jcrpe-2018-0153.R3
Original Article

Epicardial fat thickness in children with classic congenital adrenal hyperplasia

Kotb Abbass Metwalley[1], Hekma Saad Farghaly[1], Abdelrahman Abdelhamid[2]
1-Department of Pediatrics, Faculty of Medicine, Assiut University, Assiut, Egypt.
2-Department of Clinical Pathology, Qena Faculty of Medicine, South Valley University, Qena, Egypt

Corresponding author: Kotb Abbass Metwalley
Phone numbers: 0020882368373
kotb72@gmail.com
Received: 07.06.2018
Accepted: 03.10.2018

Abstract:
Objective: Epicardial fat thickness (EFT) is an emerging cardiometabolic risk factor and has been shown to be related to atherosclerosis. EFT has not been studied in the context of CAH. This study aimed to evaluate EFT in children with CAH and its relation to carotid intima media thickness (CA-IMT) and left ventricular functions.
Methods: 36 children with classic CAH were compared with 36 healthy controls. All children had confirmed CAH and received steroid substitution therapy. Patients and controls underwent anthropometric evaluation, measurement of fasting lipids, glucose, insulin, homeostasis model assessment for insulin resistance (HOMA-IR), Left ventricular functions and EFT were assessed using conventional echocardiography. Duplex ultrasonography was used to measure CA-IMT.
Results: Compared to controls, patients had higher EFT (p=0.001), HOMA-IR(p=0.001), CA- IMT (p=0.01), LVMI (p=0.001) and prolonged mitral deceleration time (DcT) (p=0.01).Abnormalities were marked in uncontrolled children on medical treatment. In multivariate analysis in children with classic CAH , EFT correlated positively with waist circumference (OR =1.9,95%Cl=1.07-1.14.p=001), 17-OHP; nmol/l (OR =1.6; 95% CI = 1.33-2.89, p = 0.05), testosterone , ng/dl(OR =1.7; 95% CI = 1.55-2.13, p = 0.01), LVMI (OR=1.14, 95%Cl=1.08-1.13, p=0.0001) , mitral DcT (OR=2.25;95% CI=1.15-2.05, p=0.01) and CA-IMT (OR=1.6 ;95% CI=1.15-2.05, p=0.01).
Conclusions: EFT may be elevated in children with classic CAH particularly those with poor control and is correlated to carotid intima media thickness, left ventricular mass and mitral deceleration time. Measurement of EFT by Echocardiography in CAH children may help to identify those at high risk of developing left ventricular dysfunction and subclinical atherosclerosis.
Keywords : Diastolic function; Echocardiography ;Epicardial fat thickness ; Left ventricular function; Left ventricular mass index; Congenital adrenal hyperplasia ; Carotid intima media thickness, Mitral deceleration time.

What is already known on this topic?
There is an increased risk for cardiac abnormalities in children with congenital adrenal hyperplasia (CAH), Epicardial fat thickness (EFT) is an emerging cardiometabolic risk factor and has been shown to be related to atherosclerosis. Up to author knowledge, no studies assessed EFT in children with CAH.

What this study adds?
EFT is higher in children with CAH than in healthy children and correlated with carotid intima media thickness, left ventricular mass and mitral deceleration time. EFT may be used a possible marker of early atherosclerosis and myocardial function in children with CAH.

Introduction:
Congenital adrenal hyperplasia (CAH) is an autosomal recessive condition resulting from mutations in enzymes required for adrenal steroid synthesis [1]. The 21-hydroxylase deficiency causes about 95% of cases [2]. CAH is commonly divided into the severe classic and the milder nonclassic form. Classic CAH is generally subdivided, depending on the extent of enzymatic impairment, into the salt-wasting (SW) form, presenting with both cortisol and aldosterone deficiency, and the simple virilizing (SV) form characterized by an isolated cortisol deficiency. Both conditions are associated with androgen excess resulting in virilization of female external genitalia [3]. Researchers have long thought that patients with 21-hydroxylase deficiency are at increased risk for cardiovascular diseases due

to the resulting high plasma levels of androgens and/or the harmful effects of glucocorticoid and mineralocorticoid treatment. 21-Hydroxylase deficiency may also have detrimental effects on vascular structures as well as ventricular systolic and diastolic function.[4,5] Obesity, hypertension, dyslipidaemia and insulin resistance have been found to be associated with both CAH itself and the treatment strategies [6].

