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Paediatric obesity and cardiovascular dysfunction: searching for early markers of damage

Background

During the past two decades, the prevalence of obesity in children has risen worldwide. Childhood obesity causes a wide range of serious complications, increasing the risk of premature morbidity and mortality and raising public-health concerns (1).

In addition, obese children are more prone to become obese adults, with an enhanced risk for cardiovascular diseases (CVD) (2,3). A cluster of cardiovascular disease risk factors as hypertension, dyslipidaemia, chronic inflammation, increased blood clotting tendency, endothelial dysfunction, and hyperinsulinaemia has been identified in children as young as 5 years of age (4). Among adolescents and young adults, the presence of cardiovascular disease risk factors correlates with asymptomatic coronary atherosclerosis, and lesions were more advanced in obese subjects (5-7). Moreover, recent studies reported that paediatric obesity is often associated with current cardiovascular abnormalities, suggesting that this issue requires immediate attention to prevent progressive cardiovascular damage.

Childhood obesity has been related to an impaired cardiac structure and function. Obese children show left atrial (LA) and left ventricular (LV) dimensions and mass significantly greater than lean controls (8-10). Moreover, greater epicardial fat has also been reported in children with obesity. Moreover, obese children and adolescents show an altered cardiac mechanics including diastolic dysfunction and systolic dysfunction at rest and during exercise (8-10, 11,12).

It is well known that atherogenesis and arterial wall damage begin during childhood. Furthermore, there is evolving evidence that clinical indicators of atherosclerosis such as CIMT, arterial stiffness, and endothelial dysfunction are altered in children with obesity, although mechanisms by which these effects are mediated have not been fully understood (13-17).

Therefore, the detection of a high cardiovascular risk profile associated to obesity during childhood would allow the early diagnosis of cardiovascular complications and the institution of preventive and therapeutic measures.

The mechanisms relating adiposity to cardiovascular dysfunction in children are difficult to ascertain (18). Obesity is a state of chronic low-level inflammation and increased oxidative stress.

Oxidative stress plays an important role in the pathogenesis of cardiovascular alterations by either triggering or exacerbating the biochemical processes accompanying endothelial dysfunction (19).

Moreover, adipose tissue acts as a secretory gland, releasing hormones and adipokines with pro- or anti-inflammatory activity (20). Clinical studies of obese adult subjects have observed an association between plasma levels of adipokines and markers of inflammation and/or oxidative stress (19).

Among various adipokines, adiponectin, the most abundant protein secreted by the adipose tissue and circulating in human plasma as multimeric forms, seems to play an important role for several reasons. First, in contrast to other adipokines, which are up-regulated in obesity, secretion of adiponectin is markedly reduced in obese subjects and in most experimental models of obesity. Second, adiponectin seems to exert mainly positive activities on metabolism, vascular tone and inflammatory reaction. Consequently, in contrast to other adipokines, which circulate in excess in obese subjects and exert unbeneficial effects when chronically elevated, deficiency rather than excess of adiponectin is implicated in obesity-associated complications. Finally, serum concentration of adiponectin is very high in comparison to other hormones and cytokines, which suggests that apart from binding to specific high-affinity receptors, this protein may also have some less specific low affinity targets (21).

Adiponectin has been associated with endothelial improvement and vascular protection through the activation of an endothelial isoform of nitric oxide (eNOS)–related signalling and with anti-inflammatory properties and antiatherogenic effects (22,23). Moreover, adiponectin plays a protective role in myocardial ischemia-reperfusion injury and myocardial hypertrophy in human studies (21) and increase systolic and diastolic function and coronary blood flow in animal models (24). Conversely, adiponectin deficiency has been reported to be predictive of future adverse cardiac events and has been associated with increased oxidative stress and inferior cardiac function recovery (21).

Thus, an impaired production of adipokines, such as in obese children, may be a key mechanism linking obesity with inflammation and oxidative stress. Moreover, adiponectin may be one of the primary signals linking inflammation, obesity and cardiovascular dysfunction.

The understanding of these complex mechanisms and the identification of possible early markers of cardiovascular damage are therefore necessary in order to establish preventive and therapeutic measures in childhood and to decrease cardiovascular morbidity and mortality in adulthood.

Aims

In the present research study we aim to examine cardiac and vascular changes in obese children and adolescents and to analyze the relationship with plasmatic adipokines, inflammation and oxidative stress markers at baseline and after 6 and 12 months of an isocaloric Mediterranen balanced diet plus an exercise training regimen.

The first main endpoint will be the difference of 10% of diastolic function between controls and obese subjects.

The second endpoint will be the variation of 20% of diastolic function in the obese group after 6 months of treatment.

The third endpoint will be the evaluation of the association between cardiovascular dysfunction and adiponectin and, inflammatory and oxidative stress plasmatic markers.

Subjects

This study is a single-centre longitudinal study. Subjects will be recruited at Division of Pediatrics, Department of Health Sciences, University of Piemonte Orientale, Novara. All subjects will be evaluated at baseline (T0) and after 6 (T1) and 12 months (T2) of an isocaloric Mediterranen balanced diet plus aerobic training. The study protocol will be in accordance with the ethical guidelines of the Declaration of Helsinki. Informed written consent will be obtained from all subjects and their parents before study.

Inclusion criteria:

- children and adolescents (6-16 years);
- obese and normal-weight (control group) according to the Italian charts;
- both genders;
- diet naïve.

Exclusion criteria:

- heart and respiratory diseases;
- hypo/hyperthyroidism;
- GH deficiency;
- type 1 or type 2 diabetes;
- use of drugs (current or past): hormonal or interfering therapies (lipid-lowering, hypoglycemic, or antihypertensive treatment);
- renal or liver failure.

At the end of the study, obese subjects will be also divided into responders and non-responders to the treatment for the post-hoc analysis.

