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Cardiovascular risk factors in adult patients with multisystem Langerhans-cell histiocytosis: evidence of glucose metabolism abnormalities

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Summary

Background: Langerhans-cell histiocytosis (LCH) is a rare disease with features of chronic inflammation and it may also induce hypopituitarism, conditions associated with an increased risk of cardiovascular diseases.

Aim: Cardiovascular and metabolic risk profile investigation in multisystem LCH patients with and without anterior pituitary deficiency.

Design: Prospective, observational study.

Methods: Fourteen adult patients with LCH, 7 with and 7 without anterior pituitary deficiency, and 42 controls matched for age, body mass index (BMI) and smoking. Cardiovascular risk factors were estimated in all subjects: glucose and lipid profile, mathematical indices of insulin resistance (IR), blood pressure, structural arterial and functional endothelial properties (intima-media thickness, brachial artery flow-mediated dilatation). Cardiovascular risk factors were estimated in the three groups studied; the effect of disease activity and/or treatment was also determined in patients with LCH.

Results: Ten patients had diabetes insipidus, and 7 anterior pituitary hormone deficiencies: 8 patients had active disease and 11 had received systemic treatment. No difference was observed between the study groups in vascular parameters, in lipid profile or in blood pressure. However, the insulin resistance index GIR was decreased in patients with LCH without anterior pituitary deficiency compared to controls ($P=0.033$). Three patients had impaired glucose tolerance and one diabetes mellitus type 2. These patients were older and had active disease; there was no association with hypopituitarism and/or previous treatment.

Conclusions: Adults patients with LCH have abnormalities of glucose metabolism that tend to occur in patients with active disease, and may be a consequence of the pro-inflammatory state.

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Introduction

Langerhans-cell histiocytosis (LCH) is a rare disease, usually affecting children, although recently it has been increasingly recognized in adults with an estimated prevalence of approximately 1:560.000 of normal population,[1,2]. However, this figure may be underestimate due to under-reporting. The time interval between the first clinical appearance of the disease and the initial diagnosis may vary from months up to several years, especially among adults with mild and common clinical signs such as skin lesions or painless bone involvement.[1,2] LCH is characterized by the aberrant proliferation of dendritic cells of the monocyte-macrophage system that resemble normal epidermal Langerhans' cells. These cells can infiltrate many sites of the body leading to either localized lesions or widespread systemic disease.[2] Although LCH has been shown to be a clonal disorder,[3] it also exhibits features of an inflammatory disease, as altered expression of cytokines and cellular adhesion molecules important for the migration and homing of Langerhans cell has been documented.[4,5] In addition, LCH shows a particular predilection for hypothalamo-pituitary axis (HPA) involvement leading to diabetes insipidus and/or anterior pituitary dysfunction in 15–50% and 5–20%, of patients respectively.[3,6–8] These percentages may be higher in adult patients with multisystem involvement, being 94% and 59%, respectively.[9]

The presence of hypopituitarism is considered as an independent risk factor for cardiovascular disease.[10] The various therapies used for the treatment of multisystem LCH—chemotherapy, radiotherapy and particularly glucocorticoids—may also adversely affect the cardiovascular system, mostly through interactions of insulin resistance (IR).[11,12] Considering the presence of inflammatory mediators and their additional role to the induction of IR,[13] it is therefore possible that patients with LCH represent a group at higher risk for cardiovascular disease through the additive effect of a number of different contributing mechanisms known to induce IR.[14] Insulin resistance, besides producing an adverse metabolic profile, can also lead to endothelial dysfunction and early vascular disease.[15] Such vascular disease can be detected by non-invasive surrogate markers, such as the investigation of intima-media thickness (IMT) for structural changes in the vasculature and flow-mediated vasodilatation (FMD) for vascular functional properties, particularly in a disease characterized by an ongoing inflammatory process.[13,16,17]

In the present study, we have therefore investigated whether patients particularly with multisystem LCH do indeed show an increased cardiovascular and metabolic risk profile compared to control subjects matched for gender, age, body mass index (BMI) and smoking habit, and whether any such changes are related to LCH *per se*, hypopituitarism or therapeutic interventions.

