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Adrenal function in adult long-term survivors of nephroblastoma and neuroblastoma

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KEYWORDS

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Late effects
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Adrenal function

Abstract Background: Adrenal insufficiency, or relative insufficiency, might partly explain increased mortality rates in nephroblastoma and neuroblastoma survivors after unilateral adrenalectomy.

Objective: To assess adrenal function and its metabolic effects in survivors after adrenalectomy.

Methods: In this cross-sectional study, 67 adult long-term survivors of nephroblastoma, 36 survivors of neuroblastoma and 49 control subjects participated. Adrenal function was assessed by a 1 µg short Synacthen-test. Levels of cortisol, adrenocorticotropic hormone (ACTH), low (LDL-C) and high-density lipoprotein-cholesterol (HDL-C), triglycerides, apolipoprotein-B, glucose and insulin were assessed in blood samples taken at baseline. In addition, cortisol levels were assessed after 30 ($t = 30$) and 60 min. Homoeostatic Model Assessment (HOMA) was calculated.

Results: Adrenal insufficiency was not present in survivors. Interestingly, baseline serum cortisol levels were higher in survivors after unilateral adrenalectomy (mean 503 nmol/l) ($N = 46$) than in survivors with both adrenals intact (mean 393 nmol/l, $P = 0.002$) ($N = 52$), and than in controls (mean 399 nmol/l, $P = 0.013$) ($N = 49$). After correcting for age, sex and use of oral oestrogens, unilateral adrenalectomy was independently associated with elevated baseline cortisol and ACTH levels. Baseline cortisol levels were positively associated with triglycerides ($P < 0.001$), LDL-C ($P = 0.004$), apolipoprotein-B ($P < 0.001$) and HOMA ($P = 0.008$).

Conclusions: No adrenal insufficiency was observed in survivors of nephroblastoma and neuroblastoma. Survivors treated with unilateral adrenalectomy had relatively high basal cortisol and ACTH levels, indicating a higher central setpoint of the hypothalamic-pituitary-adrenal

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axis. This higher setpoint was associated with lipid concentrations and insulin resistance and can therefore influence the cardiovascular risk profile in long-term survivors of nephroblastoma and neuroblastoma.

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1. Introduction

Over the past decades, childhood cancer survival rates have increased significantly. Approximately 75% of children diagnosed with cancer are cured and become long-term survivors. For children diagnosed with nephroblastoma or neuroblastoma 5-year survival is approximately 84% and 64%, respectively. Increased long-term survival has led to increasing absolute numbers of survivors: to date one in 640 young adults is a childhood cancer survivor.¹ Therefore the recognition of late effects has become more relevant. In nephroblastoma and neuroblastoma survivors, especially those treated with radiotherapy, the risk for developing late adverse events like second malignant neoplasms and pulmonary, orthopaedic and cardiovascular events, musculoskeletal late effects, cardiac toxicity, reproductive health problems and renal dysfunction is significant.^{2–4} Due to poor survival, reports on late effects in intensively treated long-term neuroblastoma survivors are mainly based on case series and small cohorts.

Although endocrine sequelae are the most common late effects,^{5–15} little is known about the long-term endocrine effects in survivors of nephroblastoma or neuroblastoma. Increased mortality rates as compared to the general age-matched population have been reported in childhood cancer survivors, which are partly related to the above-mentioned documented late effects.¹⁶ However unexpected deaths occur and to date it is unknown whether adrenal insufficiency following surgery, radiotherapy and/or chemotherapy for nephroblastoma or neuroblastoma is an important denominator of this excess mortality rate later in life. Abdominal surgery is part of the treatment for nephroblastoma and neuroblastoma. In the majority of nephroblastoma patients, unilateral total nephrectomy, often including unilateral adrenalectomy, is performed. In neuroblastoma patients, adrenalectomy is performed when the tumour is located in the adrenal gland. Bilateral adrenalectomy is associated with high morbidity and mortality rates as a consequence of adrenal insufficiency.^{17,18} It is unknown whether adrenal function after unilateral adrenalectomy declines over time by ageing, especially in survivors who were also treated with chemotherapy or local radiotherapy. We hypothesised that a subset of neuroblastoma and nephroblastoma survivors has a subnormal adrenal function and therefore performed a study on the influence of administered treatment on adrenal function in long term nephroblastoma and neuroblastoma survivors. Additionally, to

evaluate the possible altered effect of the hypothalamic-pituitary-adrenal (HPA) axis on the metabolic status in these survivors, we assessed the influence of cortisol on lipids and insulin resistance parameters.