Clinical and anthropometric variables

All subjects will undergo a thorough medical history with special attention to the presence of cardiovascular risk factors and to the family history (I or II degree) of cardiovascular diseases and a clinical and anthropometric evaluation by trained physicians, using the Italian growth charts (25, *Appendix A*). Pubertal stages will be determined by using the criteria of Marshall and Tanner (26). Height will be measured to the nearest 0.1 cm by the Harpenden stadiometer and weight with light clothing to the 0.1 kg by using a manual scale. BMI will be calculated as body weight divided by squared height (kg/m2). BMI standard deviation score (BMISDS) will be calculated with the LMS method. Waist circumference will be measured with a soft tape, midway between the lowest rib margin and the iliac crest, in the standing position (27, *Appendix B*). Hip circumference will be measured over the widest part of the gluteal region, and the waist-to-hip ratio will be calculated. Systolic (SBP) and diastolic (DBP) blood pressure will be measured three times at the left arms by using a standard mercury sphygmomanometer and the mean value will be recorded and stratified according to paediatric percentiles of National High Blood Pressure Education Program Working Group on High Blood Pressure in Children and Adolescents (28, *Appendix C*).

Biochemical and metabolic evaluation

All subjects will be evaluated after 12 hours fasting for serum C-reactive protein (CRP), total- (Tc), HDL- (HDL-c) cholesterol, triglycerides (TG), AST, ALT, uric acid, creatinine, glucose, and insulin levels. Morning urine samples will be collected for the evaluation of microalbuminuria and urine creatinine. Plasma glucose levels (mg/dl; 1 mg/dl:0,05551 mMol/liter) will be measured by the gluco-oxidase colorimetric method (GLUCOFIX, by Menarini Diagnostici, Florence, Italy). Insulin (mUI/ml; 1 mUI/ml = 7.175 pmol/l) will be measured by chemiluminescent enzyme-labelled immunometric assay (Diagnostic Products Corporation, Los Angeles, CA). Sensitivity: 2 mUI/ml. Intra- and inter-assay CV ranges: 2.5–8.3 and 4.4–8.6%. CRP (mg/dl), T-c (mg/dl; 1 mg/dl: 0.0259 mMol/l), HDL-c (mg/dl; 1 mg/dl: 0.0259 mMol/l), TG (mg/dl; 1 mg/dl: 0.0113 mMol/l), AST (U/l), ALT (U/l), uric acid (mg/dl; 1 mg/dl: 59.48 μMol/l), plasmatic creatinine (mg/dl; 1 mg/dl: 88 μMol/l) and microalbuminuria (mg/mmol urine creatinine; 1 mg/dL: 0.089 mMol/L) will be evaluated using standardized methods in the hospital's chemistry laboratory.

All obese and volunteer controls will perform an OGTT (1.75 g of glucose solution per kg, maximum 75 gr). Impaired fasting glucose (IFG) and impaired glucose tolerance (IGT) will be defined according to American Diabetes Association classifications as fasting plasma glucose of ≥100 to 125 mg/dl, and as 2-h post-OGTT glucose of ≥140 to 199 mg/dl, respectively. Also the

definition of diabetes will be performed according to the criteria of the American Diabetes Association (29). Blood samples during OGTT will be drawn for the determination of glucose and insulin every 30 minutes from 0 to 120 min. Insulin resistance will be estimated, in the basal state, by use of the homeostasis model assessment (HOMA-IR) =fasting glucose x fasting insulin/22.5. Insulin sensitivity will be estimated by the Matsuda [10,000/√(fasting glucose x fasting insulin) x (GmxIm)] and the quantitative insulin sensitivity check index (QUICKI), as defined by 1/{log[fasting insulin (μU/ml)] + log[fasting glucose (mg/dl)]} (30). The stimulus for insulin secretion in the increment in plasma glucose as insulinogenic index (INS) will be calculated as the ratio of the changes in insulin and glucose concentration from 0 to 30 min. Beta-cell compensatory capacity was evaluated by the disposition index (DI) defined as the product of the Matsuda Index and INS (31).

Determination of interleukins, TNFα, plasminogen activator inhibitor-1, and adiponectin

IL-8, IL-10, IL-6, TNFα, plasminogen activator inhibitor-1 (PAI-1), and adiponectin will be measured using specific ELISA kit (R&D Systems) at the Laboratory of Clinical Pediatrics, Department of Health Sciences, University of Piemonte Orientale, Novara.

Plasma markers of oxidative stress measurement and plasmatic NO quantification

Blood samples will be taken for 3-nitrotyrosine, malondialdehyde (MDA), ROS generation, myeloperoxidase (MPO), GSH, and SOD measurement by specific kits. NO will be quantified from blood samples by using the Griess reagent. All these measures will be performed at the Laboratory of Physiology and Experimental Surgery, Department of Translational Medicine, University of Piemonte Orientale, Novara.

Cardiac function

A paediatrician, trained in echocardiography, will perform 2D-echocardiography. Transthoracic echocardiographic measurements will be performed with a GE Vivid 7 Pro, using a 3.0-mHz transducer for two-dimensional M-mode measurements of the left ventricular mass and a linear 3.0- to 11-mHz transducer for the vascular studies according to the recommendations of the American Society of Echocardiography (32). M-mode echocardiography will be used to measure left atrial and ventricular dimensions and left ventricular wall thickness, allowing for the calculation of left ventricular mass after correction for BSA; left ventricular mass and volumes will be calculated by Schiller's formula and Simpson's rule by two-dimensional echocardiography (33). Systolic function (ejection fraction [EF] and fractional shortening [FS]) and diastolic function (early (E) and late (A)

diastolic peak flow velocities, and E/A ratio) will be measured using two-dimensional, M-mode, colour Doppler echocardiography. Interventricular septum (IVSt), left ventricular posterior wall thickness (LVPWt) and left ventricular end diastolic diameter (LVEDD) will be recorded, as well.

Aortic stiffness

The abdominal aortic diameter will be measured at maximum systolic expansion (Ds) and minimum diastolic expansion (Dd) by 2D-echocardiography. All aortic measurements will be made as previously described by Lacombe and Okubo (34,35). Aortic strain (S) will be calculated from the changes in aortic diameter using the formula (S = (Ds-Dd)/Dd). Pressure strain elastic modulus (Ep) will be calculated from S, and the changes in brachial artery pressure will be determined by the formula (Ep=(Ps-Pd)/S). Pressure strain (Ep*), normalized by diastolic pressure, will be calculated using the formula (Ep* = Ep/Pd). While S is the mean strain of the aortic wall, Ep and Ep* are the mean stiffness of the aorta. Whereas S and Ep* are dimensionless ratios, Ep has the dimension newtons per square meter (i.e. force/unit area).