Subjects and methods

Fourteen patients with LCH, 10 women, aged 37.21 ± 3.23 (mean $\pm$ standard error) years, with a body mass index (BMI) of $27.34 \pm 1.89\,kg/m^2$, were recruited. 13 had multisystem disease and one (man), only bone (vertebral) disease who nevertheless, required systemic treatment. All patients were adults (age range: 21–60 years) and were referred to a single centre in order to be evaluated for LCH, all fulfilled the diagnostic criteria for *definitive diagnosis* of LCH.[18] Of the patients, two were on thyroxine replacement, one with primary hypothyroidism (Table 1, patient 9) while the other had a thyroidectomy for a multinodular goitre, during which LCH was incidentally diagnosed in lymph nodes in addition to two foci of 0.5 cm papillary carcinoma (Table 1, patient 9). The other patients at the time of the study were not suffering from any other disease and had not received any medication known to affect carbohydrate and/or endothelial function for at least 3 months prior to the study besides the treatment for LCH (Table 1). Sex hormone replacement treatment was also discontinued 3 months prior to the study. In addition, female patients who had already conceived did not have evidence of gestational diabetes or other complication during pregnancy. Forty-two healthy subjects (30 women) matched for age, BMI and smoking habits, aged: 36.90 ± 1.72 (range: 20–59) years; BMI: $25.97 \pm 0.79\,kg/m^2$, who were in good health and volunteered to participate as controls. They were not receiving any medication known to affect carbohydrate, sex hormone metabolism and/or endothelial function (such as non-steroidal anti-inflammatory drugs, oral contraceptives or ferrous supplements) for at least 3 months prior to the study. Four premenopausal patients (Table 1, patients 6, 11, 12, 13) and all premenopausal women from the control group had regular menstruation.

LCH patients had an evaluation of the extent of the disease based on clinical, biochemical and radiological investigations. Signs and symptoms related to pathology of the HPA were recorded, and all patients underwent endocrine assessment of anterior and posterior pituitary function within 6 months of the study.[9] The state of the disease was

Table 1 Anthropometric characteristics, specific organ involvement, activity of the disease, form of systemic treatment and carbohydrate metabolism in the 14 patients with multisystem LCH studied

Patients	Age (yrs)	Sex	BMI (kg/m^2)	Pituitary deficiencies	Organ involvement	Activity of disease	Treatment (previous)	Treatment (current)	Glucose tolerance	Duration from diagnosis
1	36	M	28.1	DI, GH	Gengives, Lung	Non active	CS, RT	(-)	NGT	16 yrs
2	54	F	27.4	DI, LH-FSH	CNS, Bones	Active	CS, vinblastine	2-cda	T2DM	2 yrs
3	60	F	27.8	DI, LH-FSH	CNS, Bones	Active	(-)	AZT, MTX	IGT	5 yrs
4	35	F	26.5	DI, LH-FSH, GH	Bones, Lung	Active	HRT, CS, etoposide	AZT, MTX	NGT	11 yrs
5	21	F	20	DI, LH-FSH, GH	Bones, Skin, Lung	Non active	(-)	(-)	NGT	18 yrs
6	36	F	22.6	(-)	Bones, Skin, Lung, Gengives, Genitalia	Active	RT, CS, vinblastine, cyclosporine	MERCA, MTX	NGT	9 yrs
7	36	M	31.4	(-)	Bones	Non active	CS, vinblastine	(-)	NGT	1 yr
8	29	M	24.2	DI	Bones, Lung, LNs,	Active	CS, adriamycine vinblastine, etoposide, cyclophosphamide	AZT, MTX	NGT	15 yrs
9	20	F	20.3	DI, LH-FSH, GH	LNs, Skin,	Non active	OCP, vinblastine	(-)	NGT	11 yrs
10	54	M	25.9	DI	Gengives	Active	RT, CS	(-)	IGT	13 yrs
11	45	F	32.7	(-)	Bones, Lung, LNs	Active	(-)	(-)	IGT	2.5 months
12	30	F	17.3	(-)	Bones, Lung, Skin	Active	(-)	(-)	NGT	3 months
13	29	F	33.1	DI	CNS, Skin, Ears	Non active	CS, vinblastine, MERCA, MTX	(-)	NGT	13 yrs
14	36	F	45.6	DI, LH-FSH, GH	Lung, LNs	Non active	HRT, CS, adriamycine, TX, vinchristine, cyclophosphamide	(-)	NGT	5 yrs

