age at diagnosis, total body irradiation, abdominal radiotherapy and alkylating agent dose (AAD) score.

Table 8.2 Univariate and multivariate linear regression analyses illustrating the influence of body mass index (BMI), measures of body composition and insulin on AMH levels.

Influence of obesity, body composition and insulin on FC

There were no significant associations between FC and BMI or body composition measures (Table 8.3). Survivors with insulin and HOMA in the highest tertile had significantly lower FC than others. After adjustment for confounders, no linear or exponential association between FC and BMI or measures of body composition was found. FC did not differ significantly between quintiles of BMI or body composition (data not shown). Subsequently, FC did not differ between BMI categories. However, there were only 5 obese subjects with available FCs (data not shown). Insulin was significantly associated with FC, i.e. survivors with an insulin level in the highest tertile (>48 pmol/L) had significantly lower FC compared to survivors with insulin levels <25 pmol/L (β -6.3, p=0.013) (Table 8.3, Figure 8.2.3).

	Total follicle count N=54 (34) [a]					
	Univariate model			Multivariate model		
	β (%)	95% CI	P-value	β (%)	95% CI	P-value
Obesity (BMI ≥ 30 kg/m²)	-0.6	-7.8; 6.6	0.867	-0.9	-8.0; 6.2	0.804
Overweight (BMI 25-30 kg/m²)	-2.1	-7.7; 3.5	0.452	-2.1	-8.1; 3.8	0.471
Underweight (BMI < 18 kg/m²)	-3.0	-13.9; 7.9	0.584	-5.4	-16.4; 5.6	0.330
Body mass index	-0.03	-0.43; 0.38	0.902	-0.01	-0.42; 0.40	0.978
Total fat percentage	-0.09	-0.44; 0.26	0.614	-0.06	-0.44; 0.32	0.747
Lean body mass (kg)	0.18	-0.15; 0.51	0.266	0.20	-0.17; 0.57	0.278
Waist circumference (cm)**	-0.02	-0.24; 0.21	0.890	0.04	-0.18; 0.26	0.732
Waist – hip ratio**	-0.06	-0.37; 0.25	0.696	0.05	-0.26; 0.35	0.759
Visceral fat percentage**	-0.58	-2.44; 1.28	0.529	-0.18	-2.10; 1.75	0.851
Insulin	-0.03	-0.07; 0.00	0.083	-0.02	-0.06; 0.01	0.192
Insulin 2nd tertile (25-48 pmol/L)	-3.1	-7.8; 1.5	0.186	-3.9	-8.6; 0.8	0.104
Insulin 3rd tertile (>48 pmol/L)	-6.6*	-11.3; -2.0	0.006	-6.3*	-11.2; -1.4	0.013
HOMA	-0.98	-2.24; 0.27	0.121	-0.70	-2.00; 6.00	0.283
HOMA 2nd tertile (0.66-1.24)	-2.47	-7.22; 2.28	0.302	-3.42	-8.40; 1.57	0.175
HOMA 3rd tertile (>1.24)	-5.06*	-9.95; -0.18	0.043	-5.01	-10.26; 0.23	0.061

CI confidence interval; **HOMA** homeostatic model assessment. [a]Number of available DXA scans; *P < 0.05; **For the dependent variables waist, waist-hip ratio and visceral fat percentage, survivors treated with abdominal radiotherapy are excluded from the analysis (n=19). Corrected for age, age at diagnosis, total body irradiation, abdominal radiotherapy and AAD score.

Table 8.3 Multivariate linear regression analyses illustrating the lacking influence of body mass index (BMI), measures of body composition and insulin on total follicle count.

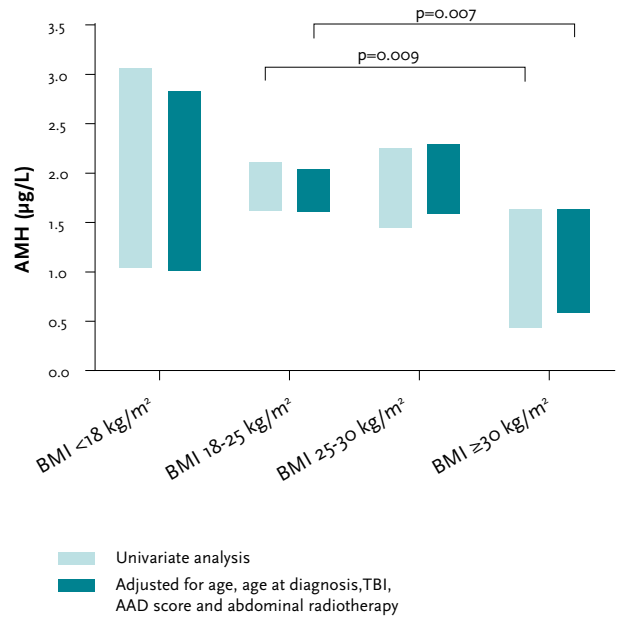

Figure 8.2.1 Anti-Müllerian hormone (AMH) in four BMI categories, univariate and after adjustment for possible confounders expressed as mean (95% CI).

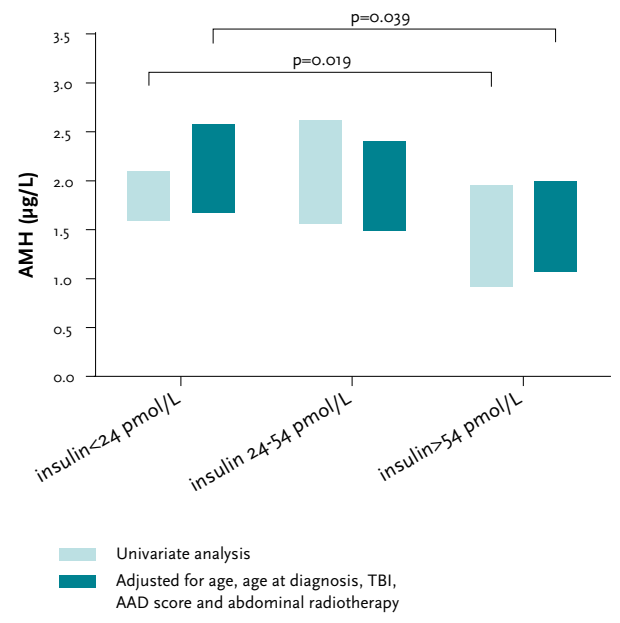

Figure 8.2.2 Anti-Müllerian hormone (AMH) in serum fasting insulin subgroups expressed as mean (95% CI).

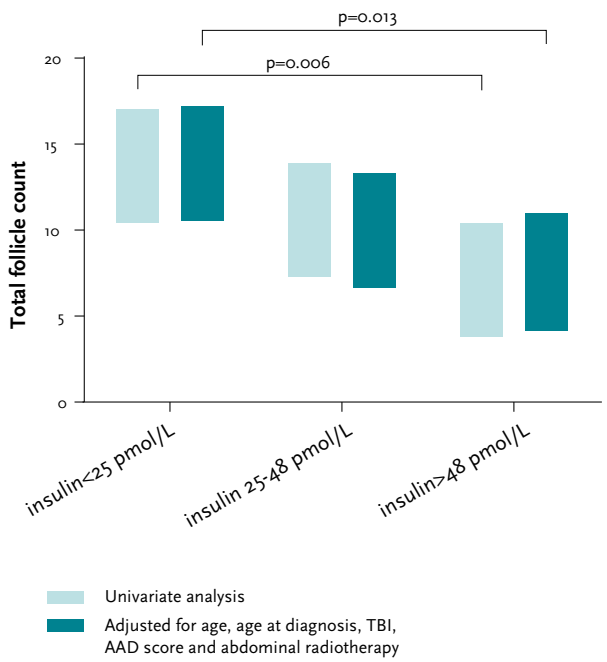

Figure 8.2.3 Total follicle count in serum fasting insulin subgroups expressed as mean (95% CI).

8.5 Discussion

The current study shows that diminished ovarian reserve, as reflected by low AMH and low FC, was independently associated with obesity and high insulin levels in female childhood cancer survivors.

Our results show that obesity is indeed independently associated with decreased AMH in female childhood cancer survivors[36, 37(p137), 220(p157)]. In the current study, total and visceral fat percentages were not associated with gonadal function, although we observed a trend to a negative association between total fat percentage and AMH levels. This may be due to a size effect or to the fact that we measured total fat percentage and not the fat distribution in different compartments. In fact, for that purpose, abdominal computer tomography, which is the gold standard for measuring intra-abdominal fat mass, is preferred over DXA[221(p157)].

Although no oral glucose tolerance tests were performed and therefore some cases in the prediabetic state might have been missed, none of the subjects was diagnosed and treated for diabetes mellitus, based on fasting glucose levels and / or medical history. Therefore, we evaluated fasting insulin levels as measure for insulin resistance and related these to ovarian reserve markers. The negative association between fasting insulin levels and AMH was previously described in women of reproductive age without PCOS[211(p156)] and was confirmed by our study among childhood cancer survivors.

We identified obesity to be negatively associated with ovarian reserve as assessed by AMH levels. There is no linear association between AMH and BMI, but above a certain threshold (in this case BMI 30 kg/m²) AMH is significantly lower as compared to normal weight subjects. The explanation for this non-linear association could be that only above a certain threshold of BMI metabolic changes do occur, including insulin resistance, leptin resistance and elevated levels of adipokines. These factors could play a role in affecting the pituitary-gonadal axis and damaging granulosa cells, although this hypothesis has never been proved. In this study, all subjects with obesity were evaluated, including one survivor treated for craniopharyngeoma and one survivor treated with high dose brain tumour irradiation (>35 Gy). In these subjects, hypothalamic obesity could not be excluded. To our knowledge, no other studies that assess a possible link between obesity and AMH have been performed in female childhood cancer survivors. In the general population, only one study among three has shown an association in women of reproductive age [210, 211(p156), 222(p157)].

Based on these results, we hypothesize that obesity influences the degree of gonadal damage in female childhood cancer survivors. However, it is also conceivable that impaired gonadal function may lead to the development of adiposity and insulin resistance. In animal models it is known that estradiol has an inhibitory effect on food intake via anorexigenic peptides that decrease meal size. In ovariectomized rats, the removal of estrogens leads to changes in meal size, obesity[223, 224(p157)], increased leptin sensitivity and decreased insulin sensitivity[225(p157)]. However, insulin resistance could also decrease granulosa cell function which could lead to reduced ovarian function and therefore lower AMH levels[212, 213(p156)]. Our hypothesis fits with the result of a study in Type 2 Diabetes Mellitus (T2DM) patients, in which AFC levels were significantly lower than in healthy controls[226(p157)], which was possibly a result of insulin resistance in the T2DM patients. Furthermore, the fact that stringent glycaemic control in diabetic patients improves menstrual cycles and fertility rates underlines the hypothesis that prolonged hyperglycemia and chronic complications of diabetes negatively affect ovarian reserve[227(p157)]. Animal models have shown that ovulation was suppressed in hyperglycemic-hyperinsulinemic conditions, due to hypovascularization, follicular atresia and eventually involution of ovaries, caused by glucotoxicity and the cytolipotoxic effect of obesity[228(p157)]. It should however be stressed that due to the cross-sectional design of the current study, cause and effect could not be distinguished.

In 105/285 cases PCOS diagnosis was verified conform the Rotterdam criteria, since these survivors also visited the gynaecological outpatient clinic. However, in the remaining 180 CCS, we were not able to distinguish between PCOS and non-PCOS subjects, because data on hyperandrogenism (clinical and biochemical) and total follicle counts (transvaginal ultrasound) were not available. Since we were not able to use the Rotterdam criteria in these remaining cases, and Dewailly et al. sug-

gested a cut-off limit of AMH > 5 µg/L to define PCOM [214(p156)], we used this marker and cut-off limit for the presence of PCOM. We recognize the limitation of this cut-off limit since we probably exclude more subjects than we would have done if we were able to exclude them based on the Rotterdam criteria. However, we believe that this is the best way to make our population as homogeneous as possible. Moreover, the remaining subjects were representative of the whole cohort of female childhood cancer survivors according to age, age at follow-up, diagnosis and treatment. Since we agree that the use of the cut-off value is of limited accuracy, we also performed subanalyses in the 105 cases that were classified based on the Rotterdam criteria. Sixteen survivors were diagnosed with PCOS and were therefore excluded from this analysis. We found a trend to an association between total fat percentage and AMH, although not significant (p=0.053). This is the same trend as found in our previous analysis. However, we did not find an association between BMI and AMH, which might be due to the underrepresentation of obese survivors in this subset (n=5). If we perform multivariate analysis in the whole group of survivors with AMH levels (n=285), we observe no significant correlations with obesity, which fits with the hypothesis that obese women with PCOS have raised AMH levels, while obese women without PCOS have lower AMH levels.

Despite the negative association between FC and serum insulin, we did not find an association between FC and obesity, in contrast to AMH. However, it should be stressed that follicle counts were available in only five obese subjects. So, power issues may be important. Larger cohorts are necessary to study this association in the future.