Carotid artery intima-media thickness (CIMT)

A vascular surgeon, blinded of the status of the patients will perform carotid sonography with the subject in supine position with a slight rotation of the neck using a linear 3.0- to 11-mHz transducer. The probe will be placed along the vessel axis to obtain a longitudinal scan of the common carotid arteries. CIMT will be measured 1.5 cm proximal to the carotid artery bifurcation, and the mean of three measurements of each artery will be reported. Data obtained will be compared with normative values, when possible (36).

Endothelium-dependent dilation of the brachial artery

Endothelium-dependent vasodilation will be measured in the right brachial artery in each subject by the same observer. After 10 min of rest, brachial artery blood flow and the diameter of the brachial artery 2 cm above the antecubital fossa will be determined by Doppler ultrasonography using a linear 3.0- to 11-mHz signal transducer. To induce hyperemia, a standard sphygmomanometer cuff will be applied to the forearm just proximal to the wrist joint and inflated to 300 mm Hg for 5 min. Blood flow and brachial artery diameter measurements will be repeated 45–60 sec after the release of sphygmomanometer pressure and will be calculated as the percentage change in brachial diameter and percentage change in flow.

Mitochondria morphology and function

Mitochondria will be isolated from monocytes. Ultrastructural analyses of mitochondria (through transmission electron microscope ZEISS EM 109) will be performed to assess morphologic mitochondrial changes (mitochondrial swelling, decrease in matrix density, possible difference in the sub-plasmalemmal and intrafibrillar sub-fraction of mitochondria, fission-fusion dynamic mitochondrial propriety, mitophagy etc). Moreover, mitochondria will be used for in vitro assays of mitochondrial oxygen consumption, complex I activity (NAD+/NADH), transmembrane potential and mitochondrial dynamic proteins expression (fusion and fission ratio through mitofusin 1 and 2 Western blot analysis). The evaluation mitochondria morphology and function will be performed at the Laboratory of Physiology and Experimental Surgery, Department of Translational Medicine, University of Piemonte Orientale, Novara.

Time course of measurements

All the evaluations previously described will be performed at baseline (T0) and after 6 (T1) and 12 months (T2) of an isocaloric Mediterranen balanced diet plus aerobic training.

Nutritional analysis and interventions

A well-trained and experienced clinical paediatric endocrinologist will assess food consumption in all subjects and will administer an isocaloric Mediterranen balanced diet in obese children. To assess food consumption, foods will be divided according to the classic basic food groups by the Italian Institute of Research on Food and Nutrition (38). Food frequencies questionnaires, validated for a wide range of ages (39), will be also completed by parents. The nutritional counselling will be performed at baseline and after 6 and 12 months, according to Italian LARN Guidelines for age and gender and the Italian food pyramid (38).

Moreover, obese subjects will undergo an exercise training regimen. Exercise will be conducted daily and will consist of 30 minutes of aerobic physical activity. Parents will record every day, on a specific questionnaire, the training performed.

Statistical analysis and power of the study

We will use an ITT analysis. The first main endpoint will be the difference of 10% of diastolic function between controls and obese. Estimates of the expected treatment effects will be obtained by using the SD of previous studies done in pediatric obesity (37). The second endpoint will be the variation of 20% of diastolic function in the obese group after 6 months of treatment. Estimates effects were obtained by using the SD of previous studies on probiotics in children (37). A sample

size of 50 individuals for each arm has been estimated to be sufficient to demonstrate those differences with a 90% power, a significance of 95%, and an estimated drop-out of 10% for the first outcome. A sample size of 125 obese individuals has been estimated to be sufficient to demonstrate those differences with a 90% power, a significance of 95%, and an estimated drop-out of 10% for the second outcome.

Data will be expressed as mean±SD, or percentages. The variation between groups will be evaluated by Student t-test. ANCOVA, correlation analysis and the stepwise regression model with two tailed probability values and 95% CI will be used when needed. $P<0.05$ will be considered significant. Statistical analyses will be performed with SPSS for Windows version 17.0.