Yr(s): year(s); M: male; F: female; DI: diabetes insipidus; GH: growth hormone deficiency; LH-FSH: gonadotrophin deficiency; CNS: central nervous system; LNs: lymph nodes; CS: glucocorticoids; RT: radiotherapy; HRT: hormone replacement treatment; OCP: oral contraceptive pill; MERCA: 6-mercaptopurine; MTX: Methotrexate; 2-cda: 2-chlorodeoxyadenosine; AZT: azathioprine; NGT: normal glucose tolerance; T2DM: type 2 diabetes mellitus; IGT: impaired glucose tolerance.

defined as either *active* or *non active* in accordance with the definition criteria of disease's state of LCH-A1 therapeutic protocol for adults of the Histiocyte Society (http://www.histio.org/site). The form of therapy (chemotherapy, radiotherapy and/or surgical treatment) that patients received during the course of the disease and the duration of the study were also recorded. Patients with documented anterior and/or posterior pituitary hormone deficiencies were receiving conventional replacement therapy except for growth hormone (GH). All subjects were advised to avoid strenuous physical activities and were on a balanced isocaloric diet for at least 4 weeks prior to the study. Current smokers were asked not to smoke one day before the haemodynamic study. The study protocol was approved by the local ethics board and informed consent was obtained from all participants.

Patients were divided into those with and without pituitary hormone deficiencies: 7 (50%) patients (4 women), aged 35.71 ± 6.02 years, had anterior pituitary involvement, while 7 patients (6 women), aged 38.00 ± 3.32 years, did not exhibit anterior pituitary involvement.

The patients as a whole group were compared to the control population. Patients were also divided according to the presence or not of carbohydrate abnormalities and then were compared to their matched control population.

Cardiovascular risk factors

Metabolic profile

The metabolic study of all patients was performed after a 10 h overnight fasting. The subjects rested for 30 min in the supine position and blood samples were collected at 08:00–9:00 h. Subsequently, 75 g of glucose were given orally in all subjects. Blood samples were obtained every 30 min for 120 min for serum glucose and insulin measurement (oral glucose tolerance test, OGTT) in LCH patients and in control subjects only after 120 min for serum glucose measurement. Weight, height, waist and hip circumference, systolic (SBP) and diastolic (DBP) blood pressure were measured. Waist circumference was obtained as the smallest circumference at the level of the umbilicus. Hip circumference was obtained as the widest circumference at the level of the buttocks. Blood pressure was measured by a mercury sphygmomanometer with the subject in sitting position, on left arm, after a rest of at least 5 min. The average of three measurements was obtained. BMI was calculated by the formula: BMI = weight (kg)/[height (m)]^2.

In the premenopausal women, all evaluations were conducted in the follicular phase of their menstrual cycle.

Haemodynamic studies

The haemodynamic study was performed, the day after the metabolic study, in a quiet, temperature-controlled (21–23°C) room following an overnight 10 h fast. Both functional and structural arterial properties were assessed by non-invasive, easily reproducible haemodynamic ultrasonographic methods, namely: IMT measurement in carotid arteries and both endothelium-dependent FMD and endothelium-independent nitrate-induced dilatation (NID) measurement in the brachial artery. IMT, FMD and NID were measured by B-Mode high-resolution ultrasound imaging (VIVID PRO; GENERAL ELECTRIC).[19] The investigator who performed the haemodynamic study was not aware about health condition of the subjects who participated in the study. The intra-observer variability for brachial diameter measurements in our laboratory was 0.08 ± 0.19 mm, respectively, while FMD variability measured on two separate days was $1.1 \pm 1\%$. An intra-observer study on IMT measurements showed an intra-class agreement of 0.89; between observers this correlation was 0.87. The same operator performed the haemodynamic study in order to avoid inter-observer variability.

Indices of insulin resistance

Insulin resistance was estimated in all the subjects by the following indices: waist-to-hip ratio (WHR), glucose-to-insulin ratio (GIR),[20] homeostasis model of assessment (HOMA) index,[21] and quantitative insulin sensitivity check index (QUICKI)[22] using basal glucose and insulin values.