2. Methods

2.1. Patients

All long-term (≥ 5 years after cessation of treatment) adult survivors of childhood nephroblastoma and neuroblastoma, treated between 1961 and 2004 in the Erasmus MC-Sophia Children's Hospital are followed prospectively at the Late Effects Registration outpatient clinic. They were invited to participate in this cross-sectional study. Informed consent was obtained according to the Helsinki declaration¹⁹ and the study was approved by the local medical ethical committee.

Of the 88 adult long-term survivors of nephroblastoma who were alive and currently living in The Netherlands, 67 participated, six were lost to follow-up, 13 refused to participate and two survivors were not able to visit the outpatient clinic at the appointed time interval. Adult survivors with neuroblastoma stage 4s who did not receive surgery, radiotherapy or chemotherapy were excluded from this study. Of the 50 eligible neuroblastoma survivors, 36 survivors participated, five were lost to follow-up, six refused to participate and three females were pregnant at the time of the study. Information on disease and treatment was obtained from our local database and medical records. Data regarding (partial) unilateral adrenalectomy were obtained from pathology reports. As to date limited normal values for young adults are available, we recruited a control group, consisting of siblings, friends or neighbours, preferably of the same sex and within an age range of 5 years of their related survivor. This group was designed as a socio-demographically similar comparison population. Information regarding smoking status and socioeconomic status (defined by the highest level of educational attainment) was collected using a questionnaire. Height was measured to the nearest millimetre using a Harpenden Stadiometer and weight was measured without shoes and clothes but in underwear to the nearest 0.1 kg with a standard clinical balance. Body mass index (BMI) was calculated as weight in kilograms divided by the squared length in metres. Survivors and controls participated in this study from October 2009 until March 2011.

2.2. ACTH stimulation test

To evaluate adrenal function, a low dose (1 µg) short ACTH stimulation-test was performed both in survivors and control subjects. ACTH stimulation-tests were started in the morning, all before 10:00 am. Subjects were resting during 1 h and during the test. Fasting blood samples were taken from one and the same intravenous-cannula at baseline, after 30 minutes ($t = 30$) and after 60 min ($t = 60$). One millilitre of Synacthen solution 250 µg/ml (Defiante Farmaceutica S.A., Portugal) was added to 249 ml of sterile solution of NaCl 0.9%. One millilitre of this solution, corresponding to 1 µg of Synacthen, was administered intravenously after the baseline blood sample was taken. Serum cortisol levels were measured at the baseline, $t = 30$ and $t = 60$. At $t = 30$, cortisol levels higher than 440 nmol/l were considered normal, adrenal insufficiency was defined as cortisol levels below 440 nmol/l at $t = 30$.²⁰ In addition, ACTH was measured in the sample taken at the baseline.

2.3. Laboratory measurements

Serum cortisol (nmol/l), adrenocorticotrophic hormone (ACTH) (pmol/l), androstenedione (adione) (nmol/l), dehydroepiandrosterone-sulphate (DHEAs) (µmol/l), insulin (p/mol/l), testosterone (nmol/l), dehydro-epiandrosterone (DHEA) (nmol/l), 17-hydroxyprogesterone (17OHP) (nmol/l), triglycerides, high-density lipoprotein-cholesterol (HDL-C), low-density lipoprotein-cholesterol (LDL-C), glucose and apolipoprotein-B (Apo-B) (g/l) were measured. Assays and intra- and inter coefficients of variation are described in [Supplementary text](#). Homoeostatic Model Assessment (HOMA) was calculated as a measure of insulin resistance.²¹