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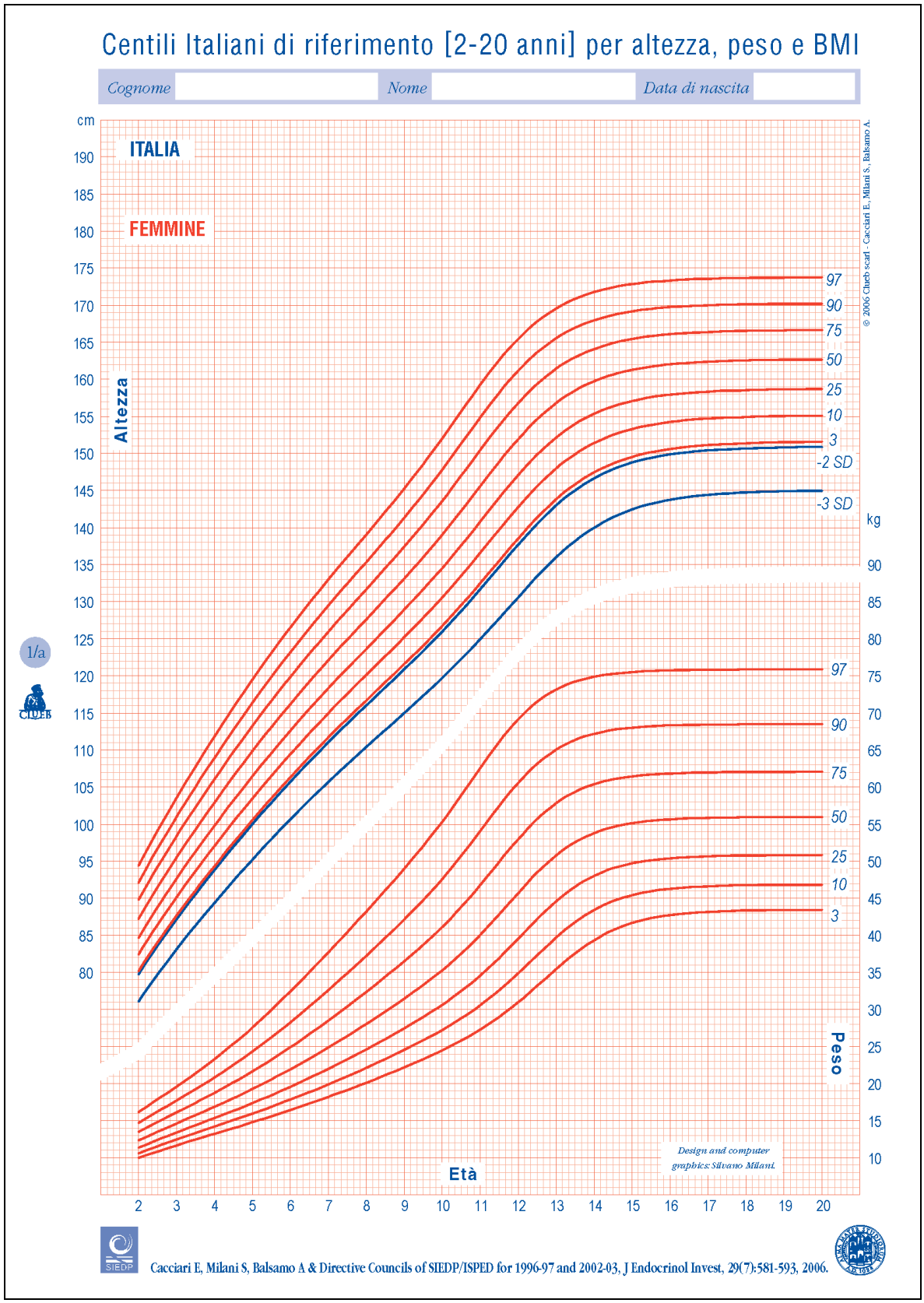
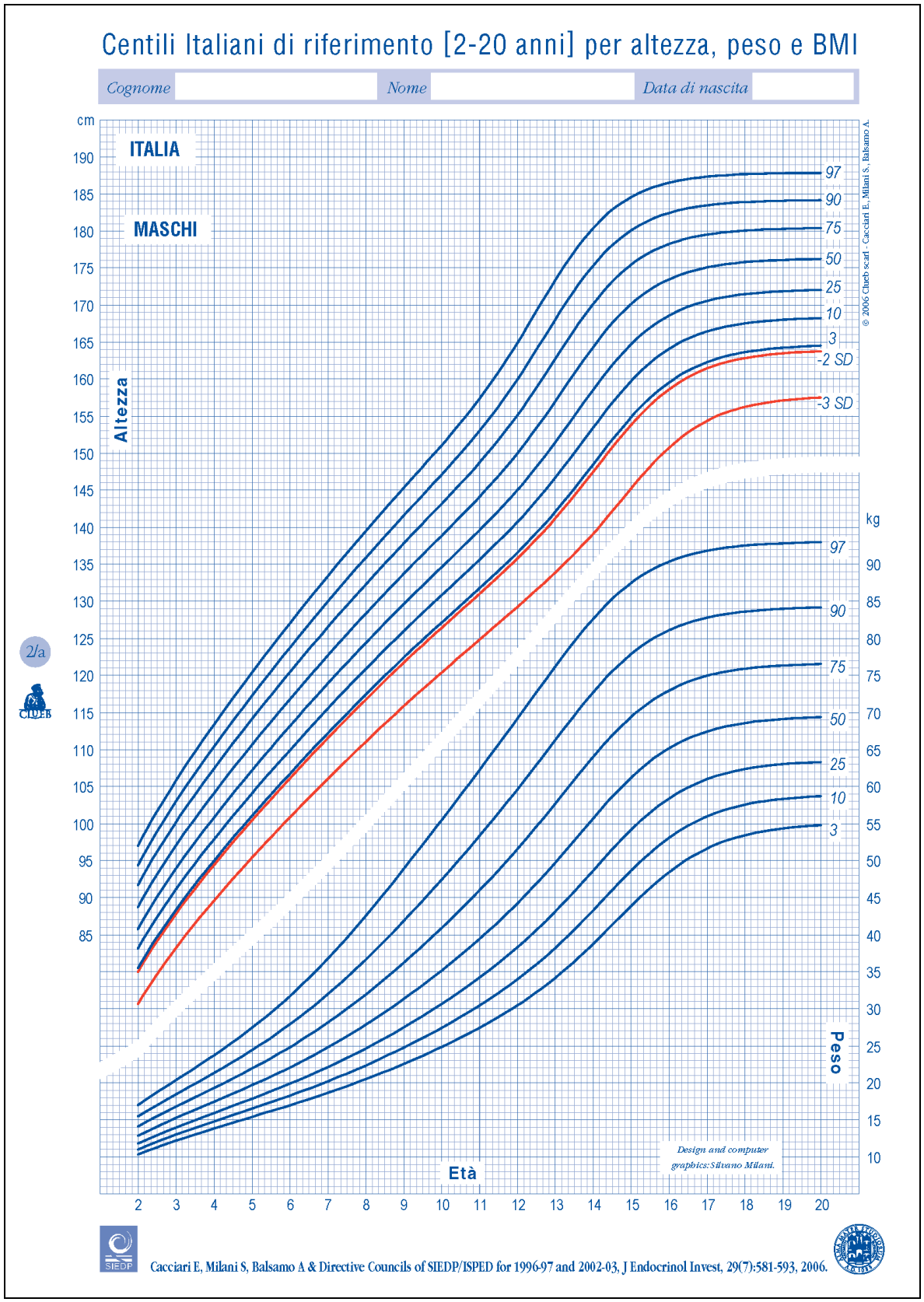
Appendix A

Italian percentiles for weight and height (2-20 years). Girls.
(Cacciari E. et al., 2006)