In healthy females AFC and AMH correlate very well[229(p157)], but the present study shows that this correlation is weak among childhood cancer survivors. This may be due to the fact that follicular AMH expression is lower in childhood cancer survivors treated with gonadotoxic therapies. To our knowledge, no large studies have been performed in female childhood cancer survivors in whom the correlation has been studied. Therefore, we cannot draw any firm conclusions based on our small subset analysis regarding the real correlation between AFC and AMH. We believe that it is important to study this association prospectively in a large nationwide cohort, such as the DCOG LATER-VEVO study[230(p158)].

We did not correct our analysis for smoking and OCP use. Smoking is linked to ovarian ageing in the general population[231(p158)]. However, we did not find a significant difference in AMH levels between smokers and non-smokers. The fact that smoking significantly influences ovarian reserve in the general population but not in female CCS may be caused by the large effect of the gonadotoxic treatment that may overshadow the influence of smoking on ovarian reserve. Whether OCP use affects AMH levels is still a matter of debate. In our study, we did not find an association between OCP and AMH. Therefore, we did not include smoking and OCP use as confounders.

Conclusion

Low serum AMH is associated with obesity and high insulin levels, and low FC with high insulin levels in a large cohort of adult female childhood cancer survivors. Furthermore, despite its highly predictive value for AFC in the healthy female population, serum AMH seems to correlate only weakly with AFC in childhood cancer survivors.

	Underweight/ Normal weight subjects BMI <25 kg/m² N=127		Overweight subjects BMI 25-30 kg/m² N=44		Obese subjects BMI ≥30 kg/m² N=20	
Age at diagnosis (yrs)	6.2 (0-16.2)		7.5 (0.4-16.1)		6.3 (1.5-15.1)	
Age at follow up (yrs)	26.3 (17.7-49.2)		28.8 (17.7-49.1)		27.8 (17.9-50.0)	
Follow up time (yrs)	18.5 (3.2-43.6)		21.2 (2.3-43.5)		18.4 (5.1-48.8)	
Diagnosis n (%)						
ALL & T-NHL	33 (26)		12 (27)		7 (35)	
Acute myeloid leukaemia	5 (4)		2 (5)		0	
B-cell non Hodgkin lymphoma	9 (7)		1 (2)		2 (10)	
Hodgkin lymphoma	11 (9)		5 (11)		2 (10)	
Bone tumour	9 (7)		4 (9)		1 (5)	
Wilms tumour	21 (17)		9 (21)		1 (5)	
Neuroblastoma	13 (10)		4 (9)		1 (5)	
Germ cell tumour	1 (1)		1 (2)		0	
Malignant mesenchymal tumour	12 (9)		2 (5)		3 (15)	
Brain tumour	6 (5)		4 (9)		3 (15)	
Other	7 (6)		0		0	
Therapy n (%)	n (%)	TCD (Gy)	n (%)	TCD (Gy)	n (%)	TCD (Gy)
Abdominal radiotherapy	13 (10)	25 (10-50)	3 (7)	12	1	12
Total body irradiation	7 (6)	8 (7-12)	6 (14)	23 (20-60)	1	40
AAD score						
0	56 (44)		27 (61)		10 (50)	
1	15 (12)		3 (7)		1 (5)	
2	18 (14)		7 (16)		1 (5)	
3	30 (24)		6 (14)		6 (30)	
4	4 (3)		0		1 (5)	
≥5	4 (3)		1 (2)		1 (5)	

Data are expressed as median (range) or frequencies (%). **ALL** Acute Lymphoblastic Leukaemia, **T-NHL** T-cell non Hodgkin lymphoma; **TCD** total cumulative dose; **AAD** alkylating agent dose.

Supplementary Table Baseline characteristics of normal weight, overweight and obese subjects.

9

CHAPTER

Gonadal function is associated with body composition in adult male survivors of childhood cancer

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Submitted

9.1 Abstract

Objective

Obesity and gonadal dysfunction are major side effects of treatment in childhood cancer survivors. In the general population, obesity has a negative influence on spermatogenesis and reproductive hormone levels. The aim was to evaluate whether altered body composition is associated with impaired gonadal function in male childhood cancer survivors.

Design

Retrospective single centre cohort study

Methods

Data of 351 male survivors of childhood cancer were analysed. Median age at diagnosis was 5.9 years (0-17.8) and median age at follow-up was 25.6 years (18.0-45.8). Main outcome measures were total and free testosterone, sex hormone-binding globulin (SHBG), inhibin B and follicle stimulating hormone (FSH). Potential risk factors were body mass index (BMI), waist circumference, waist-hip ratio and body composition measures, determined by dual energy X-ray absorptiometry (total fat percentage, lean body mass and visceral fat percentage).

Results

Free testosterone was significantly decreased in survivors with obesity (BMI ≥30 kg/m²) (β −1.8, p=0.015), high fat percentage (≥25%) (β −1.2, p=0.004), and high waist circumference (>102 cm) (β -2.0, p=0.020). Survivors with high fat percentage had significantly lower inhibin B and inhibin B / FSH ratios than survivors with normal total fat percentage in univariate and multivariate analysis after adjustment for age at follow-up, age at diagnosis, smoking, total body irradiation and abdominal irradiation and alkylating agent dose score (inhibin B: β -25, p=0.047; inhibin B / FSH ratio: β -34%, p=0.041).

Conclusion

Obesity is an independent risk factor for decreased gonadal reserve markers in a large cohort of adult male survivors of childhood cancer.

9.2 Introduction

Obesity prevalence has increased dramatically since the 1970s[201(p155)]. It has harmful effects on human health by increasing the risk of diabetes, hypertension, heart disease, stroke, and cancer[202(p155)]. In healthy men, obesity has been shown to be related to gonadal dysfunction and infertility[232, 233(p158)]. Although contradictory results have been reported, most studies found a negative association between obesity and underweight with sperm quality or quantity[232, 234, 235(p158)]. Obesity is known to be an important long-term effect of treatment for childhood cancer and occurs in 9-50% of long-term survivors, mainly depending on former treatment[36-38(p137), 48(p138)].

Gonadal dysfunction has been observed in both male and female childhood cancer survivors[83(p142), 191(p154), 218(p156), 236(p158)]. Especially former treatment with total body irradiation, abdominal irradiation and alkylating agents has previously been reported to be gonadotoxic, as represented by low inhibin B levels[83(p142)], which is considered a reliable first screening marker for spermatogenesis[237(p158)]. Inhibin B is produced by the Sertoli cells and has an inhibitory effect on the pituitary FSH secretion. Its production depends on the interaction of Sertoli cells with the germinal epithelium in the seminiferous tubules. Therefore, a low level of inhibin B is a reflection of dysfunction of the tubular compartment of the testis. In men, obesity is associated with decreased sperm quantity, motility and morphology[232, 234, 238(p158)], which might be caused by insulin resistance-related damage to the seminiferous tubules, affecting Sertoli cell function. Reduced testosterone levels might be explained by increased aromatization of androgens in the adipose tissue leading to higher circulating estradiol levels[233, 239, 240(p158)]. Additionally, low testosterone levels are thought to be the result of decreased SHBG binding activity caused by increased insulin levels. Additionally, leptin and other adipocyte-derived hormones might directly affect Leydig cell function[233, 241, 242(p159)].

We hypothesize that treatment-related obesity influences gonadal dysfunction in cancer survivors, as this association has been described in the general population previously. In survivors of childhood cancer, little is known about the influence of obesity on gonadal function. Therefore, we aim to determine the association between body composition and gonadal function as reflected by reproductive hormone levels in a representative single centre cohort of adult male survivors of childhood cancer.

9.3 Patients and Methods

Patients

A retrospective single centre cohort study was performed among male survivors who visited our outpatient clinic for long-term childhood cancer survivors. Inclusion criteria were: age ≥18 years, male sex, history of childhood cancer, cancer diagnosis between 1964 and 2005, and determination of BMI and reproductive hormones at the same moment at least 5 years after cessation of cancer treatment. Survivors treated with testosterone replacement and / or growth hormone therapy were excluded from this analysis. A written informed consent from every patient that visited the outpatient clinic was obtained according to standards of the Institutional Review Board (IRB).

Outcome measures

Peripheral serum samples were stored at -20° C until analysis. Inhibin B was used as a surrogate marker for gonadal function, since the correlation between inhibin B and sperm analyses has been reported to be strong in a subset of our cohort of childhood cancer survivors ($r = 0.54$, $r = 0.76$, respectively, $p < 0.001$)[83(p142), 237(p158)]. Inhibin B levels were measured using kits purchased from Diagnostic Systems Laboratories (Memphis, TX). Within-assay and between-assay coefficients of variation (CV) were <9%, and <15%, respectively. Serum follicle stimulating hormone (FSH), luteinizing hormone (LH) and sex hormone-binding globulin (SHBG) were determined with the Immulite assay (Siemens DPC, Los Angeles, CA). Within-assay and between-assay CV were <6% and <9%, <5% and 11%, and 4% and 5%, for FSH, LH and SHBG, respectively. Serum total testosterone levels were determined using coated-tube radioimmunoassays (Siemens DPC, Los Angeles, CA). Intra-assay and inter-assay variation coefficients were 3% and 4.5%. The reference values of LH, FSH, inhibin B, SHBG and total testosterone for male adults in our institute are 1.5-8.0 U/L, 2.0-7.0 U/l, 150-400 ng/L, 10-70 nmol/L and 10-30 nmol/l, respectively[83(p142), 243(p159)]. Free testosterone was calculated from total testosterone and SHBG[244(p159)].

Obesity variables

Follow-up data of the most recent visit included measurement of the following variables: weight, height, body mass index (BMI) calculated from height and weight[49], and waist-hip ratio (WHR), as measured by waist circumference divided by hip circumference[70(p141)]. Lean body mass (kg) and percentage of body fat were measured in a subset of survivors by dual energy X-ray absorptiometry (DXA, Lunar Prodigy, GE Healthcare, Madison, WI). Visceral fat percentage was calculated from intra-abdominal fat (kg) and total fat (kg), as measured by DXA[71(p141)]. Visceral fat percentage, waist and waist-hip ratio were not analysed in the subset of survivors treated with abdominal radiotherapy, because of the inferior value as markers for obesity in this subgroup[19(p135)]. Obesity was defined as BMI ≥30[51(p139), 245(p159)], total fat percentage ≥ 25%[246(p159)] and waist circumference >102 cm[247(p159)].

Potential confounders

Data concerning treatment protocols, disease and patient characteristics were

retrieved from the local database and were completed using the medical records where necessary. Follow-up time was defined as time since cessation of treatment. Among patients exposed to alkylating agents, the alkylating agent dose (AAD) score was calculated by determining the drug dose tertile distribution in our entire cohort of survivors and adding the tertile scores (1, 2 or 3) for each of the alkylating agents given to a particular patient as previously described by Green et al.[218, 219(p156)]. An AAD score of zero was assigned to patients not exposed to any alkylating agent.

Statistics

To evaluate the differences in hormone levels between BMI categories, analysis of variance (ANOVA) was performed. To examine the associations between obesity variables and reproductive hormone levels, we used univariate as well as multiple linear regression analysis. In multiple linear regression models, age at follow-up, age at diagnosis, current smoking status (yes or no), and treatment factors (total body irradiation, abdominal radiotherapy and AAD score) were included as confounders, based on what is known from previous studies describing the influence of treatment factors on gonadal reserve[83(p142), 248-250(p159)]. The analyses were performed in several steps. First, BMI and body composition were entered as continuous variables. Additionally, BMI was divided in 4 categories: BMI ≥30 kg/m² (obese), BMI 25-30 kg/m² (overweight), BMI 18.5-25 kg/m² (normal weight) and BMI <18.5 kg/m² (underweight) and added to the model as dummy variables with normal weight as reference category. Total fat percentage and waist circumference were divided in 2 categories: total fat % ≥25% (obese) versus total fat % <25% (non-obese) and waist circumference >102 cm (obese) versus waist circumference ≤102 cm (non-obese). Associations were expressed as standardized regression coefficients because this measure allows direct comparison of the strengths of associations between different determinants. The distribution of inhibin B / FSH ratio was normalized by ^{10}log transformation to improve the plots of the residual analyses and expressed as percentage in- or decrease compared to the reference category. P-values <0.05 (two-tailed) are considered statistically significant. SPSS 20.0 software (SPSS, Chicago, IL) was used for statistical analysis.

9.4 Results

Survivors

Data on reproductive hormone levels in combination with BMI were available in 351 out of 562 male survivors (Table 9.1), after exclusion of survivors treated with testosterone supplementation (n=10) and GH treatment (n=3). At follow-up, 11 subjects used thyroxine and thyroid function had been stable for at least 2 years in all subjects.

Median age at diagnosis was 5.9 years (0-17.8) and median age at follow-up was 25.6 years (18.0-45.8). Median follow-up time since cessation of treatment was 17.5 years (5.0-43.0). Diagnosis and treatment details are depicted in Table 9.1. Compared to excluded survivors, age at diagnosis was significantly lower (p<0.001) and ALL, Wilms tumour and neuroblastoma survivors were overrepresented, while brain tumour survivors were underrepresented in this cohort. In- and excluded survivors were comparable with regard to treatment factors (dosages of abdominal irradiation, total body irradiation and AAD score).