2.4. Statistics

Statistical analysis was performed with the Statistical Package for Social Sciences (SPSS 17.0, Chicago, IL, United States). Data are expressed as means and standard deviations, unless specified otherwise. Independent Sample t -tests were used to compare results in survivors and controls and in subgroups. The associations between cortisol levels and various treatment parameters and baseline characteristics were analysed using multiple linear regression modelling. Cortisol, ACTH, triglycerides and glucose levels were normally distributed after log-transformation. Beta's of logtransformed variables are expressed in percentages. Age and sex were added to all models and influence of BMI was evaluated as previously recommended.²² Oestrogens, as the component of hormonal contraception or as oral supplementals in oestrogen deficient women, are known to

increase serum levels of cortisol-binding globulin (CBG) resulting in higher total serum cortisol levels. Accordingly, serum cortisol concentrations do not correlate well with cortisol production rates unless the cortisol binding globulin concentration is accounted for.²³ Twenty-nine out of 73 females (9/25 controls, 20/48 survivors) in our study used oral oestrogens. Therefore, oral oestrogen use was added to the model. Furthermore, three subjects were using fluticasone (one survivor with both adrenals intact, one survivor who underwent adrenalectomy, one control subject) and one survivor who underwent adrenalectomy was using hydrocortisone cream. A subanalysis was performed excluding these survivors. In the first model we added a dummy variable for patients with intact adrenals and a dummy variable for patients who had undergone an adrenalectomy. In the second model, we added cumulative dose of anthracyclines, vincristine actinomycin, being treated with cyclophosphamide or not (dichotomous variable) and radiotherapy administered to the abdomen or to the contralateral adrenal gland. The variables smoking (smoker, non-smoker or former smoker) and socio-economic status (ordinal variable) were added to both models, but these factors were not significant. Additionally, multivariate analyses were performed to assess the influence of cortisol levels on metabolic parameters. Models were corrected for age, sex, socio-economic status, BMI, diagnosis, abdominal radiotherapy and cumulative dose of administered chemotherapy. P -values <0.05 (two-tailed) were considered statistically significant.

3. Results

3.1. Survivors and controls

Sixty-seven long-term adult nephroblastoma survivors (28 females) and 36 neuroblastoma survivors (21 females) participated in this study. Forty-eight percent of survivors had a sibling or friend willing to participate, resulting in 49 participating control subjects (23 females). Thirty-six of them were siblings and 13 were partners or friends of the survivors. The main reason for survivors not to bring a control-subject was the inability or unwillingness of siblings or neighbours to participate as they had to take a day off at work. Baseline and treatment characteristics and status of the adrenal glands are shown in [Table 1](#). In survivors who underwent adrenalectomy the tumour was located in or close to the adrenal gland, in survivors treated with partial adrenalectomy the tumour was located at the upper part of the kidney and in survivors treated without adrenalectomy the tumour was located at the lower part of the kidney. Synacthen stimulation-tests were performed in 93/103 survivors and in 48/49 controls and baseline cortisol levels were available in 101/103

Table 1
Baseline characteristics of study participants.

	Nephroblastoma		Neuroblastoma		Controls
<i>N</i>	67		36		49
Male/female	39/28		15/21		26/23
Age at follow-up (years)	30.2 (18.8–50.8)		29.6 (20.4–46.2)		32.7 (18.0–61.8)
Age at diagnosis (years)	3.3 (0.0–12.7)		0.8 (0.0–11.7)		n.a.
Follow-up time (years) ^a	26.2 (6.4–48.9)		27.8 (15.0–44.4)		n.a.
Stage					
1	32		6		n.a.
2	13		17		
3	11		8		
4	3		2		
5	1		0		
Unknown	7		3		
Surgery (<i>n</i>)	67		36		n.a.
Unilateral adrenalectomy	26		13		n.a.
Partial adrenalectomy	7		0		
No adrenalectomy	31		23		
Unknown	3		0		
Radiotherapy (<i>n</i>)	35		12		n.a.
Hemi-abdomen	20		0		n.a.
Abdomen	5		7		
Kidney	7		0		
Lung(s)	3		0		
Thorax	0		2		
Spine	0		1		
Neck	0		1		
Cumulative dose (Gy)	25 (15–40)		20 (10–30)		n.a.
Chemotherapy (<i>n</i>)	59		31		n.a.
	<i>N</i>	Cumulative dose	<i>N</i>	Cumulative dose	
Vincristin (mg/m ²)	51	22.0 (6.0–93.0)	16	22.8 (5.0–65.0)	n.a.
Actinomycin D (mg/m ²)	48	10.9 (0.1–24.8)	0	n.a.	n.a.
Anthracyclines (mg/m ²)	18	250 (100–450)	12	210 (100–315)	n.a.
Cyclophosphamide (mg/m ²)	2	3825 (250–7400)	29	7350 (3150–45990)	n.a.
Cisplatin (mg/m ²)	0	0	6	450 (270–630)	n.a.
Teniposide (mg/m ²)	0	0	6	500 (300–700)	n.a.
Dacarbazine (mg/m ²)	2	14.7 (13.5–15.8)	0	0	n.a.
Iphosphamide (mg/m ²)	2	33,000 (30,000–36,000)	0	0	n.a.