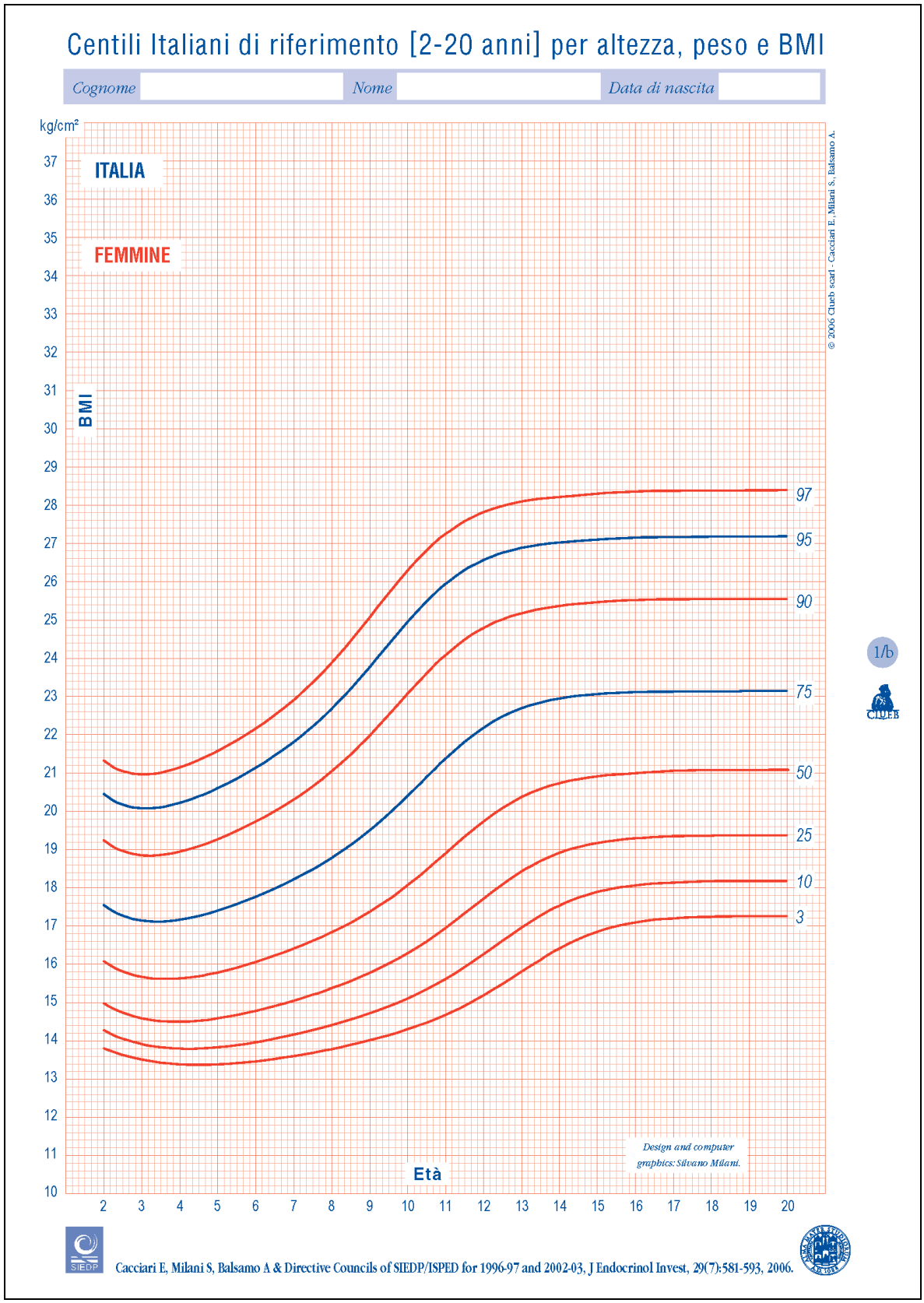
Appendix A

Italian percentiles for weight and height (2-20 years). Boys.
(Cacciari E. et al., 2006)