	Included survivors N=351		All male survivors N=562	
Age at diagnosis (yrs)	5.9 (0-17.8)		7.0 (0-18.0)	
Age at follow up (yrs)	25.6 (18.0-45.8)		NA	
Follow up time (yrs)	17.5 (5.0-43.0)		NA	
Diagnosis n (%)				
ALL & T-NHL	113 (32)		153 (27)	
Acute myeloid leukaemia	12 (3)		19 (3)	
B-cell non Hodgkin lymphoma	40 (11)		57 (10)	
Hodgkin lymphoma	34 (10)		60 (11)	
Bone tumour	18 (5)		27 (5)	
Wilms tumour	40 (11)		51 (9)	
Neuroblastoma	21 (6)		25 (4)	
Germ cell tumour	6 (2)		10 (2)	
Malignant mesenchymal tumour	28 (9)		48 (9)	
Brain tumour	19 (5)		78 (14)	
Other	20 (5)		34 (6)	
Therapy n (%)	**n (%)**	**TCD (Gy)**	**n (%)**	**TCD (Gy)**
Abdominal radiotherapy	24 (7)	30 (15-64)	34 (6)	30 (11-64)
Total body irradiation	13 (4)	12 (6-20)	23 (4)	12 (6-19.5)
Cranial radiotherapy	31 (9)	25 (25-48)	65 (12)	25 (10-40)
Brain tumour irradiation (BRT)	28 (8)	36 (10-108)	56 (10)	40 (18-108)
AAD score				
0	139 (40)		260 (46)	
1	44 (13)		60 (11)	
2	58 (17)		72 (13)	
3	86 (25)		131 (23)	
4	14 (4)		20 (4)	
≥5	10 (3)		19 (3)	

Data are expressed as median (range) or frequencies (%). **ALL** Acute Lymphoblastic Leukaemia; **T-NHL** T cell non Hodgkin Lymphoma; **AAD** score alkylating agent dose score; **NA** not applicable.

Table 9.1 Baseline characteristics of survivors included in this study.

	Total group N=351	BMI ≥ 30 N=20	BMI 25-30 N=98	BMI 18.5-25 N=219	BMI <18.5 N=14
inhibin B (ng/L)	139 (66-221)	125 (46-199)	137 (69-230)	140 (67-209)	104 (20-88)
FSH (U/L)	5.1 (3.1-11.3)	3.8 (2.9-9.0)	5.2 (3.2-10.1)	5.2 (3.2-11.9)	5.0 (3.8-22.6)
inhibin B/FSH ratio	30.8 (6.6-65.7)	31.9 (9.4-63.0)	29.2 (7.3-76.1)	32.3 (7.3-63.3)	25.4 (0.7-49.5)
Total T (nmol/L)	15.7 (12.9-19.0)	13.8 (10.3-16.3)*	14.8 (12.3-18.5)	16.2 (13.2-19.7)	17.6 (13.7-19.8)
Free T (nmol/L)	10.4 (8.7-12.6)	9.4 (7.6-10.8)*	10.3 (8.8-12.9)	10.5 (8.7-12.8)	11.5 (8.4-12.9)
SHBG (nmol/L)	26.8 (20.3-33.8)	21.9 (15.0-29.3)	23.7 (18.0-30.2)*	28.3 (22.0-36.4)	35.8 (29.2-40.7)
LH (U/L)	3.7 (2.6-5.5)	3.9 (2.2-5.4)	3.5 (2.5-4.9)	3.8 (2.7-5.7)	3.1 (1.5-7.0)

Data are expressed as median (interquartile ranges). **BMI** body mass index, expressed as kg/m²; **SHBG** sex hormone-binding globulin; **FSH** follicle stimulating hormone; **LH** luteinizing hormone. *P<0.05 as compared to survivors with BMI 18.5-25 kg/m².

Table 9.2 Median levels of inhibin B, inhibin B/FSH ratio, total & free testosterone and SHBG in the total group and different BMI categories.

	Free testosterone N=341 (224)[a]					
	Univariate model			Multivariate model		
	β	95% CI	P-value	β	95% CI	P-value
Body mass index (BMI)	-0.15	-0.23; -0.06	0.001	-0.1	-0.2; -0.01	0.029
Obesity (BMI ≥ 30 kg/m²)	-2.0	-3.5; -0.6	0.006	-1.8	-3.2; -0.3	0.015
Overweight (BMI 25-30 kg/m²)	-0.4	-1.1; 0.4	0.364	0.04	-0.7; 0.8	0.927
Underweight (BMI < 18.5 kg/m²)	-0.2	-2.0; 1.7	0.872	-0.7	-2.4; 1.1	0.462
Total fat percentage	-0.11	-0.16; -0.06	<0.001	-0.08	-0.13; -0.03	0.002
Total fat percentage ≥25%	-1.6	-2.4; -0.8	<0.001	-1.2	-2.0; -0.4	0.004
Lean body mass (kg)	0.01	-0.04; 0.07	0.624	-0.01	-0.06; 0.05	0.861
Waist circumference (cm)[b]	-0.08	-0.12; -0.05	<0.001	-0.06	-0.10; -0.02	0.001
Waist circumference > 102 cm[b]	-2.6	-4.4; -0.9	0.003	-2.0	-3.7;-0.3	0.020
Waist – hip ratio[b]	-0.15	-0.21; -0.09	<0.001	-0.10	-0.17; -0.04	0.002
Visceral fat percentage[b]	-0.21	-0.50; 0.08	0.159	0.12	-0.21; 0.45	0.479

CI confidence interval; [a]number of available DXA scans; [b]survivors treated with abdominal radiotherapy are excluded from this analysis (n=21); Model was adjusted for age at follow-up, age at diagnosis, current smoking status (yes/no), total body irradiation, abdominal radiotherapy and alkylating agent dose score.

Table 9.3 Univariate and multivariate linear regression analyses illustrating the influence of body mass index (BMI) and measures of body composition on free testosterone.

Endocrine function

Median inhibin B of the total group was 139 ng/L (interquartile range (IQR) 66-221). Median inhibin B / FSH ratio was 30.8 (IQR 6.6-65.7). The spearman correlation coefficient (ρ) between inhibin B and inhibin B / FSH ratio was 0.93 (P<0.001). Median total testosterone was 15.7 nmol/L (IQR 12.9-19.0). Median free testosterone was 10.4 nmol/L (IQR 8.7-12.6) and median SHBG was 26.8 nmol/L (IQR 20.3-33.8). Twenty subjects (6%) were defined as obese by BMI (≥ 30 kg/m²), 98 (28%) as overweight (BMI 25-30 kg/m²), 14 (4%) as underweight (BMI <18.5 kg/m²) and 219 (62%) had normal weight. In Table 9.2, hormone levels are presented per BMI category. Subjects with BMI <18.5 kg/m² appeared to have lower inhibin B levels (median 104 ng/L) than normal weight subjects (140 ng/L), however this difference did not reach significance. Obese subjects had significantly lower total and free testosterone levels than normal weight subjects (13.8 versus 16.2, p=0.010 and 9.4 versus 10.5, p=0.040). Overweight subjects had significantly lower SHBG levels than normal weight subjects (23.7 versus 28.3, p=0.001).

Influence of obesity and body composition on free testosterone

In univariate analysis, free testosterone was significantly inversely associated with body mass index, total fat percentage, waist and waist-hip ratio (Table 9.3). After adjustment for age at follow-up, age at diagnosis, current smoking status (yes/no), TBI, abdominal radiotherapy and AAD score, free testosterone was significantly inversely associated with BMI (β –0.1, p=0.029), total fat percentage (β -0.08, p=0.002), waist circumference (β -0.06, p=0.001) and waist-hip ratio (β -0.1, p=0.002), but not with visceral fat percentage. Survivors with obesity, defined by BMI ≥30 (β -1.8, p=0.015), total fat percentage ≥25% (β -1.2, p=0.004) or waist circumference >102 cm (β -2.0, p=0.020) had significantly lower free testosterone than survivors without obesity. For total testosterone, similar, but stronger associations were observed (Supplementary Table 9.1).

Influence of obesity and body composition on sex hormone-binding globulin

Sex hormone-binding globulin levels were significantly inversely associated with body mass index, and all measures of body composition in uni- and multivariate analysis, including age at follow-up, age at diagnosis, current smoking status (yes/no), TBI, abdominal radiotherapy and AAD score: BMI (β –1.0, p<0.001), total fat percentage (β -0.5, p<0.001), lean body mass (β –0.3, p=0.003), waist circumference (β -0.3, p<0.001), waist-hip ratio (β -0.4, P<0.001) and visceral fat percentage (β -3.2, p<0.001). Survivors with obesity, defined by BMI ≥30 (β -7.6, p=0.003), total fat percentage ≥25% (β -6.6, p<0.001) or waist circumference >102 cm (β -7.6, p<0.001) had significantly lower SHBG than survivors without obesity (Table 9.4).

	SHBG N=344 (225)[a]					
	Univariate model			Multivariate model		
	β	95% CI	P-value	β	95% CI	P-value
Body mass index	-0.9	-1.2; -0.6	<0.001	-1.0	-1.3; -0.7	<0.001
Obesity (BMI ≥ 30 kg/m²)	-6.3	-11.4; -1.2	0.016	-7.6	-12.5; -2.6	0.003
Overweight (BMI 25-30 kg/m²)	-5.2	-7.9; -2.5	<0.001	-5.7	-8.4;-3.1	<0.001
Underweight (BMI < 18.5 kg/m²)	4.2	-2.3;10.6	0.205	3.6	-2.6; 9.9	0.254
Total fat percentage	-0.5	-0.6; -0.3	<0.001	-0.5	-0.7; -0.3	<0.001
Total fat percentage ≥25%	-6.7	-9.7; -3.7	<0.001	-6.6	-9.6; -3.5	<0.001
Lean body mass (kg)	-0.3	-0.5; -0.1	0.004	-0.3	-0.5; -0.1	0.003
Waist circumference (cm)[b]	-0.3	-0.4; -0.2	<0.001	-0.3	-0.5; -0.2	<0.001
Waist circumference > 102 cm[b]	-5.7	-12.0; 0.6	0.076	-7.6	-13.6; -1.5	0.014
Waist – hip ratio[b]	-0.3	-0.5; -0.1	0.004	-0.4	-0.6; -0.2	<0.001
Visceral fat percentage[b]	-1.9	-3.0; -0.9	<0.001	-3.2	-4.3; -2.0	<0.001

CI confidence interval; [a]number of available DXA scans; [b]survivors treated with abdominal radiotherapy are excluded from this analysis (n=21). Model was adjusted for age at follow-up, age at diagnosis, current smoking status (yes/no), total body irradiation, abdominal radiotherapy and alkylating agent dose score.

Table 9.4 Univariate and multivariate linear regression analyses illustrating the influence of body mass index (BMI) and measures of body composition on sex hormone-binding globulin (SHBG).

	inhibin B N=340 (226)[a]					
	Univariate model			Multivariate model		
	β	95% CI	P-value	β	95% CI	P-value
Body mass index	-0.2	-3.0; 2.6	0.863	-1.3	-4.0; 1.3	0.317
Obesity (BMI ≥ 30 kg/m²)	-7	-54;39	0.761	-10	-52; 31	0.626
Overweight (BMI 25-30 kg/m²)	5.0	-20; 30	0.689	0.4	-22; 23	0.971
Underweight (BMI < 18.5 kg/m²)	-3.7	-61; 53	0.897	2.8	-48; 54	0.914
Total fat percentage	-2.2	-3.7; -0.6	0.006	-1.9	-3.3; -0.5	0.010
Total fat percentage ≥25%	-30	-57; -4	0.026	-25	-49; -0.4	0.047
Lean body mass (kg)	2.3	0.5; 4.0	0.011	0.1	-1.6; 1.8	0.882
Waist circumference (cm)[b]	-1.0	-2.1; 0.2	0.106	-1.0	-2.1; 0.1	0.087
Waist circumference > 102 cm[b]	-44	-101; 14	0.135	-44	-97; 8	0.096
Waist – hip ratio[b]	-2.1	-4.1; -0.2	0.034	-2.1	-4.1; -0.1	0.042
Visceral fat percentage[b]	-5.2	-14.1; 3.8	0.254	-0.7	-9.8; 8.7	0.905

CI confidence interval; [a]number of available DXA scans; [b]survivors treated with abdominal radiotherapy are excluded from this analysis (n=21). Model was adjusted for age at follow-up, age at diagnosis, current smoking status (yes/no), total body irradiation, abdominal radiotherapy and alkylating agent dose score.

Table 9.5 Univariate and multivariate linear regression analyses illustrating the influence of body mass index (BMI) and measures of body composition on serum inhibin B.