Data expressed as median (range).

^a Time after cessation of treatment. n.a. = not applicable, Gy = gray.

survivors and in all controls. In 3/103 survivors information regarding adrenalectomy was not available, they were therefore excluded in the relevant analysis.

3.2. Baseline levels

Mean baseline cortisol level in nephroblastoma and neuroblastoma survivors was 450 nmol/l and in controls 399 nmol/l ($P = 0.141$). Two survivors (one nephroblastoma and one neuroblastoma) and two control subjects had cortisol levels higher than 1000 nmol/l at baseline. Baseline cortisol levels were not different between nephroblastoma or neuroblastoma survivors (mean 461 versus mean 429, $P = 0.44$, Appendix Table 1). Interestingly, baseline serum cortisol levels were higher in survivors after unilateral adrenalectomy compared with survivors without adrenalectomy ($P = 0.002$), and also higher when compared with controls ($P = 0.013$) (Fig. 1a). The percentage of oestrogen users was not significantly different

between the two survivor subgroups ($P = 0.203$). After correcting for confounders the effect of adrenalectomy remained significant ($P = 0.010$) in the total group (Table 2), in the subanalysis excluding females on oral oestrogens ($\beta = 28.3$, $P = 0.002$) and in the subanalysis excluding survivors who were currently using corticosteroids ($\beta = 20.0$, $P = 0.019$). Cumulative dose of anthracyclines ($\beta = -0.0002$, $P = 0.995$), vincristine ($\beta = -0.4$, $P = 0.121$) and actinomycin ($\beta = 0.8$, $P = 0.297$), being treated with cyclophosphamide ($\beta = -3.3$, $P = 0.723$), abdominal radiotherapy ($\beta = -2.3$, $P = 0.789$) and radiotherapy administered to the contralateral adrenal gland ($\beta = 17.2$, $P = 0.244$) were not significant.

Mean ACTH levels were 5.3 pmol/l both in controls and in survivors with both adrenals intact, and were 7.8 pmol/l in survivors after adrenalectomy. After correcting for age, sex, oral oestrogen use and BMI, adrenalectomy was positively associated with baseline ACTH levels both in the total group ($\beta = 49.2$,