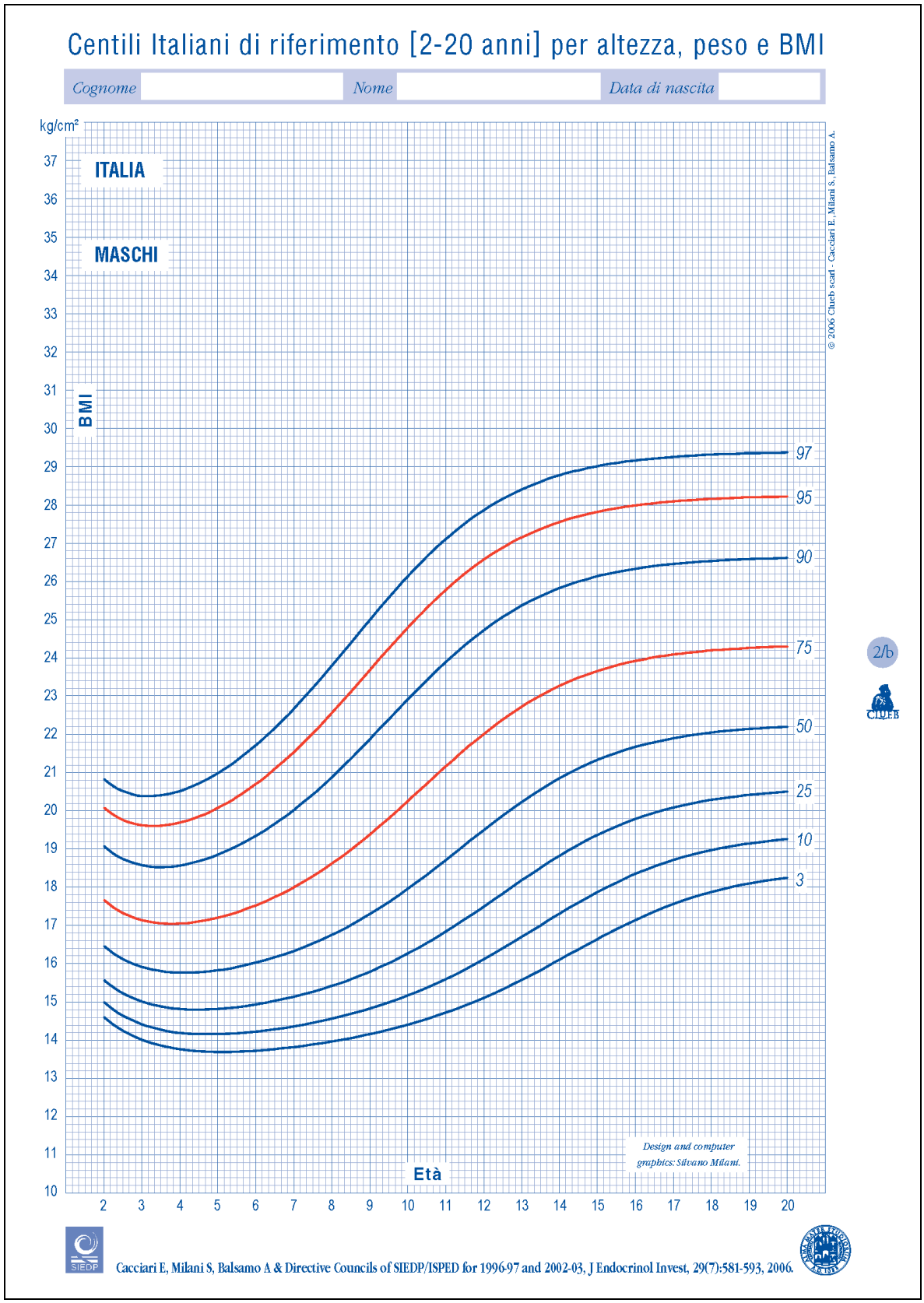
Appendix A

Italian percentiles for BMI (2-20 years). Girls.
(Cacciari E. et al., 2006)



Appendix A

Italian percentiles for BMI (2-20 years). Boys.
(Cacciari E. et al., 2006).



Appendix B

Waist circumference, 90° percentile.
(McCarthy HD. et al., 2001).

BOYS		GIRLS	
Age (years)	**Waist circumference (cm) 90° percentile**	**Age (years)**	**Waist circumference (cm) 90° percentile**
5	55,6	5	55,4
6	57,1	6	57,0
7	58,8	7	58,7
8	60,9	8	60,4
9	63,2	9	62,0
10	65,6	10	63,6
11	67,9	11	65,4
12	70,4	12	67,3
13	73,1	13	69,1
14	76,1	14	70,6
15	79,0	15	71,7
16	81,8	16	72,6

Appendix C

Blood pressure percentiles. Girls.
(National High Blood Pressure Education Program Working Group on High Blood Pressure in Children and Adolescents, 2004).

BP Levels for Girls by Age and Height Percentile

Age, y	BP Percentile	SBP, mm Hg							DBP, mm Hg						
		Percentile of Height							Percentile of Height						
		5th	10th	25th	50th	75th	90th	95th	5th	10th	25th	50th	75th	90th	95th
1	50th	83	84	85	86	88	89	90	38	39	39	40	41	41	42
	90th	97	97	98	100	101	102	103	52	53	53	54	55	55	56
	95th	100	101	102	104	105	106	107	56	57	57	58	59	59	60
	99th	108	108	109	111	112	113	114	64	64	65	65	66	67	67
2	50th	85	85	87	88	89	91	91	43	44	44	45	46	46	47
	90th	98	99	100	101	103	104	105	57	58	58	59	60	61	61
	95th	102	103	104	105	107	108	109	61	62	62	63	64	65	65
	99th	109	110	111	112	114	115	116	69	69	70	70	71	72	72
3	50th	86	87	88	89	91	92	93	47	48	48	49	50	50	51
	90th	100	100	102	103	104	106	106	61	62	62	63	64	64	65
	95th	104	104	105	107	108	109	110	65	66	66	67	68	68	69
	99th	111	111	113	114	115	116	117	73	73	74	74	75	76	76
4	50th	88	88	90	91	92	94	94	50	50	51	52	52	53	54
	90th	101	102	103	104	106	107	108	64	64	65	66	67	67	68
	95th	105	106	107	108	110	111	112	68	68	69	70	71	71	72
	99th	112	113	114	115	117	118	119	76	76	76	77	78	79	79
5	50th	89	90	91	93	94	95	96	52	53	53	54	55	55	56
	90th	103	103	105	106	107	109	109	66	67	67	68	69	69	70
	95th	107	107	108	110	111	112	113	70	71	71	72	73	73	74
	99th	114	114	116	117	118	120	120	78	78	79	79	80	81	81
6	50th	91	92	93	94	96	97	98	54	54	55	56	56	57	58
	90th	104	105	106	108	109	110	111	68	68	69	70	70	71	72
	95th	108	109	110	111	113	114	115	72	72	73	74	74	75	76
	99th	115	116	117	119	120	121	122	80	80	80	81	82	83	83
7	50th	93	93	95	96	97	99	99	55	56	56	57	58	58	59
	90th	106	107	108	109	111	112	113	69	70	70	71	72	72	73
	95th	110	111	112	113	115	116	116	73	74	74	75	76	76	77
	99th	117	118	119	120	122	123	124	81	81	82	82	83	84	84
8	50th	95	95	96	98	99	100	101	57	57	57	58	59	60	60
	90th	108	109	110	111	113	114	114	71	71	71	72	73	74	74
	95th	112	112	114	115	116	118	118	75	75	75	76	77	78	78
	99th	119	120	121	122	123	125	125	82	82	83	83	84	85	86
9	50th	96	97	98	100	101	102	103	58	58	58	59	60	61	61
	90th	110	110	112	113	114	116	116	72	72	72	73	74	75	75
	95th	114	114	115	117	118	119	120	76	76	76	77	78	79	79
	99th	121	121	123	124	125	127	127	83	83	84	84	85	86	87
10	50th	98	99	100	102	103	104	105	59	59	59	60	61	62	62
	90th	112	112	114	115	116	118	118	73	73	73	74	75	76	76
	95th	116	116	117	119	120	121	122	77	77	77	78	79	80	80
	99th	123	123	125	126	127	129	129	84	84	85	86	86	87	88
11	50th	100	101	102	103	105	106	107	60	60	60	61	62	63	63
	90th	114	114	116	117	118	119	120	74	74	74	75	76	77	77
	95th	118	118	119	121	122	123	124	78	78	78	79	80	81	81
	99th	125	125	126	128	129	130	131	85	85	86	87	87	88	89
12	50th	102	103	104	105	107	108	109	61	61	61	62	63	64	64
	90th	116	116	117	119	120	121	122	75	75	75	76	77	78	78
	95th	119	120	121	123	124	125	126	79	79	79	80	81	82	82
	99th	127	127	128	130	131	132	133	86	86	87	88	88	89	90
13	50th	104	105	106	107	109	110	110	62	62	62	63	64	65	65
	90th	117	118	119	121	122	123	124	76	76	76	77	78	79	79
	95th	121	122	123	124	126	127	128	80	80	80	81	82	83	83
	99th	128	129	130	132	133	134	135	87	87	88	89	89	90	91
14	50th	106	106	107	109	110	111	112	63	63	63	64	65	66	66
	90th	119	120	121	122	124	125	125	77	77	77	78	79	80	80
	95th	123	123	125	126	127	129	129	81	81	81	82	83	84	84
	99th	130	131	132	133	135	136	136	88	88	89	90	90	91	92
15	50th	107	108	109	110	111	113	113	64	64	64	65	66	67	67
	90th	120	121	122	123	125	126	127	78	78	78	79	80	81	81
	95th	124	125	126	127	129	130	131	82	82	82	83	84	85	85
	99th	131	132	133	134	136	137	138	89	89	90	91	91	92	93
16	50th	108	108	110	111	112	114	114	64	64	65	66	66	67	68
	90th	121	122	123	124	126	127	128	78	78	79	80	81	81	82
	95th	125	126	127	128	130	131	132	82	82	83	84	85	85	86
	99th	132	133	134	135	137	138	139	90	90	90	91	92	93	93
17	50th	108	109	110	111	113	114	115	64	65	65	66	67	67	68
	90th	122	122	123	125	126	127	128	78	79	79	80	81	81	82
	95th	125	126	127	129	130	131	132	82	83	83	84	85	85	86
	99th	133	133	134	136	137	138	139	90	90	91	91	92	93	93