Assay methods

Blood samples were centrifuged immediately and serum was stored at −20°C until assayed. The samples were assayed within 12 months of their collection. Glucose (GLU, Glucose LR, GOD-PAP; Linear Chemicals, Barcelona, Spain), total cholesterol (TC, CHOLESTEROL, Clinical Chemistry, Abbott Laboratories, Abbott Park, IL 60064, USA), HDL-cholesterol (HDL, ULTRA HDL, Clinical Chemistry, Abbott Laboratories, Abbott Park, IL 60064, USA), triglycerides (TRIGLYCERIDE, Clinical Chemistry, Abbott Laboratories, Abbott Park, IL 60064, USA), insulin (INS-Irma, Biosource Europe SA, Nivelles, Belgium) were measured by commercial kits. LDL-cholesterol (LDL) was calculated by the Friedewald equation. The HDL-to-LDL

ratio (HLR) was also calculated. All patients underwent clinical examination and basal endocrine assessment of anterior and posterior pituitary function, as previously described.[9]

Statistical analysis

The results are reported as mean values ± standard error (SE). Statistical significance in the results was accepted at a P-value < 0.05. Normal distribution of continuous variables was assessed by applying the non-parametric Kolmogorov–Smirnov test: all parameters studied were normally distributed. Analysis of variance (ANOVA) was used for comparisons between the three study groups. The data are presented after Bonferroni correction for multiple comparisons. Correlations between variables were evaluated in the LCH group by Pearson's correlation since all variables were normally distributed. An independent sample, two-tailed t-test was used for comparisons between patients with multisystem LCH and the control group. The Mann–Whitney U test was used for the comparisons between the subgroups of LCH patients and their matched controls. Correlations between categorical variables were estimated by the chi-square test. Analysis was performed using SPSS (Statistical Package for the Social Sciences, version 11.01; SPSS, Chicago, IL, USA) for Windows XP (Microsoft).

Results

The specific characteristics of LCH patients, including extent of the disease, are shown in Table 1. The control group did not differ from both patients group in age or BMI by study design. No difference was observed in waist circumference or WHR between groups (Table 2). The three groups did not differ in smoking habits ($P = 1.00$).

The duration of the disease from diagnosis in patients with LCH was 8.53 ± 1.66 (range: 0.20–18) years. Ten (71%) patients had diabetes insipidus (DI) and 7 patients (50%) had anterior pituitary involvement; 3 (21%) had a single pituitary hormone deficiency and 4 (29%) multiple pituitary hormone deficiencies [6 patients (43%) had gonadotrophin, and 5 (36%) had GH deficiency]. Eleven patients had previously received systemic therapy; 9 patients had received treatment with glucocorticoids that was discontinued at least 6 months before the study. Eight patients were considered as having active disease and 5 of them were receiving treatment at the time of the study (Table 1). The duration of the disease in patients with LCH and pituitary deficiency was 9.71 ± 2.26 years and in patients with LCH without pituitary deficiency 7.35 ± 2.52 years ($P = 0.46$).

Cardiovascular risk factors profiles

No difference was observed between the three groups studied in vascular parameters, in lipid profile or in blood pressure. However, the insulin resistance index GIR was decreased in patients with LCH without anterior pituitary deficiency compared to controls ($P = 0.033$).

Following an OGTT, in 4 patients with normal fasting glucose, abnormalities of carbohydrate metabolism were revealed; one patient developed diabetes mellitus type 2 (7%) and 3 (21%) impaired glucose tolerance (IGT) (Tables 1 and 2). Three of these patients had diabetes insipidus and two anterior pituitary hormone deficiencies. Two patients had received treatment with glucocorticoids, and two systemic treatments with various agents. The patient with diabetes mellitus type 2 had received 6 monthly courses with 2-chlorodeoxyadenosine (2-cda), the last course 8 months before the study, while a patient with IGT was receiving azathioprine and methotrexate at the time of the study (Table 1). Patients with abnormal glucose metabolism were older (53.25 ± 3.09 years) compared to patients with normal glucose metabolism (30.33 ± 2.32 years; $P = 0.008$). All four patients who had abnormalities in glucose metabolism had active disease; there was a trend for an association between activity of the disease and the presence of abnormal glucose metabolism, but this was not formally statistically significant ($P = 0.08$). No association between current treatment and the presence of abnormal glucose metabolism was found ($P = 0.48$).