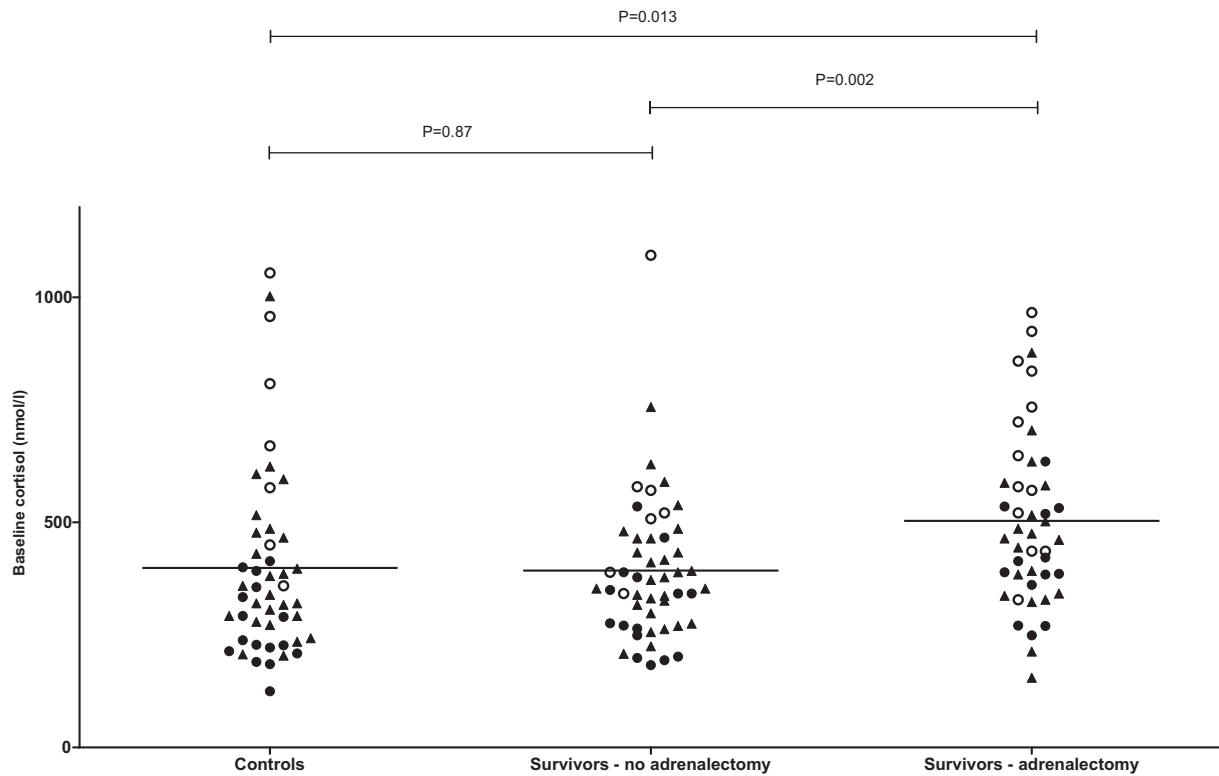


Fig. 1a. Baseline cortisol levels. Data shown as means and individual values. ▲ Males. ● Females no oral oestrogens. ○ Females oral oestrogens.

Table 2
Influence of determinants on serum cortisol levels in survivors and controls.

Model 1 Variable	Cortisol $t = 0^a$			Cortisol $t = 30^b$			Cortisol $t = 60^c$		
	β (%)	95% CI	<i>P</i> -value	β (%)	95% CI	<i>P</i> -value	β (%)	95% CI	<i>P</i> -value
Age (per 10 years)	-0.4	-1.0, 0.3	0.291	0.1	-0.3, 0.4	0.644	0.2	-0.2, 0.07	0.339
Sex (male)	24.3	9.0, 41.8	0.001	0.5	-6.3, 7.9	0.890	-0.8	-9.5, 8.7	0.859
Oestrogen use	85.7	55.6, 121.7	<0.001	33.6	21.0, 47.4	<0.001	49.2	31.4, 69.2	<0.001
BMI	-0.1	-1.7, 1.4	0.851	0.8	-0.1, 0.16	0.068	0.3	-1.4, 0.8	0.581
Survivor no adrenalectomy	0.9	-12.0, 15.7	0.897	-2.2	-9.2, 5.4	0.568	-1.9	-10.8, 7.9	0.700
Survivor adrenalectomy	20.8	4.6, 39.4	0.010	3.9	-3.8, 12.2	0.332	9.9	-0.5, 21.2	0.063

CI = confidence interval.

^a $t = 0$ = baseline.

^b $t = 30$ = 30 min after administration of Synacthen.

^c $t = 60$ = 60 min after administration of Synacthen.

$P = 0.006$), in the subanalysis excluding females on oral oestrogens ($\beta = 39.5$, $P = 0.036$) and in the subanalysis excluding survivors who were currently using corticosteroids ($\beta = 46.4$, $P = 0.012$). Adding the cumulative dose of various chemotherapeutic agents and radiotherapy to the model did not affect the results (data not shown).