Appendix C

Blood pressure percentiles. Boys.
(National High Blood Pressure Education Program Working Group on High Blood Pressure in Children and Adolescents, 2004).

BP Levels for Boys by Age and Height Percentile

Age, y	BP Percentile	SBP, mm Hg							DBP, mm Hg						
		Percentile of Height							Percentile of Height						
		5th	10th	25th	50th	75th	90th	95th	5th	10th	25th	50th	75th	90th	95th
1	50th	80	81	83	85	87	88	89	34	35	36	37	38	39	39
	90th	94	95	97	99	100	102	103	49	50	51	52	53	53	54
	95th	98	99	101	103	104	106	106	54	54	55	56	57	58	58
	99th	105	106	108	110	112	113	114	61	62	63	64	65	66	66
2	50th	84	85	87	88	90	92	92	39	40	41	42	43	44	44
	90th	97	99	100	102	104	105	106	54	55	56	57	58	58	59
	95th	101	102	104	106	108	109	110	59	59	60	61	62	63	63
	99th	109	110	111	113	115	117	117	66	67	68	69	70	71	71
3	50th	86	87	89	91	93	94	95	44	44	45	46	47	48	48
	90th	100	101	103	105	107	108	109	59	59	60	61	62	63	63
	95th	104	105	107	109	110	112	113	63	63	64	65	66	67	67
	99th	111	112	114	116	118	119	120	71	71	72	73	74	75	75
4	50th	88	89	91	93	95	96	97	47	48	49	50	51	51	52
	90th	102	103	105	107	109	110	111	62	63	64	65	66	66	67
	95th	106	107	109	111	112	114	115	66	67	68	69	70	71	71
	99th	113	114	116	118	120	121	122	74	75	76	77	78	78	79
5	50th	90	91	93	95	96	98	98	50	51	52	53	54	55	55
	90th	104	105	106	108	110	111	112	65	66	67	68	69	69	70
	95th	108	109	110	112	114	115	116	69	70	71	72	73	74	74
	99th	115	116	118	120	121	123	123	77	78	79	80	81	81	82
6	50th	91	92	94	96	98	99	100	53	53	54	55	56	57	57
	90th	105	106	108	110	111	113	113	68	68	69	70	71	72	72
	95th	109	110	112	114	115	117	117	72	72	73	74	75	76	76
	99th	116	117	119	121	123	124	125	80	80	81	82	83	84	84
7	50th	92	94	95	97	99	100	101	55	55	56	57	58	59	59
	90th	106	107	109	111	113	114	115	70	70	71	72	73	74	74
	95th	110	111	113	115	117	118	119	74	74	75	76	77	78	78
	99th	117	118	120	122	124	125	126	82	82	83	84	85	86	86
8	50th	94	95	97	99	100	102	102	56	57	58	59	60	60	61
	90th	107	109	110	112	114	115	116	71	72	72	73	74	75	76
	95th	111	112	114	116	118	119	120	75	76	77	78	79	79	80
	99th	119	120	122	123	125	127	127	83	84	85	86	87	87	88
9	50th	95	96	98	100	102	103	104	57	58	59	60	61	61	62
	90th	109	110	112	114	115	117	118	72	73	74	75	76	76	77
	95th	113	114	116	118	119	121	121	76	77	78	79	80	81	81
	99th	120	121	123	125	127	128	129	84	85	86	87	88	88	89
10	50th	97	98	100	102	103	105	106	58	59	60	61	61	62	63
	90th	111	112	114	115	117	119	119	73	73	74	75	76	77	78
	95th	115	116	117	119	121	122	123	77	78	79	80	81	81	82
	99th	122	123	125	127	128	130	130	85	86	86	88	88	89	90
11	50th	99	100	102	104	105	107	107	59	59	60	61	62	63	63
	90th	113	114	115	117	119	120	121	74	74	75	76	77	78	78
	95th	117	118	119	121	123	124	125	78	78	79	80	81	82	82
	99th	124	125	127	129	130	132	132	86	86	87	88	89	90	90
12	50th	101	102	104	106	108	109	110	59	60	61	62	63	63	64
	90th	115	116	118	120	121	123	123	74	75	75	76	77	78	79
	95th	119	120	122	123	125	127	127	78	79	80	81	82	82	83
	99th	126	127	129	131	133	134	135	86	87	88	89	90	90	91
13	50th	104	105	106	108	110	111	112	60	60	61	62	63	64	64
	90th	117	118	120	122	124	125	126	75	75	76	77	78	79	79
	95th	121	122	124	126	128	129	130	79	79	80	81	82	83	83
	99th	128	130	131	133	135	136	137	87	87	88	89	90	91	91
14	50th	106	107	109	111	113	114	115	60	61	62	63	64	65	65
	90th	120	121	123	125	126	128	128	75	76	77	78	79	79	80
	95th	124	125	127	128	130	132	132	80	80	81	82	83	84	84
	99th	131	132	134	136	138	139	140	87	88	89	90	91	92	92
15	50th	109	110	112	113	115	117	117	61	62	63	64	65	66	66
	90th	122	124	125	127	129	130	131	76	77	78	79	80	80	81
	95th	126	127	129	131	133	134	135	81	81	82	83	84	85	85
	99th	134	135	136	138	140	142	142	88	89	90	91	92	93	93
16	50th	111	112	114	116	118	119	120	63	63	64	65	66	67	67
	90th	125	126	128	130	131	133	134	78	78	79	80	81	82	82
	95th	129	130	132	134	135	137	137	82	83	83	84	85	86	87
	99th	136	137	139	141	143	144	145	90	90	91	92	93	94	94
17	50th	114	115	116	118	120	121	122	65	66	66	67	68	69	70
	90th	127	128	130	132	134	135	136	80	80	81	82	83	84	84
	95th	131	132	134	136	138	139	140	84	85	86	87	87	88	89
	99th	139	140	141	143	145	146	147	92	93	93	94	95	96	97