The whole group of patients was compared with the control group (Table 3). A trend towards marginally higher levels of insulin was observed in LCH patients ($P = 0.09$), with lower levels of fasting glucose ($P = 0.01$). The GIR index was statistically lower in LCH patients compared to controls ($P < 0.001$). When the four patients with the abnormal carbohydrate metabolism and their matched control subjects were excluded from the analysis, the GIR index was still statistically lower in LCH patients ($P = 0.02$) (Table 3).

No statistical significant difference in any parameter studied was observed among controls and the patients who were receiving treatment during the study and those not treated, or among the patients who had systemic treatment and those never treated, or among patients with and without active disease (data not shown).

Table 2 Characteristics and cardiovascular risk factors profile between the three studied groups, LCH patients with pituitary deficiency, LCH patients without pituitary deficiency and controls. Statistical significance is shown after Bonferroni correction

	Patients with pituitary deficiency ($n=7$)	Patients without pituitary deficiency ($n=7$)	Control groups ($n=42$)	*P*-value		
				Patients with pituitary deficiency vs. C	Patients without pituitary deficiency vs. C	Patients with pituitary deficiency vs. patients without pituitary deficiency
Age (yrs)	35.71 ± 6.02	38.00 ± 3.32	37.21 ± 3.23	1.00	1.00	1.00
BMI (kg/m^2)	27.97 ± 3.21	26.73 ± 2.24	27.34 ± 1.89	1.00	1.00	1.00
Waist (cm)	93.21 ± 6.71	93.00 ± 7.00	87.33 ± 2.50	1.00	1.00	1.00
GLU 0′ (mmol/l)	4.20 ± 0.20	4.43 ± 0.29	4.83 ± 0.10	0.053	0.37	1.00
INS 0′ (pmol/l)	61.20 ± 10.08	80.28 ± 15.12	52.14 ± 5.10	1.00	0.15	0.86
TC(mmol/l)	4.89 ± 0.20	5.30 ± 0.21	4.78 ± 0.154	1.00	0.59	1.00
HDL(mmol/l)	1.43 ± 0.09	1.56 ± 0.19	1.43 ± 0.05	1.00	1.00	1.00
Triglycerides (mmol/l)	1.00 ± 0.15	1.44 ± 0.44	0.93 ± 0.07	1.00	0.11	0.47
LDL (mmol/l)	3.00 ± 0.17	3.05 ± 0.28	3.34 ± 0.15	0.95	1.00	1.00
HLR	0.49 ± 0.06	0.52 ± 0.57	0.48 ± 0.37	1.00	1.00	1.00
WHR	0.88 ± 0.02	0.92 ± 0.48	0.90 ± 0.08	1.00	1.00	1.00
GIR	8.59 ± 1.25	6.70 ± 0.85	12.78 ± 0.93	0.17	**0.03**	1.00
HOMA	1.95 ± 0.36	2.78 ± 0.66	1.91 ± 0.20	1.00	0.39	0.76
QUICKI	0.353 ± 0.011	0.339 ± 0.015	0.358 ± 0.004	1.00	0.55	1.00
Brachial artery diameter (mm)	3.43 ± 0.34	3.94 ± 0.41	3.61 ± 0.09	1.00	0.80	0.61
FMD (%)	7.02 ± 1.81	7.94 ± 2.13	5.73 ± 0.56	1.00	0.63	1.00
IMT (mm)	0.64 ± 0.06	0.53 ± 0.05	0.54 ± 0.02	0.32	1.00	0.50
NID (mm)	15.78 ± 3.11	13.07 ± 2.82	17.04 ± 2.59	1.00	1.00	1.00
SBP (mmHg)	102.86 ± 4.96	113.57 ± 3.81	115.41 ± 2.57	0.16	1.00	0.61
DBP (mmHg)	71.86 ± 2.28	74.86 ± 2.93	75.05 ± 1.82	1.00	1.00	1.00

Data as means ± SE; $P<0.05$ as statistically significant (in bold); BMI: body mass index; GLU 0′: fasting glucose; INS 0′: fasting insulin; TC: total cholesterol; HDL: HDL-cholesterol; LDL: LDL-cholesterol; HLR: HDL-to-LDL ratio; WHR: waist-to-hip ratio; GIR: glucose-to-insulin ratio; HOMA: HOMA index; QUICKI: index QUICKI; FMD: flow-mediated dilatation; IMT: intima-media thickness; SBP: systolic blood pressure; DBP: diastolic blood pressure.