Influence of obesity and body composition on inhibin B

Inhibin B was significantly inversely associated with total fat percentage and waist-hip ratio and positively associated with lean body mass in univariate analysis (Table 9.5). After adjustment for age at follow-up, age at diagnosis, current smoking status (yes/no), TBI, abdominal radiotherapy and AAD score, inhibin B was independently and inversely associated with total fat percentage (β -1.9, p=0.010) and waist-hip ratio (β -2.1, p=0.042), but not with lean body mass. Survivors with obesity, defined by total fat percentage ≥25% (β -25, p=0.047) had significantly lower inhibin B than survivors without obesity

In univariate analysis, inhibin B / FSH ratios were significantly inversely associated with total fat percentage and waist-hip ratio and positively associated with lean body mass (Supplementary Table 9.2). After adjustment for confounders, the inhibin B / FSH ratio was significantly inversely associated with total fat percentage (β (%) -2.8, p=0.018), but not with waist-hip ratio or lean body mass. Survivors with obesity, defined by total fat percentage ≥ 25% (β (%) -34, p=0.041) had significantly lower inhibin B/FSH ratio than survivors without obesity.

9.5 Discussion

The current study in adult male survivors of childhood cancer describes the association between two major side effects of treatment, namely obesity and gonadal dysfunction. We observed that obesity was independently associated with low levels of testosterone and inhibin B in male childhood cancer survivors.

In the current study, an inverse association between BMI and testosterone was found which is in line with previous reports in the general population[232, 233(p158), 239, 240(p159), 251(p160)]. However, most previous studies are based on BMI as marker for obesity, while it was recently shown that BMI is an inaccurate measure for total body fat since obesity was underestimated in 39% of the general population and in 52% of childhood cancer survivors[43(p138), 252(p160)]. Waist circumference, representing the amount of intra-abdominal fat, is a slightly better marker, but the most accurate method for total body fat measurement is dual energy X-ray absorptiometry (DXA)[241(p159)]. In the present study, we were able to include data of total body fat measured by DXA in a substantial subset of the survivors and found that obesity, represented by total fat percentage, was an independent risk factor for low total and free testosterone.

In the general population, the association between BMI and inhibin B is controversial. A negative association between BMI and inhibin B was reported several times[233, 234(p158), 253(p160)], whereas one study did not find a relationship[254(p160)] and one study described both high and low BMI to be associated with low inhibin B levels[232(p158)]. The current study clearly describes an independent relationship between obesity, represented by high total fat percentage or high waist-hip ratio, and low

Inhibin B levels in male survivors of childhood cancer. Additionally, low inhibin B levels were observed in underweight subjects. However, no significant relation between underweight and inhibin B levels was found, probably due to the small number of subjects in this BMI category.

We hypothesize that obesity negatively affects gonadal function in male survivors of childhood cancer. This may be due to reduced androgen levels, which can be induced by higher aromatase activity in overweight and obese men. P450 aromatase cytochrome converts testosterone to estradiol, is expressed at high levels in white adipose tissue and is responsible for the key step in the biosyntheses of estrogens[(p159)241, 255(p160)]. Unfortunately, estradiol levels were not available in our male survivors, but we showed that testosterone levels were low in obese men, which supports this hypothesis.

Recently, in female childhood cancer survivors, we also found that obesity and insulin resistance are associated with ovarian failure, as reflected by a decreased Anti-Müllerian hormone (AMH) levels and reduced follicle counts[256(p160)]. In previous studies regarding men, a negative or no association between obesity and insulin resistance with gonadal function has been found. Unfortunately, no data of fasting insulin were available in our cases. However, it is well known that insulin resistance is a major determinant of decreased SHBG levels, by its inhibition of SHBG production in the liver[257(p160)]. We found a strong relationship between low levels of SHBG and obesity, which is probably secondary to insulin resistance. The negative influence of obesity on gonadal function might thus be secondary to insulin resistance, which relation has previously been investigated in animal studies. Hyperglycaemic conditions in diabetic rats affected Sertoli cell function directly, due to atrophy of the seminiferous tubules, followed by depletion of germ cells, or indirectly due to damage to the Leydig cells affecting testosterone dependent sperm production[258, 259(p160)]. In the present study, we hypothesized that obesity negatively influences male reproductive capacity, based on knowledge from previous studies in the general population. However, due to the cross-sectional design, we cannot exclude that in childhood cancer survivors hypogonadism may be a determinant rather than a consequence of obesity. Therefore, future studies investigating this relationship in childhood cancer survivors should focus on longitudinal data.

We were not able to include sperm analysis in the survey. Nevertheless, the correlation between inhibin B values and sperm analyses was reported to be strong ($r = 0.54$, $r = 0.76$, respectively, $p < 0.001$)[(p142)83, 237(p158)], indicating that it is justified to use inhibin B as a surrogate marker for gonadal function. Moreover, we measured testosterone to provide complete information on reproductive hormone status, and also found a significant negative correlation between total fat percentage and free testosterone.

Conclusion

Obesity is an independent risk factor for decreased gonadal reserve markers in a large cohort of adult male survivors of childhood cancer.

	Total testosterone N=351 (226)[a]					
	Univariate model			Multivariate model		
	β	95% CI	P-value	β	95% CI	P-value
Body mass index	-0.4	-0.5; -0.2	<0.001	-0.3	-0.5; -0.2	<0.001
Obesity (BMI ≥ 30 kg/m²)	-3.8	-6.1; -1.4	0.002	-3.7	-6.0; -1.4	0.002
Overweight (BMI 25-30 kg/m²)	-1.5	-2.7; -0.2	0.019	-1.1	-2.3; 0.1	0.075
Underweight (BMI < 18.5 kg/m²)	0.5	-2.2; 3.3	0.713	0.05	-2.6; 2.7	0.972
Total fat percentage	-0.2	-0.3; -0.2	<0.001	-0.2	-0.3; -0.1	<0.001
Total fat percentage ≥25%	-3.6	-4.7; -2.1	<0.001	-2.9	-4.2; -1.6	<0.001
Lean body mass (kg)	-0.04	-0.1; 0.1	0.441	-0.1	-0.2; 0.0	0.169
Waist circumference (cm)[b]	-0.2	-0.2; -0.1	<0.001	-0.1	-0.2; -0.1	<0.001
Waist circumference > 102 cm[b]	-4.5	-7.4; -1.6	0.003	-4.1	-6.9; -1.2	0.005
Waist – hip ratio[b]	-0.3	-0.4; -0.2	<0.001	-0.2	-0.3; -0.1	<0.001
Visceral fat percentage[b]	-0.6	-1.0; -0.1	0.020	-0.4	-0.9; 0.2	0.198

CI confidence interval; [a]number of available DXA scans; [b]survivors treated with abdominal radiotherapy are excluded from this analysis (n=21). Model was adjusted for age at follow-up, age at diagnosis, current smoking status (yes/no), total body irradiation, abdominal radiotherapy and alkylating agent dose score.

Supplementary Table 9.1 Univariate and multivariate linear regression analyses illustrating the influence of body mass index (BMI) and measures of body composition on total testosterone levels.

	Inhibin B / FSH ratio (%) N=341 (226)[a]					
	Univariate model			Multivariate model		
	β (%)	95% CI	P-value	β (%)	95% CI	P-value
Body mass index	2.1	-3.2; 7.7	0.440	0.3	-4.2; 4.9	0.912
Obesity (BMI ≥ 30 kg/m²)	2.9	-57; 149	0.949	1.5	-50; 106	0.968
Overweight (BMI 25-30 kg/m²)	4.6	-35; 67	0.852	-1.3	-33; 45	0.951
Underweight (BMI < 18.5 kg/m²)	-50	-83; 46	0.202	-45	-77; 30	0.171
Total fat percentage	-4.0	-6.8; -1.2	0.006	-2.8	-5.0; -0.5	0.018
Total fat percentage ≥25%	-46	-67; -12	0.014	-34	-55; -1.7	0.041
Lean body mass (kg)	5.1	1.7; 8.5	0.003	-0.4	-3.1; 2.3	0.753
Waist circumference (cm)[b]	-1.9	-3.9; 0.2	0.076	-1.5	-3.3; 0.4	0.113
Waist circumference > 102 cm[b]	-56	-84; 24	0.122	-53	-80; 12	0.088
Waist – hip ratio[b]	-3.2	-5.2; -0.2	0.037	-2.2	-4.4; 0.9	0.141
Visceral fat percentage[b]	-13	-26; 2	0.091	-3.0	-16; 13	0.711

FSH follicle stimulating hormone; **CI** confidence interval; [a]number of available DXA scans; [b]Survivors treated with abdominal radiotherapy are excluded from this analysis (n=21); Model was adjusted for age at follow-up, age at diagnosis, current smoking status (yes/no), total body irradiation, abdominal radiotherapy and alkylating agent dose score.

Supplementary Table 9.2 Univariate and multivariate linear regression analyses illustrating the influence of body mass index (BMI) and measures of body composition on inhibin B / FSH ratio.

10

CHAPTER

General Discussion and Conclusions

The increasing survival rates after childhood cancer treatment emphasize the need for follow-up of late effects, including specialized care. One of the major side effects is obesity, which in the general population contributes to increased morbidity and mortality. Higher prevalence of obesity among childhood cancer survivors (CCS), suggests that prior diagnosis and treatment factors strongly influence body composition. It is conceivable that altered body composition is not only directly caused by cancer treatment, but also indirectly by other treatment-related complications (Figure 10.1). Additionally, obesity itself may contribute to the development of other sequelae. So far, systematic studies on these determinants and sequelae in one and the same cohort are not available. Therefore, in this project we aimed to:

- Evaluate the value of current obesity measures in adult CCS
- Define determinants of altered body composition in this group
- Evaluate the influence of obesity on gonadal reserve in adult CCS

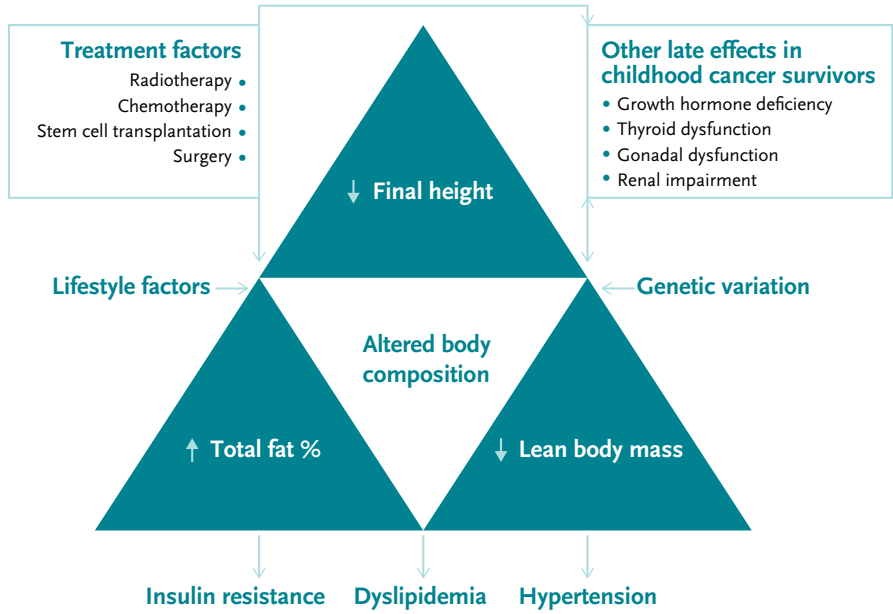


Figure 10.1 Determinants & sequelae of altered body composition in childhood cancer survivors.

10.1 Markers for obesity in childhood cancer survivors

Body mass index (BMI) is the most generally used obesity marker world-wide and is a significant predictor of metabolic health risk[12(p135)]. The advantage of BMI is

its easy measurement, which makes it possible to evaluate obesity in large cohorts, based on self-reported questionnaires[260](p161). Besides the fact that self-reported BMI is subject to misclassification bias, there is a world-wide debate about the value of BMI defining obesity. Formulation of cut-off points for BMI in CCS are based on international data and linked to the widely accepted adult cut-off points for obesity, e.g. 30 kg/m² [245](p159). In the general population, it was recently observed that obesity is underestimated in around 30% of subjects, using BMI instead of total fat percentage as measured by dual energy X-ray absorptiometry (DXA)[43](p138), the gold standard for body composition measurement. Using DXA, we showed that this misclassification is even more dramatically in CCS, suggesting that current cut-off limits for BMI do not reflect true obesity prevalence in CCS. Waist circumference has been described many times as a more potent surrogate marker for obesity than BMI, since it represents the abdominal distribution of fat, which is highly associated with insulin resistance and metabolic health risk and it is therefore included in the internationally standardized metabolic syndrome criteria[261](p161). In our cohort of CCS, we also found waist-hip ratio to be a better predictor for obesity. However, this measurement was not useful in a substantial subgroup of CCS treated with abdominal irradiation, due to deformation of the abdominal region. In this group, total fat percentage measured by DXA, instead of waist circumference was needed to assess the metabolic syndrome[19](p135). However, although DXA is the gold standard for true body composition, it is important to realize that it is expensive and time-consuming, making it unavailable as standard measurement at the late effects outpatient clinic. Therefore we endeavoured to define a surrogate marker for body composition, which is sensitive and easy to measure. Insulin-like growth factor-I (IGF-I) is responsible for linear growth during childhood and has for a long time been used as marker for growth hormone secretory status[74-76](p141). However, the value of IGF-I for predicting GHD has been shown to be limited in CCS, since normal IGF-I levels do not exclude growth hormone deficiency (GHD), particularly after treatment with cranial radiotherapy (CRT), which causes neurosecretory dysfunction [78](p142). Nevertheless, GHD and subsequently low IGF-I levels are highly correlated with alterations in body composition, particularly abdominal fat accumulation[262](p161). An inverse relation between IGF-I and visceral fat depot was earlier described in ALL survivors[46](p138), but was not confirmed in our study. These conflicting results may be due to the fact that Janiszewski and co-workers assessed intra-abdominal fat by the gold standard, which is computed tomography (CT)[221](p157), whereas we used DXA. Although DXA is the gold standard for total body fat measurement, its value for the assessment of visceral fat mass is comparable to that of waist circumference[221](p157). In our study, IGF-I was positively correlated with lean body mass and negatively with total fat mass, however correlations were weak. In addition, a limitation of both studies was that total, rather than free or bio-active IGF-I as a surrogate for growth hormone action was used, which is not representative for IGF-I activity since a large part of total IGF-I is inactivated due to its binding to IGF binding proteins[263](p161).