Current advances in the PhD program

Actually, 35 obese subjects who met the inclusion criteria, have been recruited at Division of Pediatrics, Department of Health Sciences, University of Piemonte Orientale, Novara. All these patients have been evaluated at baseline (T0).

In this population, 18 subjects were females (51.4%), 10 children were prepubertal (28,6%) and the mean age was of 11.3 ± 2.5 years.

All the children underwent clinical and anthropometric evaluation. The mean height was 150.8 ± 2.5 cm, the mean weight was 69.4 ± 2.5 kg, the mean BMI was 29.8 ± 2.5 kg/m^2 and the mean BMI z-score was 2.25 ± 2.52. 25 subjects (74.3%) showed a systolic or a diastolic blood pressure above the 90th percentile for age and sex and all the subjects (100%) showed a waist circumference above the 90th percentile for age and sex.

Biochemical and metabolic evaluation, included an OGTT, was performed in all the patients. 15 children (42.9%) showed an HDL-cholesterol below the 10th percentile and in 7 subjects (20%) triglycerides were above the 90th percentile for age and sex. Impaired fasting glucose (IFG) was detected in 2 children (5.7%) and impaired glucose tolerance (IGT) was found in 4 patients (11.4%). None of the children showed diabetes according to the criteria of the American Diabetes Association. 17 subjects (48.6%) met the International Diabetes Federation (IDF) criteria for metabolic syndrome and 13 patients (37.1%) met the criteria for metabolic syndrome proposed by the National Cholesterol Education Program (NCEP).

The day of the OGTT, at baseline, serum and plasma samples were collected for the determination of IL-8, IL-10, IL-6, TNFα, plasminogen activator inhibitor-1, and adiponectin**,** plasma markers of oxidative stress and plasmatic NO quantification, and isolation of mitochondria from monocytes.

All the 35 obese children underwent a transthoracic 2D-echocardiography. Left atrial and ventricular dimensions and left ventricular wall thickness have been recorded and left ventricular mass was calculated. The mean left ventricular mass index was 65.4 ± 15.2 g/m^2 and the mean left ventricular mass z-score was -0.05 ± 1.25. Regarding systolic function, the mean ejection fraction (EF) was 69.3 ± 8.6% and the mean fractional shortening (FS) was 39.3 ± 6.9%. Regarding diastolic function, the mean E/A ratio was 1.76 ± 0.54. Interventricular septum (z-score, mean ± SD: 0.10 ± 1.0), left ventricular posterior wall thickness (z-score, mean ± SD: 0.45 ± 0.95) and left ventricular end diastolic diameter (z-score, mean ± SD: -0.99 ± 1.00) were recorded, as well.

A vascular echography was performed in all the subjects enrolled in the study. The abdominal aortic diameter was measured at maximum systolic expansion (Ds) and minimum diastolic expansion (Dd). The mean aortic strain was 0.29 ± 0.16. Also pressure strain elastic modulus was (Ep) and pressure strain (Ep*) were calculated. Carotid artery intima-media thickness (CIMT) was measured

in each children as well as endothelium-dependent dilation of the brachial artery, and femoral artery diameters.

Pubblications during PhD program

1. Cadario F, Savastio S, Magnani C, Cena T, Pagliardini V, Bellomo G, Bagnati M, Vidali M, Pozzi E, Pamparana S, Zaffaroni M, **Genoni G**, Bona G. High prevalence of vitamin d deficiency in native versus migrant mothers and newborns in the north of Italy: a call to act with a stronger prevention program. PLoS One, 2015; 10(6):e0129586, doi:10.1371/journal.pone.0129586. eCollection 2015

2. Monzani A, **Genoni G**, Cassone R, Binotti M, Ferrero F, Bona G. Hyperexcitability as the main sign of neonatal hypoxia. Minerva Pediatr, 2015; 67(3):276-277

3. De Rienzo F, Mellone S, Bellone S, Babu D, Fusco I, Prodam F, Petri A, Muniswamy R, De Luca F, Salerno M, Momigliano-Richardi P, Bona G, Giordano M; **Italian Study Group on Genetics of CPHD**. Frequency of genetic defects in combined pituitary hormone deficiency: a systematic review and analysis of a multicentre Italian cohort. Clin Endocrinol (Oxf), 2015; doi: 10.1111/cen.12849 [Epub ahead of print]. As a coauthor listed in the acknowledgements

Congress during PhD program

1. ESPE congress 2015 - **MODERATED POSTER SECTION** (October 2015) **Genoni G**, Esposito S, Agarla V, Monzani A, Castagno M, Raviolo S, Petri A, Prodam F, Bellone S, Bona G. "SHOX deficiency: clinical, radiological signs and value of screening scores"

2. Corso "Le cardiopatie congenite: dalla diagnosi alla profilassi respiratoria" (September 2015) – **Partecipant**

Courses and Seminars

- July 28, 2015. **Le cellule staminali nel danno renale acuto e nel trapianto di rene** (Prof Cantaluppi)

- July 9, 2015. **Miniworkshop on biotechnology for dermatology** (Prof Isidoro, Dr Rolin, Dr Lihoreau)