3.3. Normal controls

To confirm that 440 nmol/l is a correct cut-off value for serum cortisol at $t = 30$ in subjects of this age group, 47 control subjects were evaluated. Mean cortisol level at $t = 30$ in our controls was 662 nmol/l (range 417–1200). Ninety-six percent ($n = 45$) of controls had serum

cortisol levels higher than 440 nmol/l, 4% ($n = 2$) of controls had levels below 440 nmol/l (419 nmol/l and 430 nmol/l). These subjects were further investigated using an insulin tolerance test and then reached a peak cortisol level of >550 nmol/l, indicating that adrenal function in these subjects was sufficient. Fifteen percent of controls had cortisol levels at $t = 30$ below 500 nmol/l, versus 5% of survivors.

3.4. Adrenal insufficiency

In order to evaluate adrenal insufficiency, cortisol levels at $t = 30$ after administration of Synacthen were analysed. None of the nephroblastoma or neuroblastoma survivors had cortisol levels below 440 nmol/l at $t = 30$

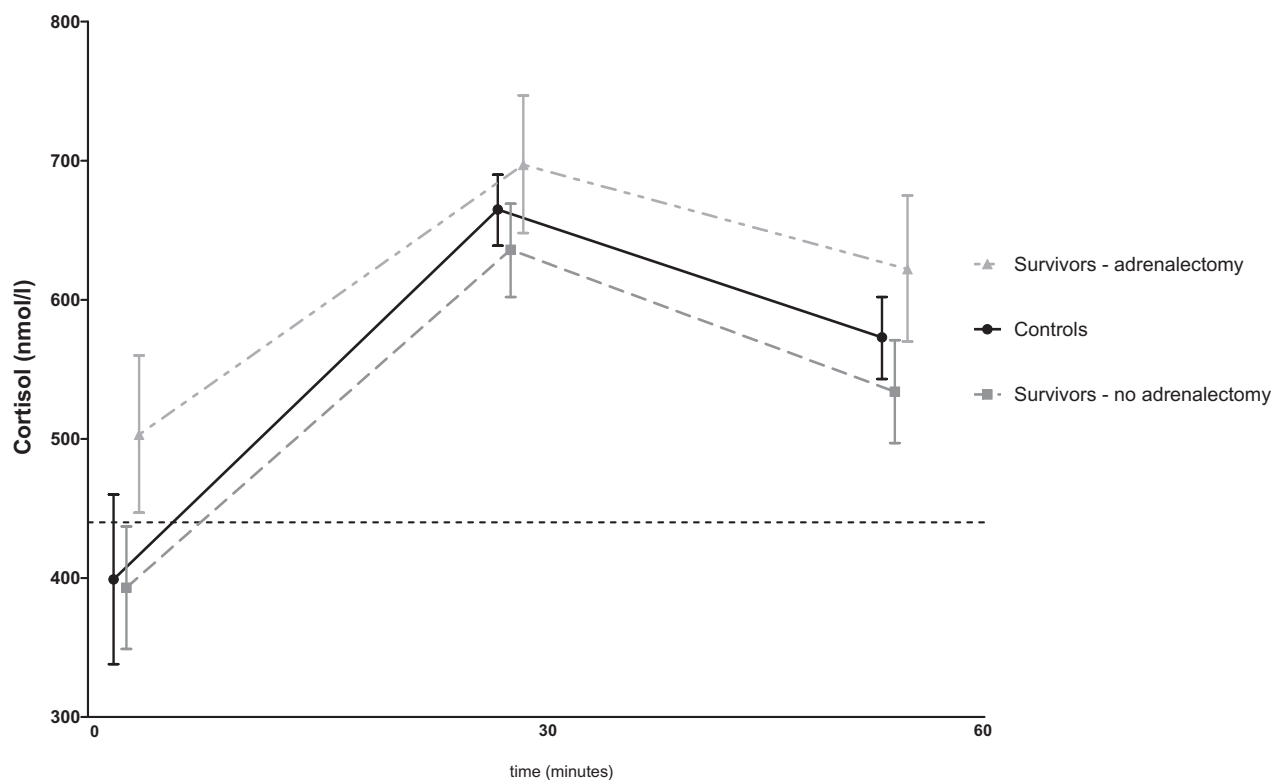


Fig. 1b. Synacthen-test. Data shown as mean (95% confidence interval). To avoid overlapping spaces are introduced on the x-axis between subgroups.