10.2 Determinants of altered body composition

Endocrine factors

The detrimental changes of body composition after CRT and brain tumour irradiation represented a significantly loss of height, increase in total fat mass and reduced lean body mass, while especially after high dose CRT visceral fat percentage was increased. This thesis describes the first study that emphasizes the severity of these alterations, by showing that even after 15 years of follow-up, total fat percentage increased more rapidly than in the general population, suggesting a persistent treatment effect. Only one comparable study was performed describing a more rapid increase in BMI 25 years after diagnosis, especially after high dose CRT[48](p138). CRT contributes to hypothalamic-pituitary damage, followed by GHD and dysregulation of energy balance (Figure 10.2).

The growth hormone (GH) axis is the most vulnerable to irradiation and GHD is usually the only neuro-endocrine deficiency due to irradiation of the hypothalamic-pituitary axis with dosages below 30 Gray (Gy)[68](p141). GHD occurs in a time- and dose-dependent manner, i.e. the higher the irradiation dose and the longer the interval from treatment, the greater the risk[68](p141). The prevalence of GHD among CCS varies from 30% associated with an irradiation dose of 18-24 Gy (mainly acute lymphoblastic leukaemia (ALL) survivors treated with CRT) to 100% when the irradiation dosage exceeds 50 Gy (survivors of head-and neck tumours or brain tumours, who have received local irradiation)[66](p140), [264](p161). Although only a small proportion of our cohort was tested for GHD by using stimulation tests, the vast majority of those diagnosed with GHD were brain tumour survivors treated with high irradiation dosages (range 35-54 Gray). IGF-I is synthesized in the liver and secreted into the blood under the control of GH and is responsible for linear growth during childhood. IGF-I and final height were found to be significantly lower in all survivors, but more dramatically in those treated with CRT, brain tumour irradiation or total body irradiation (TBI), which is explained by irradiation-induced pituitary damage. During adulthood, GHD and subsequent low IGF-I levels are associated with reduced quality of life[265-267](p161) and metabolic changes, represented by decreased lean body mass and bone density[268, 269](p161), abdominal fat accumulation[270, 271](p162) and raised plasma lipids[272]. In addition to low IGF-I levels and deterioration in IGF-I stimulated glucose transport in muscle, high visceral fat percentage and reduced physical activity might contribute to insulin resistance in GHD subjects[271](p162). Furthermore, those with abdominal obesity exhibit greater risk of hypertension, type 2 diabetes, dyslipidemia, and mortality[12](p135), [19](p135), [273](p162).

Hypothalamic obesity particularly plays a role in survivors of brain tumour who have received high irradiation dosages and/or undergone surgery near the sellar region, by damage to the ventromedial hypothalamus [274](p162). Normally, the ventromedial hypothalamus receives and interprets vagal and hormonal (ghrelin, insulin

and leptin) signals, generated from the gastrointestinal tract and adipose tissue. In response, efferent signals stimulate the sympathetic nervous system enhancing lipolysis, heat and physical activity, or the parasympathetic nervous system increasing insulin secretion. Hypothalamic obesity is caused by damage to the ventromedial hypothalamus (VMH), leading to insulin hypersecretion, leptin resistance, hyperphagia due to reduced ghrelin's suppression of hunger and compromised energy expenditure[274, 275](p162).

Ghrelin, the so-called 'hunger hormone', is released in the stomach in fasting conditions, while in conditions of a positive energy balance like hyperglycaemia and obesity, ghrelin levels are low. It plays an important role in the neurohormonal regulation of food intake and energy homeostasis[112](p145). The predominant form of ghrelin, which is unacylated, is associated with improved metabolic state, including reduced fat mass, favourable lipid profile and improved insulin sensitivity[115-118](p146). In order to give more insight into the physiology of desacyl ghrelin (DAG) in lean and obese subjects, we performed a pilot study among adult CCS undergoing an insulin tolerance test. DAG was significantly higher in lean subjects and rapidly decreased after insulin infusion, while obese subjects seemed to be relatively DAG resistant as no DAG change was observed. Another study among GHD and obese subjects found that after DAG infusion glucose tolerance significantly improved[116, 121](p146). The positive interaction between DAG and insulin metabolism opens future possibilities for treatment strategies for obesity and other insulin resistance diseases, which will be of use in the growing cohort of CCS, who are at higher risk for obesity.

Survivors who had been treated with TBI presented with underweight, contributable to a substantial loss of lean body mass, which finding was supported by others[27](p136), [260](p161). In this group, GHD may play a role, although to a lesser extent due to the relatively low irradiation dose the hypothalamic-pituitary axis is exposed to. In survivors of myeloid leukaemias treated with TBI and stem cell transplantation (SCT), primary hypothyroidism was found in 55% and gonadal dysfunction in 100% of subjects as well as high waist-hip ratio and increased levels of insulin and triglycerides in contrast to survivors treated with only chemotherapy. Comparable results concerning the effect of TBI in combination with SCT on the endocrine system have been reported, with gonadal failure being the most frequent followed by hypothyroidism and GHD[80](p142), [84](p142), [87](p143), [94](p143). Hypothyroidism contributes to obesity because T_3 regulates energy metabolism and thermogenesis and plays a crucial role in lipid and glucose metabolism, food intake, and the oxidation of fatty acids[276](p162). The mechanisms of by which hypogonadism is associated with body composition will be discussed in paragraph 10.3. Studies concerning the effect of SCT whether or not combined with TBI on the metabolic syndrome emphasized the increased risk to develop all separate components including hypertriglyceridemia, hyperinsulinemia, abdominal obesity and hypertension and an increased risk of diabetes[81](p142), [277-279](p162). Interestingly, we observed that acute myeloid leukaemia survivors treated with only chemotherapy did not show any metabolic or endocrine disorders except for the fact that markers for gonadal reserve were lower than controls, contributable to high dosages of cyclophosphamide. Since TBI is omitted in most of the current treatment protocols, and replaced by high dosed chemotherapy combinations, this finding is promising, although larger studies are needed to confirm the less detrimental effects of high dose chemotherapy combinations replacing TBI.

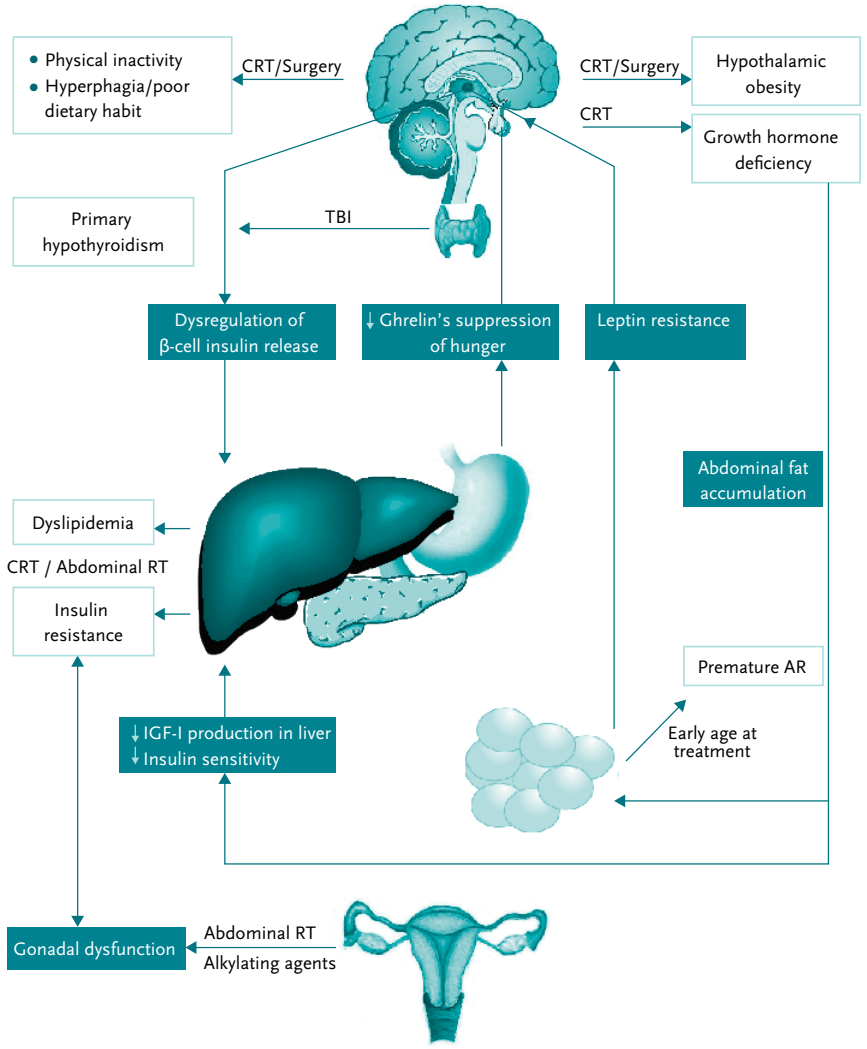


Figure 10.2 Mechanisms contributing to the development of obesity & altered body composition among childhood cancer survivors.

Non-endocrine factors

Besides endocrine dysfunction, direct irradiation-related toxicity to the skeleton might contribute to substantial loss of height and lean mass as observed after TBI. Survivors treated with TBI show a disproportionate short stature due to the direct effect of radiation on the vertebral growth plate and irradiation of the long bones[280, 281(p163)].

It is remarkable that when final height of CCS was compared to the normal population, even survivors treated without irradiation were significantly shorter than the normal population. In addition, non-irradiated survivors presented with comparable BMI, but altered body composition, represented by a higher fat mass and lower lean body mass than Dutch references, although changes were less dramatically than in irradiated survivors. This shows again the limited role of BMI in predicting true body composition.

A younger age at cancer treatment negatively influences final height attainment[281, 282(p163)] and might contribute to early adiposity rebound. Adiposity rebound represents the second period in life in which a rapid increase of body fat takes place, which normally occurs between 5 and 7 years. The timing of this process is relevant since early adiposity rebound is strongly correlated with a substantially increased risk of adult obesity. Because excess weight gain is typically during treatment of ALL and the peak incidence of ALL occurs around 3-5 years of age, premature adiposity rebound might be an important denominator of altered body composition in individuals surviving ALL[283(p163)].

There is still much unknown about the effects of specific chemotherapy regimen on body composition changes, although a profound effect of combination chemotherapy in general has been observed[284(p163)]. Recently, Vandecruys and co-workers reported a modest but significant loss in final height in ALL survivors treated with only chemotherapy[285(p163)]. Other childhood cancer survivors treated with chemotherapy and radiotherapy have more severe growth restriction than if treated with radiation only[24(p136)]. Height loss as a consequence of treatment with anthracyclines has been suggested, although findings were not confirmed[286(p163)]. Prior treatment with anthracyclines and alkylating agents has been associated with underweight, although the mechanism is not clear[260(p161), 287(p163)]. Authors suggested that underlying health problems might have confounded these outcomes, however, the relation with anthracyclines was observed repeatedly. Temporary height loss and obesity was found in ALL survivors treated with chemotherapy only, including central nervous system (CNS) prophylaxis and glucocorticoids, but eventually normal height was reached due to catch up growth and BMI was normalized, suggesting a temporary effect of treatment[17(p135), 26, 27(p136), 45(p138), 288(p163)]. We showed that at 18 years of follow-up, height and fat mass were not negatively influenced by prior corticosteroid treatment. Except for corticosteroids, we did not investigate the influence of separate chemotherapeutics on body composition, due to the heterogeneity of our cohort.

A remarkable finding was that in Wilms tumour survivors treated with nephrectomy, height was comparable to that of the normal population and was significantly higher than that of a comparable group of non-irradiated survivors of other cancer types, who had significantly lower stature than Dutch references. Additionally, IGF-I levels in non-irradiated Wilms survivors were comparable to Dutch references many years after nephrectomy, in contrast to non-irradiated survivors of other cancer types, in which IGF-I was significantly lower than references. There is convincing evidence that IGF-I levels increase after nephrectomy in both children and adults, associated with hypervascularization and regeneration of the contralateral kidney[132-134(p148), 148(p149)]. We suggest that increased IGF-I levels may contribute to a faster catch-up growth resulting in better attainment of final height. Another mechanism that might contribute to significant taller stature is the with Wilms tumour associated growth factor excess[142(p149), 155(p150)]. Since this is the first study on long-term follow-up reporting these remarkable results, larger studies are needed to confirm this.