Table 3 Cardiovascular risk factors profile in the patients LCH as a unique group and as subgroups according to their carbohydrate metabolism and their matched control population

	All patients compared to controls			Patients with normal glucose tolerance compared to matched controls			Patients with abnormal glucose tolerance compared to matched controls		
	LCH patients ($N=14$)	Controls ($N=42$)	P	LCH patients ($N=10$)	Controls ($N=30$)	P	LCH patients ($N=4$)	Controls ($N=12$)	P
GLU 0′ (mmol/l)	4.32 ± 0.17	4.83 ± 0.10	**0.01**	4.22 ± 0.23	4.77 ± 0.12	0.06	4.55 ± 0.11	5.00 ± 0.16	0.28
GLU 120′ (mmol/l)	6.59 ± 0.76	5.40 ± 0.26	0.16	5.19 ± 0.56	5.18 ± 0.33	0.99	9.71 ± 0.93	5.98 ± 0.29	**0.004**
INS 0′ (pmol/l)	70.02 ± 8.88	52.14 ± 5.10	0.09	69.36 ± 12.03	53.76 ± 6.00	0.25	71.58 ± 10.74	46.98 ± 10.26	0.08
TC (mmol/l)	5.09 ± 0.14	4.78 ± 0.154	0.27	5.00 ± 0.19	4.68 ± 0.20	0.32	5.33 ± 0.13	5.03 ± 0.22	0.32
HDL (mmol/l)	1.50 ± 0.11	1.43 ± 0.05	0.52	1.48 ± 0.12	1.45 ± 0.07	0.89	1.53 ± 0.25	1.36 ± 0.08	0.86
triglycerides (mmol/l)	1.22 ± 0.23	0.93 ± 0.07	0.17	1.28 ± 0.31	0.87 ± 0.07	0.24	1.09 ± 0.12	1.08 ± 0.17	0.84
LDL (mmol/l)	3.03 ± 0.16	3.34 ± 0.15	0.22	2.98 ± 0.2	3.25 ± 0.19	0.45	3.13 ± 0.27	3.58 ± 0.1	0.19
HLR	0.51 ± 0.39	0.48 ± 0.37	0.71	0.51 ± 0.04	0.48 ± 0.04	0.66	0.51 ± 0.10	0.39 ± 0.03	0.19
Waist circumference (cm)	87.33 ± 2.50	87.33 ± 2.50	0.24	89.31 ± 6.33	85.18 ± 3.70	0.93	100.75 ± 4.82	91.00 ± 2.31	0.14
WHR	0.90 ± 0.22	0.90 ± 0.08	0.99	0.89 ± 0.03	0.90 ± 0.08	0.06	0.92 ± 0.04	0.84 ± 0.02	0.08
GIR	7.71 ± 0.79	12.78 ± 0.93	**<0.001**	7.87 ± 1.08	12.03 ± 0.95	**0.02**	7.37 ± 1.11	15.22 ± 2.43	**0.050**
HOMA	2.33 ± 0.36	1.91 ± 0.20	0.31	2.30 ± 0.51	1.94 ± 0.24	0.74	2.41 ± 0.37	1.81 ± 0.44	0.15
QUICKI	0.347 ± 0.009	0.358 ± 0.004	0.27	0.351 ± 0.013	0.357 ± 0.006	0.74	0.337 ± 0.008	0.360 ± 0.010	0.15
FMD (%)	7.48 ± 1.34	5.73 ± 0.56	0.17	7.56 ± 1.65	6.24 ± 0.68	0.17	7.31 ± 2.65	4.45 ± 0.99	0.47
IMT (mm)	0.59 ± 0.04	0.54 ± 0.02	0.36	0.57 ± 0.04	0.50 ± 0.02	0.10	0.61 ± 0.09	0.65 ± 0.04	0.60
SBP (mmHg)	105.80 ± 3.72	115.41 ± 2.57	0.90	106.00 ± 4.23	113.10 ± 2.87	0.30	105.00 ± 11.00	121.73 ± 5.30	0.30
DBP (mmHg)	73.00 ± 1.79	75.05 ± 1.82	0.59	72.88 ± 1.91	75.27 ± 2.31	0.85	73.50 ± 6.50	74.45 ± 2.78	0.92