Table 3
Influence of baseline cortisol levels on metabolic parameters.

	Cortisol (per 100 mmol/l)		
	β	95% Confidence interval	P-value
Glucose (%) ^a	0.90	-0.00, 0.02	0.138
Insulin (pmol/l) ^a	1.86	-4.52, 8.23	0.57
HOMA ^a	0.06	0.02, 0.10	0.008
Triglycerides (%)	10.52	6.61, 14.45	<0.001
HDL-C (mmol/l)	-0.02	-0.05, 0.00	0.105
LDL-C (mmol/l)	0.09	0.03, 0.15	0.004
Apo-B (g/l)	0.04	0.02, 0.06	<0.001

Corrected for: age, sex, SES, smoking status, diagnosis, body mass index, abdominal radiotherapy, cumulative dose of anthracyclines, vincristin, actinomycin and cyclophosphamide.

HOMA = Homoeostatic Model Assessment, HDL-C = high-density lipoprotein-cholesterol, LDL-C = low-density lipoprotein-cholesterol.

^a Subjects with diabetes excluded.

(Fig. 1b). Serum cortisol levels at $t = 30$ were not different between long-term survivors of nephroblastoma, neuroblastoma or controls. However, serum cortisol levels at $t = 30$ and $t = 60$ were significantly higher in survivors after adrenalectomy compared with survivors with both adrenals intact (Appendix Tables 1 and 2). Concordantly, mean increase of cortisol level after Synacthen administration was lower in survivors after adrenalectomy (210 nmol/l) compared with both controls (mean 266, $P = 0.034$) and survivors with both adrenals intact (mean 262, $P = 0.032$).

3.5. Other adrenal steroids

DHEA, DHEAs, adione and 17-OHP were measured both in males and females, testosterone was only measured in females, all at baseline, and after 30 and 60 min. All steroids in males followed a pattern similar to that of cortisol. Steroids in females (oestrogen users and non-oestrogen users) followed a somewhat less clear pattern. For most steroids, baseline levels in adrenalectomised patients were higher than in non-adrenalectomised patients. However, no significance was reached (Appendix Table 3).

3.6. Influence of baseline cortisol levels on metabolic parameters

Baseline cortisol levels were positively associated with triglyceride ($P < 0.001$), LDL-C ($P = 0.004$) and Apo-B ($P < 0.001$) levels, but not with insulin or glucose levels. However, association of baseline cortisol levels with HOMA was significant ($P = 0.008$) (Table 3).

4. Discussion

The present study describes adrenal function in very long-term (mean 27 years) adult survivors of childhood nephroblastoma and neuroblastoma, in a substantial subset of whom unilateral adrenalectomy was performed. To our knowledge, this is the first study in such

a relatively large cohort of nephroblastoma and neuroblastoma survivors. We observed that all nephroblastoma and neuroblastoma survivors, regardless of the presence of both or one adrenal gland(s), had adequate adrenal function. It is therefore unlikely that adrenal dysfunction contributes to the unexplained mortality rate in childhood cancer survivors.

However, we unexpectedly found baseline cortisol and ACTH levels to be higher in survivors treated with adrenalectomy compared with survivors treated without adrenalectomy, and compared with healthy controls. Other treatment components did not affect the increased basal cortisol and ACTH levels. In the normal population, cortisol levels are known to be highly dependent on physical (e.g. physical activity) or psychosocial (e.g. emotional arousal) stress. It is possible that participating in this study was more stressful for survivors, because of their medical history, than for healthy controls. Raised cortisol levels have been previously reported in childhood cancer survivors after cranial irradiation.²⁴ It could be that this is explained by damaging the hypothalamus or pituitary, or that raised cortisol levels are a more universal finding in childhood cancer survivors, either explained by physical or psychosocial stress. However, if that would be the explanation, similar cortisol and ACTH levels would then have been expected in survivors treated without adrenalectomy. The hypothesis of a higher 'setpoint' of the adrenal system in cases after adrenalectomy is therefore more reasonable. The presence of both high ACTH levels and high cortisol levels in survivors after adrenalectomy suggests that the feedback system has been altered. A possible explanation for this higher setpoint could be that it is achieved by a feedback loop of the neural pathways, which might be damaged during surgery. Such a mechanism has been described for the thyroid gland, which is known to have neural connections with the hypothalamic suprachiasmatic and paraventricular nuclei. Although the specific role of autonomic innervation of the thyroid gland in terms of endocrine regulation remains to be elucidated, it is very likely that these connections contribute to the coordination of metabolic control.²⁵ Although the pathogenesis of this possible higher setpoint is not clear yet, it is important to realise that the presence of elevated cortisol levels can influence the metabolic system in this subset of survivors. Cortisol plays a role in gluconeogenesis, suppresses the immune system, and is involved in fat, protein and carbohydrate metabolism. Higher prevalence of coronary calcification in young and middle aged adults has been described in subjects with more flattened cortisol slopes, but not with average cortisol levels.²⁶ In a large Dutch epidemiologic study total cortisol exposure was associated with higher prevalence of atherosclerosis as indicated by the number of plaques in the carotid arteries.²⁷ We found baseline serum cortisol levels to be positively associated with lipid concentrations and insulin resistance. This indicates that the higher