An important treatment-related effect which is not of endocrine origin, but substantially affects height attainment and metabolic health is renal impairment due to nephrotoxic treatment[31(p137), 289(p164)]. Long-term follow-up studies among CCS who are at substantial risk for loss of renal function are lacking and therefore we cross-sectionally investigated renal impairment and associated risk factors for nephrotoxicity in this group. In our relatively young cohort (median age 26.9 years) 2.8% had an estimated GFR <60 ml/minute per 1.73 m², while 32% had an estimated GFR between 60 and 90 ml/minute per 1.73 m². In the normal population, estimated GFR decreases with age[175(p152)], indicating that this may reflect an emerging problem in the future. It should however be stressed that the results in this study are based on one measurement, while the definition of chronic kidney disease is based on renal impairment for at least 3 months[144(p149)]. Future studies should therefore focus on longitudinal data, to be able to assess CKD stage in CCS. Particularly at risk for glomerular damage and proteinuria 18 years after treatment, are those treated with nephrectomy whether combined with abdominal irradiation or high dosages of ifosfamide and cisplatin. Tubular dysfunction is associated with high dosages of ifosfamide and hypertension. Interestingly, we did not find a long-term effect of methotrexate, which has been reported to be nephrotoxic[193, 194(p154)]. Hypertension was present in 23.4% of survivors and 31.4% in renal tumour survivors, compared to 15.1% in the Dutch population[290(p164)]. Other follow-up studies in CCS reported remarkable lower percentages of 14% and 19%, using the same definition for hypertension[199(p154), 282(p163)]. The higher prevalence in our cohort might be explained by the larger interval since treatment and is alarming, since it may suggest an increasing prevalence of hypertension among young CCS. Besides hypertension, which might be a cause as well as a complication of renal failure, the other components of the metabolic syndrome, represented by obesity, dyslipidemia and insulin resistance are associated with kidney disease due to glomerular changes and microvascular damage[31(p137)]. Considering the greater risk of the metabolic syn-

drome among CCS[18](p135) and the high cardiovascular morbidity associated with renal failure[167](p151), CCS should be monitored closely, particularly because hypertension is often a disguised symptom.

Due to the retrospective design of most of the studies presented in this thesis, we were not able to include objective data on physical activity and dietary intake, which are important denominators of obesity and body composition[291-293](p164). In general, survivors are less likely to be active than non-cancer controls, represented by less than half of survivors engaging in regular physical activity[294-296](p164), although some studies reported more healthful patterns among particularly younger survivors[297, 298](p164). Methodologically well-described studies on dietary habits in CCS are lacking. Although the general finding is that diets are unhealthy, to our knowledge no studies using controls have been described. Particularly in brain tumour survivors, reduced energy expenditure and hyperphagia as a result of hypothalamic damage is of influence on obesity, although among these survivors lifestyle improvements do not seem to result in weight loss due to the overruling effect of resistance to leptin and ghrelin effects and insulin hypersecretion[274, 275](p162), [299](p164).

10.3 The influence of obesity & altered body composition on gonadal reserve

Gonadal failure is a major complication after treatment with alkylating agents[300](p165), TBI and abdominal or testicular irradiation[83](p142), [191](p154), [249](p159). Besides a detrimental effect on quality of life, due to subfertility and estrogen deficiency-related complaints in women, gonadal failure is associated with osteoporosis, obesity and insulin resistance. In the normal population obesity is related to subfertility[203, 205](p155). In CCS, both obesity and gonadal failure are major treatment-related complications, but the relation, as is observed in the general population, has never been reported. We studied whether altered body composition would be of influence on gonadal function, independent of treatment factors in both male and female CCS.

In female CCS, accelerated loss of primordial follicles as a result of gonadal damage may lead to premature ovarian failure. Ovarian reserve is determined by the measurement of anthral follicle count (AFC) during intra-vaginal ultrasonography. Anti-Müllerian hormone (AMH) is a sensitive marker for ovarian reserve[206](p155), [301](p165) and is stable during and in between menstrual cycles. In the general population, AMH correlates well with AFC, as it reflects gonadal reserve[302](p165). Consequently, low AMH levels might be predictive for depletion of the primordial follicle pool in CCS. Being obese and insulin resistant were independently associated with low AMH levels, whereas only insulin resistance was associated with low total follicle count, which was measured in a subgroup of CCS. The mechanism behind this association however is not completely clear. One hypothesis is that removal of functional ovaries and subsequent estradiol deficiency leads to obesity and insulin resistance as was observed in animal models[223, 224](p157). Another, more profound explanation is that adiposity and insulin resistance affect granulosa cell function, thereby reducing ovarian function[212, 213](p156), [226](p157). A remarkable finding was that in CCS a weak correlation between AMH and AFC was observed in contrast to that in the general population, suggesting a reduced quality rather than quantity of the follicles. The use of AMH as sensitive marker for predicting AFC in CCS should be reconsidered in larger CCS cohorts.

Gonadal function in men is determined by testosterone production in the Leydig cells, and by semen quality, as produced by the Sertoli cells. A reliable first screening marker which corresponds well with sperm quality is inhibin B, which is also produced by the Sertoli cells[237](p158). Whereas Sertoli cell function is vulnerable to gonadotoxic treatment, Leydig cells are relatively resistant to this treatment, which explains findings of previous studies that reported normal testosterone levels while sperm quality and inhibin B were impaired after treatment with alkylating agents[83](p142), [300](p165). In our male CCS, we found an independent association between obesity, defined by high total fat percentage, and gonadal failure represented by low levels of inhibin B and testosterone. Large epidemiological studies in the general population have described a profound negative effect of obesity on testicular production of testosterone, which is hypothesized to be a result of high aromatase activity as expressed in white adipose tissue which converts testosterone into estradiol[232, 233](p158). The relation with inhibin B is not so frequently described although obese men are known to have reduced sperm quality.

As is observed in the general population, we found an independent association between gonadal dysfunction and obesity and we hypothesized that obesity negatively affects gonadal function, independent of treatment effects. These findings emphasize the severity of obesity-related impaired health status of the growing CCS population.

10.4 Perspectives

The aim of this thesis was to improve the understanding of factors contributing to and evolving from obesity in CCS in order to design intervention and prevention strategies. Both physical activity and dietary intake are important targets for intervention treatments, since obesity and cardiovascular morbidity will be reduced or prevented[303](p165), leading to improved quality of survival and reduced morbidity-related costs. Current literature on health intervention in CCS is scarce, but shows significant improvements on knowledge and self-efficacy, quality of life and metabolic parameters, including BMI, waist circumference and insulin resistance, even on short term[304-306](p165).

In the near future, a large nationwide study will start in 7000 long-term survivors in the Netherlands, the so-called Dutch Childhood Oncology Group (DCOG) - LATER

Q2008. In this study the prevalence and risk factors of long-term treatment-related complications will be assessed, including obesity, metabolic syndrome, endocrine late effects, secondary malignancies, cardiotoxicity, infertility, neurocognitive and psychosocial late effects. The fact that participants will be actively recruited makes this study unique compared to many other large nationwide studies, using questionnaires to assess late effects.

10.5 Conclusions

The main findings of this thesis are summarized in Figure 10.3.

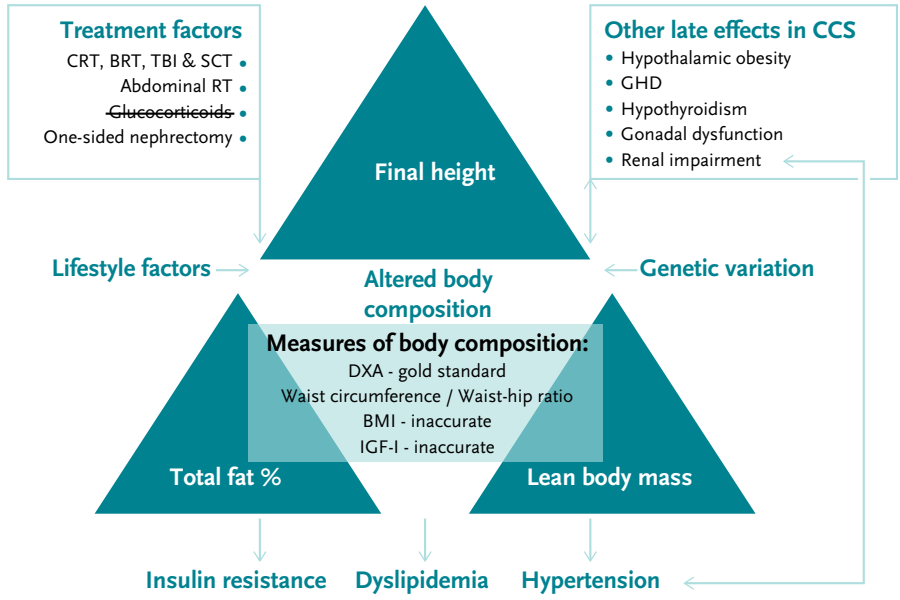


Figure 10.3 Determinants and sequelae of altered body composition in childhood cancer survivors.

The current cut-off limit for BMI, the most generally used obesity measure, was inaccurate since it underestimated obesity in more than half of CCS. Waist-hip ratio was more reliable since it represents the abdominal distribution of fat, which is strongly and independently associated with obesity-related morbidity. Insulin-like growth factor-I, which in the normal population is highly correlated with visceral fat mass, did not show to be of additive value in the prediction of altered body composition in CCS.

The detrimental changes of body composition that were found in all CCS, but more dramatically after treatment with CRT, brain tumour irradiation and TBI, included a significant loss of height, increase of total fat mass and reduced lean body mass, while especially after high dose CRT treatment visceral fat percentage was increased. Endocrine factors, represented by hypothalamic obesity, GHD, hypothyroidism and gonadal dysfunction, as well as non-endocrine factors, including irradiation-induced damage to the musculoskeletal system and renal impairment, may contribute to these alterations. We observed no effect of prior corticosteroids treatment at very long-term follow-up. Wilms tumour survivors treated with nephrectomy had significantly taller stature than all other survivors and was comparable to that of the general population. Lifestyle, including dietary behaviour and physical activity, as well as genetic variation play a substantial role in obesity development, but was not evaluated in this thesis.

Gonadal dysfunction and obesity are major complications after treatment, affecting general health. In the normal population gonadal function is affected by obesity. In CCS obesity and insulin resistance appeared to be independently associated with gonadal dysfunction, irrespective of treatment effect.

CCS are at increased risk for impaired general health related to obesity, i.e. hypertension, dyslipidemia and insulin resistance, which increases the risk of cardiovascular morbidity. Future studies should focus on the effects of intervention and treatment strategies in CCS, particularly in high-risk groups, described in this thesis.

11

CHAPTER

Summary & Samenvatting

11.1 Summary

In the Netherlands, approximately 600 children are newly diagnosed with cancer every year. Due to improvement of treatment protocols, survival has increased substantially over the last decades, leading to a rapidly growing cohort of childhood cancer survivors (CCS). As a result, treatment-related long-term complications have become increasingly important. Obesity is one of the major complications contributing to increased morbidity and mortality in the general population. The higher prevalence of obesity among CCS suggests an important role of former diagnosis and treatment factors. The aim of this thesis was to evaluate the value of current obesity markers in adult CCS and to study both determinants and sequelae of altered body composition in this group.

Obesity measures in childhood cancer survivors

Body mass index is the most generally used instrument for the definition of obesity. However, it is inaccurate since it does not distinguish between lean mass and fat mass. Recent literature shows that in the general population, obesity as measured by BMI is underestimated in 30% of subjects, as compared to total fat percentage measured by dual energy X-ray absorptiometry (DXA). By the same comparison as described in **Chapter 2**, we found an even more dramatic underestimation of obesity in CCS of 52%. Waist-hip ratio appeared to be a more reliable marker, but it should be taken into account that this is not useful in a large subgroup of survivors that was previously treated with abdominal irradiation. Since BMI is only a crude marker for true body composition and waist-hip ratio is not useful in a large subgroup of CCS, the aim of the study described in **Chapter 3** was to investigate the value of IGF-I as surrogate marker for alterations in body composition in adult CCS. Besides its use as marker for growth hormone secretory status, insulin-like growth factor-I (IGF-I) is highly related to body composition, particularly visceral fat percentage. Previously administered cranial radiotherapy (CRT) and brain tumour irradiation were associated with increased total fat mass, whereas only in CRT survivors visceral fat percentage was significantly higher than non-irradiated survivors. Treatment with total body irradiation (TBI) was associated with underweight, illustrated by a substantial loss of lean body mass. Nevertheless, IGF-I appeared to be of limited value as screening marker for altered body composition since the associations with body composition measures were significant but weak.