Data as means ± SE; $P < 0.05$ as statistically significant (in bold); GLU: glucose; INS: insulin; TC: total cholesterol; HDL: HDL-cholesterol; LDL: LDL-cholesterol; HLR: HDL-to-LDL ratio; WHR: waist-to-hip ratio; GIR: glucose-to-insulin ratio; HOMA: HOMA index; QUICKI: index QUICKI; FMD: flow-mediated dilatation; IMT: intima-media thickness; SBP: systolic blood pressure; DBP: diastolic blood pressure.

Correlation analysis

In LCH patients, correlation analyses revealed known inter-correlations between cardiovascular risk factors. Insulin resistance indices were inter-correlated: GIR was positively related to QUICKI ($r=0.868$, $P<0.001$) and negatively to BMI ($r=-0.633$, $P=0.020$), HOMA ($r=-0.844$, $P<0.001$), waist circumference ($r=-0.586$, $P=0.020$); QUICKI was negatively related to HOMA ($r=-0.943$, $P<0.001$); GLU was positively related to INS ($r=0.765$, $P=0.02$); BMI ($r=-0.57$, $P=0.042$), waist circumference ($r=-0.671$, $P=0.017$); FMD was positively related to HDL ($r=0.601$, $P=0.039$); HDL was negatively related to triglycerides ($r=-0.680$, $P=0.007$); SBP was negatively related to GIR ($r=-0.586$, $P=0.035$) and positively to TC ($r=0.573$, $P=0.032$) and to DBP ($r=0.809$, $P<0.001$).

Discussion

Although patients with LCH combine several independent risk factors for cardiovascular disease, their exact prevalence is unknown mainly due to the rarity of the disease and the difficulty in conducting large epidemiological studies. Screening for surrogate markers of cardiovascular disease offers an alternative way to detect early vascular disease[13,16,17] and identify whether these patients are at higher risk for cardiovascular disease. The results of this study reveal a small but significant increased risk for metabolic abnormalities in adult patients with LCH; specifically, a high prevalence of abnormalities of glucose metabolism was demonstrated, with 29% of patients showing either impaired glucose tolerance (21%) or diabetes mellitus type 2 (7%). In addition, one index of IR demonstrated resistance values in patients with LCH compared to controls, but no other difference was detected between groups or subgroups in the other indices of IR, arterial vascular properties parameters, lipid serum concentrations or arterial blood pressure.

There are only a few previous case reports documenting abnormalities of glucose metabolism in patients with LCH.[23–28] Although these patients were not studied into detail it was claimed that pancreatic involvement by the disease process with secondary insulin deficiency[24] might be responsible. In the presence of hepatic involvement, glucose intolerance may also occur as a consequence of decreased hepatic glucagon degradation[29,30] or the development of portal hypertension.[31] While in the present study, subclinical hepatic and/or pancreatic involvement by LCH cannot be excluded in patients with LCH, the finding of altered glucose metabolism with advancing age is in accordance with the previous cases reported with development of diabetes mellitus type 2 in adult LCH patients without hepatic and/or pancreatic involvement.[24–26] Although there were variations in the results using different estimates of insulin resistance, this might reflect the inherent limitation of these indices that are calculated using mathematical formulae to precisely estimate insulin resistance.[32] Patients with abnormalities of carbohydrate metabolism were not particularly obese and did not have any other known risk factors for diabetes mellitus type 2 or cardiovascular diseases.[14] It is therefore likely that the presence of insulin resistance could lead to abnormalities of carbohydrate metabolism, particularly in the presence of other contributing factors, such as advancing age or treatment with corticosteroids. This could be enhanced further by the activity of the disease, as an association that almost reached statistical significance between disease activity and abnormalities of glucose metabolism was demonstrated.