setpoint of the HPA axis may influence metabolic and cardiovascular parameters in long-term childhood cancer survivors and are therefore important confounders for future studies.

In our study, adrenal function was assessed by a low-dose ACTH stimulation test. This test is considered to be superior to the 250 µg short ACTH stimulation test, as 250 µg is a supraphysiological dose. This may lead to overstimulation of partially atrophied adrenals, thereby producing a deceptively adequate cortisol response.^{20,28} So far, no consensus has been reached regarding the cut-off value for peak cortisol levels after the low-dose ACTH stimulation test. According to a meta-analysis by Kazlauskaitė et al. cortisol levels below 440 at 30 min after administration of Synacthen are predictive of hypothalamic-pituitary-adrenal insufficiency.²⁰ The lack of a standard cortisol assay method explains at least partly the variability in diagnostic cortisol thresholds reported across studies. For that reason and also because large control groups of young adults tested for adrenal insufficiency are lacking, we felt that an age-matched control group consisting of healthy subjects was mandatory to strengthen our study. Results of the Synacthen-test in our control subjects confirm that 440 nmol/l is a correct cut-off value in this group of survivors and controls. Moreover, as 73% of the control subjects in this study were relatives of the survivors, the possibly confounding effect of genetic variation is negligible.

Although 17-hydroxy-progesterone, andione, DHEA, DHEAs and testosterone have, compared with cortisol, only minor importance in the diagnosis of primary adrenal insufficiency, their levels as a reflection of normal response to the low dose stimulation test provide further proof of sufficient adrenal function and are in line with our observed serum cortisol levels. In our study, levels of these steroids correlated well with cortisol in males, however in females this correlation was less strong. This might be explained by the fact that we did not perform the Synacthen-test on the same day of the menstrual cycle of the females. It is known that the menstrual cycle yields a varying ovarian contribution to the concentration of these steroids.²⁹

In conclusion, long-term nephroblastoma and neuroblastoma survivors after unilateral adrenalectomy are not at risk for adrenal insufficiency. However, we found their baseline cortisol and ACTH levels to be elevated compared with controls and survivors without adrenalectomy. This higher setpoint can induce consequences for the metabolic system and cardiovascular risk in these survivors, which is suggested by our findings of elevated lipid levels and increased insulin resistance. Our study indicates that further studies on the exact mechanism behind the altered setpoint of the adrenal system after adrenalectomy are warranted. Detailed information on the status of the adrenal glands after surgery is therefore of value in those studies. Moreover, studies on

the influence of this higher baseline setting on metabolic and cardiovascular parameters in subsets of long-term adult survivors of childhood cancer are relevant.

Conflict of interest statement

M. van Waas, R. Pieters, J.P. van Eck, M.M. van Noesel, F.H. de Jong, S.J.C.M.M. Neggers and M.M. van den Heuvel-Eibrink have no conflict of interest to declare. A.J. van der Lely received travel grants and consultancy fees from Pfizer Incorp, Novartis Pharma and Ipsen Pharma.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at [doi:10.1016/j.ejca.2012.02.046](https://doi.org/10.1016/j.ejca.2012.02.046).

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