Determinants of altered body composition in childhood cancer survivors

Survivors at risk for alterations in body composition and endocrine dysfunction are those treated with TBI as conditioning regimen for stem cell transplantation (SCT) diagnosed with (recurrent) acute lymphoblastic leukemia (ALL) or acute myeloid

leukemia (AML). Currently, intensive chemotherapy has become the standard treatment for AML and has replaced SCT in most of the treatment protocols. Therefore, endocrine functions and components of the metabolic syndrome were evaluated in survivors of myeloid leukaemias treated with chemotherapy only and compared to those treated with TBI and SCT and to controls (**Chapter 4**). Except for low markers of gonadal reserve, no other endocrine or metabolic disorders were found in survivors treated with chemotherapy only, in contrast to TBI survivors in whom gonadal failure and primary hypothyroidism as well as high waist-hip ratio and increased levels of insulin and triglycerides were observed.

A hormone which plays an important role in energy homeostasis and glucose metabolism is ghrelin, the so-called 'hunger hormone'. It is released in the stomach in fasting conditions, while in conditions of a positive energy balance like hyperglycaemia and obesity, ghrelin levels are low. The unacylated form, desacyl ghrelin (DAG), is associated with improved metabolic state, represented by increased insulin sensitivity and reduced fat mass. In order to give more insight into the physiology of desacyl ghrelin (DAG) in lean and obese subjects, we performed a pilot study among CCS undergoing an insulin tolerance test (**Chapter 5**). We observed that DAG was significantly higher in lean subjects and rapidly decreased after insulin infusion, while obese subjects seemed to be relatively DAG resistant since no DAG change was observed. The positive interaction between DAG and insulin metabolism may be of use for future treatment strategies for obesity and other insulin resistance diseases.

An important treatment-related effect which is not of endocrine origin, but substantially affects height attainment and metabolic health is renal impairment, which in CCS is a result of nephrotoxic treatment. Information concerning treatment-related nephrotoxicity in very long-term survivors is limited. In a large cohort, we defined risk factors for renal impairment 18 years after treatment (**Chapter 7**). We found that glomerular impairment was associated with former treatment with one-sided nephrectomy (whether or not combined with abdominal irradiation) and high cumulative dosages of cisplatin and ifosfamide. Albuminuria was related to combination treatment of nephrectomy and abdominal radiotherapy. Persisting tubular damage was particularly exhibited in survivors with hypertension and those treated with high dosages of ifosfamide. Treatment with cyclophosphamide and metothrexate was not related to long-term nephrotoxicity. Additionally, we found that particularly renal tumour survivors were at increased risk for hypertension, which is often a disguised symptom, emphasizing the need for close follow-up.

One-sided nephrectomy, as part of standard treatment in Wilms tumour patients, is followed by increased IGF-I levels, which are associated with linear growth during childhood. We hypothesized that Wilms tumour survivors show better catch up growth and attainment of final height due to increased IGF-I levels after nephrectomy. In the study described in **Chapter 6**, final height and IGF-I levels of Wilms tumour survivors were compared with data of Dutch references and other survivors. Wilms tumour survivors had significantly taller stature than non-irradiated survivors of other cancer types. Both height and IGF-I levels were comparable to that of the general population, in contrast to all other survivors who had significantly lower height and IGF-I levels than Dutch references.

Obesity and Gonadal reserve in childhood cancer survivors

Obesity and gonadal dysfunction are both major complications of childhood cancer treatment. Particularly survivors treated with TBI, abdominal irradiation and alkylating agents are at substantial risk of gonadal dysfunction. In the general population, obesity has a negative influence on female fertility. We observed that, independent of gonadotoxic treatment, also in female CCS, obesity and insulin resistance were associated with impaired ovarian reserve, as reflected by decreased levels of anti-Müllerian hormone (AMH) and reduced follicle count. A remarkable finding described in **Chapter 8** was that in contrast to its highly predictive value for antral follicle count (AFC) in the healthy female population, serum AMH did not seem to correlate as well with AFC in CCS. This may suggest that AMH reflects follicle quality, rather than quantity. In healthy men, obesity has a negative influence on spermatogenesis and reproductive hormone levels. In CCS, both obesity and gonadal dysfunction are treatment-related complications and have a high impact on general health. In male CCS, we found that, independent of gonadotoxic treatment, obesity, as represented by high total fat percentage, was associated with low levels of testosterone and inhibin B, as marker for spermatogenesis (**Chapter 9**).

In **Chapter 10** the findings of the thesis are summarized and discussed in the context of the current literature. Firstly, the use of current obesity measures in CCS is questioned. Secondly, determinants of altered body composition are described, including treatment factors which directly, or indirectly via other treatment-related disorders, affect final height, total fat mass and lean body mass. Furthermore, the negative influence of obesity on gonadal function in CCS is discussed. Lastly, the need for future studies is emphasized. The DCOG-LATER Q2008 is a large nationwide study which will start in the near future, evaluating late effects among 7000 long-term survivors. The chapter ends with general conclusions.

11.2 Samenvatting

In Nederland worden er ongeveer 600 kinderen per jaar gediagnosticeerd met kanker. Dankzij de verbeterde behandelingsprotocollen zijn de overlevingscijfers snel gestegen. Op dit moment zijn er in Nederland ongeveer 7000 overlevenden van kinderkanker. Het vroeg herkennen en behandelen van late effecten als gevolg van de behandeling is daarom van groot belang. Obesitas is één van de late effecten waarvan bekend is dat het in de normale populatie bijdraagt aan verhoogde morbiditeit en mortaliteit. Het feit dat de prevalentie van obesitas hoger is onder overlevenden van kinderkanker, laat zien dat vroegere diagnose en behandeling hierin een belangrijke rol spelen. Het doel van dit proefschrift was om determinanten en gevolgen te beschrijven van obesitas en veranderde lichaamssamenstelling, met als doel risicogroepen te definiëren waarop toekomstige interventies en behandelingsstrategieën gericht dienen te worden.

Meetinstrumenten voor obesitas in overlevenden van kinderkanker

Body mass index (BMI) is de meest gebruikte maat voor obesitas, maar is onbetrouwbaar omdat er geen onderscheid wordt gemaakt tussen spiermassa en vetmassa. In de normale populatie werd obesitas, gemeten met BMI, onderschat in 30% van de mensen. Hierbij werd de afkapwaarde van BMI vergeleken met die van de totale vetmassa, gemeten door middel van een dual energy X-ray absorptiometry (DXA) scan. In de studie beschreven in **hoofdstuk 2** toonden we op dezelfde wijze aan dat in overlevenden van kinderkanker deze onderschatting nog groter is, namelijk 52%. Taille-heup ratio bleek een betere maat te zijn, maar is niet toepasbaar in een grote groep overlevenden die met abdominale bestraling is behandeld. Omdat het onhaalbaar is om in iedere overlevende een DXA scan uit te voeren, onderzochten we in **hoofdstuk 3** de waarde van insuline-achtige groeifactor-I (IGF-I) als surrogaatmarker voor veranderingen in de lichaamssamenstelling in volwassen overlevenden van kinderkanker. Naast het gebruik als marker voor groeihormoon afgifte, is IGF-I namelijk sterk gerelateerd aan lichaamssamenstelling, in het bijzonder visceraal of intra-abdominaal vetpercentage. Vergeleken met niet bestraalde overlevenden, hadden overlevenden behandeld met craniale bestraling (CRT) en lokale bestraling voor een hersentumor significant lagere IGF-I waardes en meer totale vetmassa, terwijl alleen CRT overlevenden een hoger visceraal vetpercentage hadden. Behandeling met totale lichaamsbestraling (TBI) werd geassocieerd met ondergewicht, te wijten aan een aanzienlijk verlies van spiermassa. IGF-I bleek van beperkte waarde als marker voor veranderde lichaamssamenstelling, omdat de correlaties met totaal vetpercentage en spiermassa zwak waren.

Determinanten van veranderde lichaamssamenstelling in overlevenden van kinderkanker

Overlevenden die een verhoogd risico hebben op veranderingen in lichaamssamenstelling en endocriene afwijkingen zijn zij die behandeld zijn met TBI en stamceltransplantatie (SCT) hetgeen een behandeling is voor met name acute myeloide leukemie (AML). Tegenwoordig is deze zware behandeling vervangen door intensieve chemotherapie in de meeste behandelingsprotocollen. Daarom hebben we in **hoofdstuk 4** endocriene functies en componenten van het metabool syndroom bestudeerd in overlevenden van myeloïde leukemie, die alleen behandeld zijn met chemotherapie, en vergeleken met patiënten die behandeld zijn met totale lichaamsbestraling in combinatie met stamceltransplantatie en met gezonde controlepersonen. Behalve verlaagde vruchtbaarheidsmarkers (anti-müllerian hormoon in vrouwen en inhibine B in mannen) werden geen endocriene of metabole stoornissen gevonden in deze groep, in tegenstelling tot de met TBI behandelde groep, in wie gonadale dysfunctie, primaire hypothyreoïdie, een verhoogde taille-heupratio en significant hogere spiegels van insuline en triglyceriden dan gezonde controlepersonen werden gevonden.

Een hormoon dat een belangrijke rol speelt in glucose homeostase en energie metabolisme is het zogenaamde 'honger hormoon' ofwel ghreline. Het komt in geacyleerde en ongeacyleerde vorm voor in de circulatie en met name de ongeacyleerde vorm, genaamd desacyl ghrelin (DAG) lijkt een positief effect te hebben op insulinesensitiviteit en het metabool systeem, terwijl de geacyleerde vorm, genaamd acylated ghrelin (AG) juist geassocieerd is met insulineresistentie. Om meer inzicht te geven in de fysiologie van de ongeacyleerde vorm van ghreline in verschillende metabole condities voerden wij een pilot studie uit in overlevenden van kinderkanker die een insulinetolerantie test ondergingen (**hoofdstuk 5**). We vonden dat DAG significant hoger was in overlevenden met een normaal gewicht en dat DAG sneller afnam in respons op de insuline-infusie, terwijl in de overlevenden met obesitas de DAG concentratie nagenoeg niet veranderde. De positieve relatie tussen DAG en insuline metabolisme kan van nut kan zijn voor de toekomstige behandelingsstrategieën voor obesitas en andere insulineresistentie ziekten.

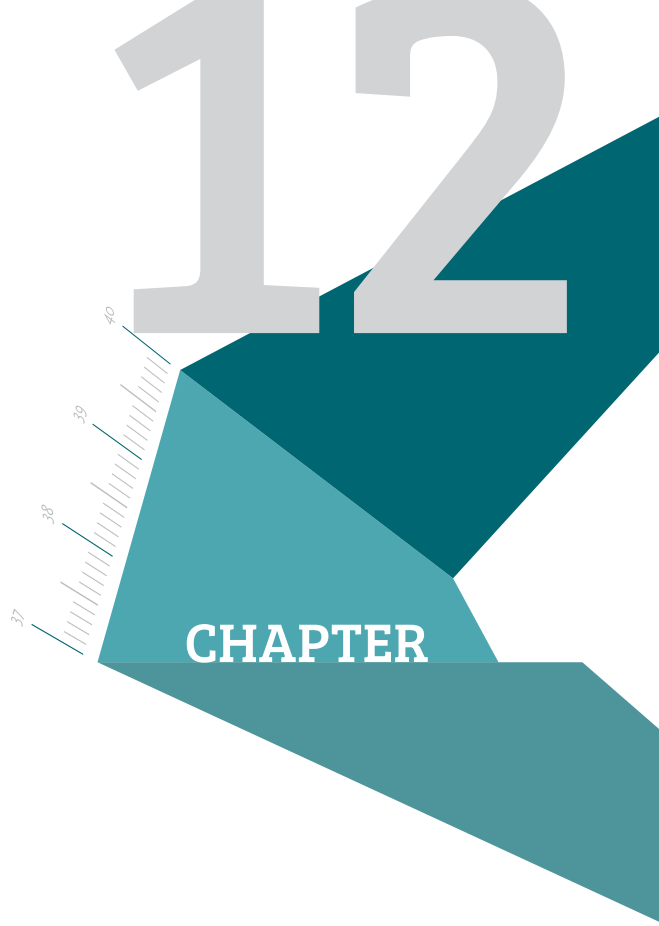
Een belangrijke lange termijn-complicatie die niet van endocriene aard is maar wel een belangrijke invloed heeft op groei en componenten van het metabool syndroom is de nierfunctie. Informatie met betrekking tot de prevalentie en risicofactoren van nierschade ten gevolge van de vroegere behandeling is beperkt. In **hoofdstuk 7** wordt een studie beschreven waarin de risicofactoren voor nierschade 18 jaar na therapie zijn onderzocht in overlevenden van kinderkanker. We vonden dat zij die behandeld zijn met eenzijdige nefrectomie, al dan niet in combinatie met abdominale bestraling en hoge doseringen cisplatine en ifosfamide, een verhoogd risico hebben op het ontwikkelen van nierschade. Vroegere behandeling met cyclofosfamide en methotrexaat was niet gerelateerd aan nierschade op lange termijn. Daarnaast vonden we dat voornamelijk overlevenden van een niertumor een verhoogd risico hadden op hypertensie.