Notably, no particular significant alterations in the surrogate cardiovascular risk factors studied were detected. Partial or complete hypopituitarism has been associated with increased IMT values and the presence of atherosclerotic plaques in the carotid arteries as well as with an increased number of deaths from cardiovascular disorders besides adequate hormone replacement therapy.[33] However, this has not been investigated in depth in various causes of hypopituitarism other than pituitary adenomas.[33] Although lack of GH replacement has been thought to account for some of these findings,[34] women with hypopituitarism on conventional replacement treatment, other than GH, had no alterations of IMT, number of plaques in the carotid arteries or other ultrasonographic parameters of cardiac function.[35] Similarly, in the present study patients with LCH had no abnormalities in endothelial function and arterial structural properties compared to controls, irrespective of the presence of anterior pituitary hormone deficiencies. It is probable that such changes may not have been manifest yet due to the relatively young age of the patients included in the present study. An additional factor for this finding might be the short duration from the diagnosis of the disease, as well as the fact that the changes might not have been detected because of the small number of patients studied. Alternatively, considering the common molecular mechanisms that have been thought to operate in inflammatory and insulin-signalling pathways leading to IR,[36] the inflammatory process in patients with LCH might not attain the appropriate level to induce

damage to arterial bed properties. Additionally, patients with or without treatment or with or without active disease did not differ between them as well as with the control group (data not shown). The real impact of the treatments used for LCH, and the role of the activity of the disease in insulin resistance induction, has yet to be clarified from larger studies since no association was revealed in the present study.

The presence of glucose metabolism abnormalities in patients with LCH without associated alterations of any vascular bed properties presents an intriguing finding, as several studies have demonstrated a close association between IR and alterations of vascular bed properties.[15] This could imply limitations of the methodology employed to study vascular bed studies and/or calculate indices of IR; however, the former is less likely as a number of previous studies support the methodology used.[19] Although the presence of IR was evident in only one of the indices employed, the 28% prevalence of abnormalities of glucose metabolism with adequate insulin increments following the OGTT in patients with LCH is suggestive of an insulin resistant state. However, in agreement with the present study, a recent publication showed that when mechanisms of IR vary, the endothelial function may be affected differently.[37] Another theoretical possibility is that since the cytokine milieu in patients with LCH is different,[4,5] the presence of anti-inflammatory cytokines may exert a protective effect to the vascular bed but not to insulin sensitivity. Interestingly, previous systemic treatment did not seem to exhibit any effect in any of the cardiovascular risk factors studied, in spite of the fact that 64% of the patients had previously received treatment with corticosteroids. However, it is difficult to estimate the effect that these therapies could exert on vascular bed properties from this study due to its retrospective nature.

There are some potential limitations to our study and therefore its findings have to be interpreted with caution. Besides the small number of patients studied, LCH patients are subject to a variable number of potentially confounding factors and the interpretation of the results is difficult. The population of LCH patients studied was heterogeneous with a number of them having already received previous treatment with corticosteroids, chemotherapeutic agents and/or hormone replacement. In addition, patients were studied at different phases during the course of the disease when the inflammatory milieu and its influence on vascular bed could be different. The effect that these factors could exert on vascular properties is not known and could account for the absence of alterations of vascular bed properties besides the presence of insulin resistance. However, LCH is a rare disease, and it is difficult to obtain a large cohort of adult patients from a single centre.

In conclusion, this study demonstrates that patients with multisystem LCH tend to show insulin resistance, since a significant number may develop abnormalities of glucose metabolism. Our data suggest that this is related to the disease process as such rather than being secondary to pituitary hormone deficiency or modalities of therapy, although these findings need to be replicated in larger studies. Rare diseases such as LCH and their sequelae are difficult to investigate at a single centre, and often multicentre and international studies are necessary to provide the statistical power to support their findings. Nevertheless, studies from a single institution have the advantage of using uniform protocols and assessment techniques in a relatively ethnically homogeneous population. In spite of its limitations, the present study highlights the importance of screening and follow-up of patients with LCH for the presence of cardiovascular risk factors such as glucose metabolism abnormalities that could lead to an increased risk of cardiovascular disease later in life.

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