Eenzijdige nefrectomie, als onderdeel van de standaard behandeling van Wilms tumor, is geassocieerd met een stijgende IGF-I concentratie. In kinderen is het IGF-I verantwoordelijk voor lineaire groei. In **hoofdstuk 6** hebben we de IGF-I concentratie en eindlengte beschreven van Wilms tumor overlevenden en vergeleken met data van overlevenden van andere kankersoorten en met die van de Nederlandse bevolking. Wilms tumor overlevenden bereikten een significant langere eindlengte dan een vergelijkbare groep overlevenden van andere kankersoorten die niet bestraald waren. Eindlengte en IGF-I waren vergelijkbaar met die van de Nederlandse bevolking, in tegenstelling tot alle andere overlevenden, van wie de eindlengte en het IGF-I significant lager waren dan gezonde Nederlanders. Onze hypothese is dat Wilms overlevenden een betere inhaalgroei vertonen dankzij verhoogde IGF-I spiegels na nefrectomie, waardoor ze uiteindelijk eindigen met een hogere eindlengte, die vergelijkbaar is met die van gezonde Nederlanders.

Obesitas en gonadale reserve in overlevenden van kinderkanker

Obesitas en verminderde vruchtbaarheid zijn beide belangrijke complicaties van de vroegere behandeling en hebben een negatieve invloed op de kwaliteit van leven. Verminderde vruchtbaarheid komt voornamelijk voor bij overlevenden die behandeld zijn met totale lichaamsbestraling, abdominale bestraling en alkylerende middelen. In de algemene bevolking heeft obesitas een negatieve invloed op de vruchtbaarheid. In de studie beschreven in **hoofdstuk 8** vonden we dat onafhankelijk van de schadelijke effecten van vroegere behandeling, obesitas en insulineresistentie geassocieerd waren met lagere AMH-spiegels en verminderde ovariële reserve. Een opvallende bevinding was dat AMH, wat in de normale bevolking een betrouwbare marker is voor eicelvoorraad, in overlevenden van kinderkanker niet goed correleerde met het aantal follikels in de antrale fase. In mannelijke overlevenden van kinderkanker, vonden we dat, onafhankelijk van gonadotoxische behandeling, obesitas was geassocieerd met een laag testosteron en laag inhibine B, als marker voor spermatogenese (**hoofdstuk 9**).

In de discussie (**hoofdstuk 10**) worden de bevindingen van dit proefschrift samengevat en besproken in de context van de literatuur. Allereerst wordt het gebruik van de huidige obesitasmaten in overlevenden van kinderkanker bediscussieerd. Vervolgens worden de determinanten van veranderde lichaamssamenstelling beschreven, voornamelijk de verschillende soorten behandeling die direct of indirect via andere behandelingsgerelateerde aandoeningen invloed uitoefenen op de eindlengte, totale vetmassa en spiermassa. Daarnaast wordt de mogelijke invloed van veranderde lichaamssamenstelling op gonadaal falen in de normale populatie en in overlevenden besproken. Ten slotte wordt de noodzaak voor toekomstige interventiestudies benadrukt. Het hoofdstuk eindigt met de conclusies van dit proefschrift.



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13

CHAPTER

About the author

13.1 Curriculum vitae

Karin Blijdorp werd geboren op 8 januari 1984 in Leiden. Na het behalen van haar gymnasiumdiploma aan de Gereformeerde Scholengemeenschap Rotterdam in 2002, startte zij in hetzelfde jaar met de studie Geneeskunde aan de Erasmus Universiteit in Rotterdam, na toelating via de decentrale selectie. In 2007 studeerde ze af op het effect van hemofiltratie bij neonaten aan extracorporele membraanoxygenatie (ECMO) op de intensive care afdeling van het Sophia kinderziekenhuis, onder begeleiding van prof.dr Cransberg en prof.dr. Tibboel. Haar laatste 8-weekse coschap liep ze op de kinderafdeling in het Diakonessenhuis in Paramaribo, Suriname. Naast haar studie heeft ze een jaar lang plaatsgenomen in het dagelijks bestuur van het Nederlands Studenten Orkest (NSO) als penningmeester. Samen met 6 medestudenten organiseerde ze een orkesttournee in binnen- en buitenland. Nadat ze in juni 2009 haar artsexamen behaalde, werkte ze als arts-assistent op de kinderafdeling in het Maasstad ziekenhuis in Rotterdam, waar ze een jaar eerder met succes haar oudste coschap had afgerond. In januari 2010 begon ze onder begeleiding van prof. dr. A.J. van der Lelij, dr. S.J.C.M.M. Neggers en dr. M.M. van den Heuvel-Eibrink met haar PhD-project, waarvan het onderzoek beschreven in dit boek het resultaat is. Tijdens haar PhD-project volgde ze een research master bij de Netherlands Institute for Health Sciences (NIHES) en in augustus 2012 ontving ze haar master of science in Clinical Epidemiology. In januari 2014 zal ze starten met de opleiding tot internist-endocrinoloog in het Erasmus Medisch Centrum Rotterdam.

13.2 List of publications

1. **Blijdorp K**, Cransberg K, Wildschut ED, Gischler SJ, Houmes R, Wolff ED, Tibboel D. Haemofiltration in newborns treated with extracorporeal membrane oxygenation: a case-comparison study. *Crit Care. 2009; 13(2):R48.*

2. **Blijdorp K**, van den Heuvel-Eibrink MM, Pieters R, Boot AM, Sluimer JP, van der Lely AJ, Neggers SJ. The limited screening value of insulin-like growth factor-I as a marker for alterations in body composition in very long-term adult survivors of childhood cancer. *Pediatr Blood Cancer. 2012; 59(4):711-6.*

3. **Blijdorp K**, van den Heuvel-Eibrink MM, Pieters R, Boot AM, Delhanty PJ, van der Lely AJ, Neggers SJ. Obesity is underestimated using body mass index and waist-hip ratio in long-term adult survivors of childhood cancer. *PLoS One. 2012; 7(8):e43269.*

4. Van Waas M, Neggers SJ, Uitterlinden AG, **Blijdorp K**, van der Geest IM, Pieters R, van den Heuvel-Eibrink MM. Treatment factors rather than genetic variation determine metabolic syndrome in childhood cancer survivors. *Eur J Cancer. 2013; 49(3):668-75.*

5. **Blijdorp K**, van Waas M, van der Lely AJ, Pieters R, van den Heuvel-Eibrink MM, Neggers SJ. Endocrine sequelae and metabolic syndrome in adult long-term survivors of childhood acute myeloid leukaemia. *Leuk Res. 2013; 37(4):367-71.*

6. **Blijdorp K**, Dekkers IA, Cransberg K, Pluijm SM, Pieters R, Neggers SJ, van den Heuvel-Eibrink MM. Long-term nephrotoxicity in adult survivors of childhood cancer. *Clin J Am Soc Nephr. 2013; 8(6): 922-29.*

7. **Blijdorp K**, van Dorp W, Laven JS, Pieters R, Visser JA, van der Lely AJ, Neggers SJ, van den Heuvel-Eibrink MM. Decreased ovarian function is associated with obesity in very long-term female survivors of childhood cancer. *Eur J Endocrinol. 2013; 168(6):905-12.*

8. **Blijdorp K**, van der Lely AJ, van den Heuvel-Eibrink MM, Huisman TM, Themmen AP, Delhanty PJ, Neggers SJ. Desacyl ghrelin is influenced by changes in insulin concentration during an insulin tolerance test. *Growth Horm IGF Res 2013; in press.*

9. **Blijdorp K**, van Dorp W, Laven JS, Pieters R, de Jong FH, Pluijm SM, van der Lely AJ, van den Heuvel-Eibrink MM, Neggers SJ. Gonadal function is associated with obesity in very long-term male survivors of childhood cancer. *Submitted*

10. **Blijdorp K**, van den Heuvel-Eibrink MM, Pieters R, Pluijm SM, Wagner A, Segers H, van der Lely AJ, Neggers SJ. Final height and insulin-like growth factor-I in adult survivors of Wilms tumour. *Submitted*

11. Khajeh L, **Blijdorp K**, Ribbers GM, Dippel DW, van Kooten F, Neggers SJ. Hypopituitarism after subarachnoid hemorrhage (SAH), an update. *Submitted*

12. **Blijdorp K**, Khajeh L, van Kooten F, Sneekes E, Heijenbrok M, van den Berg-Emons HJ, van der Lely AJ, Ribbers GM, Neggers SJ. Diagnostic value of a ghrelin test for the diagnosis of growth hormone deficiency after subarachnoid hemorrhage. *Submitted*

13. Dekkers IA, **Blijdorp K**, Pieters R, Cransberg K, Neggers SJ, van den Heuvel-Eibrink MM. A patient with chronic renal insufficiency due to childhood cancer treatment. *Submitted*

13.3 PhD Portfolio

Name PhD student:	Karin Blijdorp
Erasmus MC Departments:	Medicine - section Endocrinology & Paediatric Oncology/ Haematology
Research School:	Netherlands Institute of Health Sciences - Clinical Epidemiology
PhD period:	January 2010 - June 2013
Promotor:	prof.dr. A.J. van der Lelij
Supervisors:	dr. S.J.C.M.M. Neggers & dr. M.M. van den Heuvel-Eibrink

General academic courses

2010	BROK ('Basiscursus Regelgeving Klinisch Onderzoek')
2010	Minicourse Methodology of Clinical Research and preparation of grant applications
2012	Research Integrity
2012	English Biomedical Writing and Communication

Research skills

2010-2012	Master of Science Clinical Epidemiology NIHES Including elective/in depth courses: • Cancer Epidemiology • Analysis of Growth Data • Quality of Life Measurement
2010-2013	Weekly research meeting Quality of Life and Toxicity of Care Working Group

(Inter)national conferences - oral presentations

The limited screening value of insulin-like growth factor-I as a marker for alterations in body composition in very long-term adult survivors of childhood cancer

2011	European Symposium on Late Complications after Childhood Cancer (ESLCCC), Amsterdam
2011	53rd ASH Annual Meeting, San Diego, USA

Obesity is underestimated using body mass index and waist-hip ratio in long-term adult survivors of childhood cancer

2012	Science days, Antwerp, Belgium
2012	12th International conference on long term complications of treatment of children and adolescents for cancer, Williamsburg, USA

Desacyl ghrelin is influenced by changes in insulin concentration during an insulin tolerance test

2013	Dutch Endocrine Meeting, Noordwijkerhout

Long-term nephrotoxicity in adult survivors of childhood cancer

2013	13th International conference on long term complications of treatment of children and adolescents for cancer, Memphis, USA

Decreased ovarian function is associated with obesity in adult female survivors of childhood cancer

2013	13th International conference on long term complications of treatment of children and adolescents for cancer, Memphis, USA

(Inter)national conferences - poster presentations

The limited screening value of insulin-like growth factor-I as a marker for alterations in body composition. In very long-term adult survivors of childhood cancer

2012	14th European Congress of Endocrinology, Florence, Italy
2012	The endocrine Society's 94th Annual meeting & Expo, Houston, USA

Obesity is underestimated using body mass index and waist-hip ratio in long-term adult survivors of childhood cancer

2012	14th European Congress of Endocrinology, Florence, Italy
2012	The endocrine Society's 94th Annual meeting & Expo, Houston, USA
2012	44th Congress of the International Society of Paediatric Oncology (SIOP), London, UK

Endocrine sequelae and metabolic syndrome in adult long-term survivors of childhood acute myeloid leukaemia

2012	54th ASH Annual Meeting, Atlanta, USA

Desacyl ghrelin is influenced by changes in insulin concentration during an insulin tolerance test

2013	Science days, Antwerp, Belgium

Decreased ovarian function is associated with obesity in adult female survivors of childhood cancer

2013	15th European Congress of Endocrinology, Copenhagen, Denmark
2013	The endocrine Society's 95th Annual meeting & Expo, San Francisco, USA

Gonadal function is associated with body composition in adult male survivors of childhood cancer

2013 13th International conference on long term complications of treatment of children and adolescents for cancer, Memphis, USA
2013 The endocrine Society's 95th Annual meeting & Expo, San Francisco, USA

Final height and insulin-like growth factor-I in adult survivors of Wilms tumour

2013 13th International conference on long term complications of treatment of children and adolescents for cancer, Memphis, USA
2013 45th Congress of the International Society of Paediatric Oncology, Hong Kong, China

Symposia, seminars & workshops

2010 2nd National DCOG LATER Conference 'Guidelines and late effects research for childhood cancer survivors', Rotterdam
2010 Paediatric Oncology Symposium *"Who Cares"*, Rotterdam
2010-2013 Bi-annual Regional Endocrinology Meeting, Rotterdam
2010, 2012 36th and 38th Erasmus Endocrinology Course, Noordwijkerhout
2010-2011 Annual Erasmus MC Course in Clinical Neuro-Endocrinology, Rotterdam

Teaching activities

2011 Teaching data managers SKION LATER , Utrecht
2011 Erasmus MC Course in Clinical Neuro-Endocrinology, Rotterdam
2011-2012 Supervising Master's theses:
Mw. D. van Klaveren
Mw. I.A. Dekkers

13.4 Dankwoord

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