Few studies have utilized carotid intima-media thickness (CA-IMT) to asses vascular structural changes in children with CAH.[4,5,7] A hindrance to the wider use of CA-IMT measurements in the pediatric population is the lack of standardization of CA-IMT values in this age group[8]. Epicardial fat thickness (EFT) is a layer of adipose tissue surrounding the heart and coronary vessels which measured by ultrasound with a simple noninvasive procedure [9]. EFT is a reliable and sensitive marker of cardiovascular risk and has become an emerging target for therapeutic and medical interventions [10].Since there are no published data on EFT in children with CAH in the literature, The aim of this study is to evaluate the EF measurement and its relation to CA-IMT and left ventricular function in a cohort of children with classic congenital adrenal hyperplasia.

Patients and Methods

Subjects

This cross-sectional controlled study included 36 children (11 males and 25 females) (mean age=13.7±2.4 years) with a confirmed diagnosis of classic CAH [4]. Diagnosis was made based on clinical signs and biochemical assessment (elevated ACTH, 17 hydroxy-progesterone, androstenedione, and testosterone, in addition to low cortisol). Salt wasting was diagnosed in patients with frank hyponatraemia and hyperkalaemia accompanied by low plasma aldosterone and elevated rennin concentrations[11]. Patients were included if they were on glucocorticoid therapy for a minimum of 5 years. They were recruited during the period between January and December 2017 from the Pediatric Endocrinology Unit at Assiut University Children Hospital, Assiut, Egypt. Patients who had chest deformities, chronic lung disease, poor echo window, pericardial and/or pleural effusion on transthoracic echocardiography were excluded from the study. Thirty six healthy children matched for age, gender, pubertal status, and socioeconomic status as control subjects for statistical comparisons were recruited from the General Pediatric Outpatient Clinic of Assiut Children's University Hospital. None of the controls were hypertensive and none were smokers, on any medication, or had a chronic illness. Controls were attending the outpatient clinic either because of minor illness or accompanying their sick siblings. All individuals had classical CAH with 21-hydroxylase deficiency (salt wasting, SW: n=30; simple virilising, SV: n=6) and received glucocorticoid substitution therapy with hydrocortisone (HC; n=30), prednisone (PR; n=6). Prednisone dose was converted to hydrocortisone as 20 mg of hydrocortisone equals to 5 mg of prednisone [12]. Salt-wasting patients were also on 9-alphafludrocortisone therapy at a dose of 50–100 ug/m^2/day. The adequacy of steroid therapy was monitored periodically during follow-up visits every 3–6 months by clinical parameters such as signs of androgen excess , growth curves , bone age and hormonal assay [13]. Patients were divided into two groups according to the degree of control on medical treatment, children with acceptable disease control and children with poor disease control, based on the previously mentioned data [14].

The study protocol was approved by the local Ethics Committee of Assiut Children University Hospital, Assiut , Egypt **(approval number : 312/2017)** and also by Ethics Committee of Faculty of Medicine, Assiut , Egypt in accordance to the Declaration of Helsinki, and written informed consents were obtained from the parents of all participants.

Methods :

All patients and controls were subjected to a full history-taking as well as a thorough clinical examination. Demographic and clinical data were collected as follows: age, gender, duration of treatment, type and dose of steroids, blood pressure (BP), height, weight, and body mass index (BMI). Systolic BP (SBP) and diastolic BP (DBP) were measured in all subjects in the right arm with a standard sphygmomanometer by the same operator. Height and weight were measured using a wall-mounted stadiometer and a calibrated weight scale, and the child was wearing the underwear only. BMI was calculated using the following formula: BMI=weight (kg)/height (m)2. BMI was expressed as standard deviation scores (SDSs) using the Egyptian Growth Reference Data [15] Waist circumference was measured at the midpoint between the lower edge of the ribs in the midaxillary line and the top of the iliac crest by the same clinician. Waist to-height ratio was then calculated as an index of visceral adiposity. Pubertal status was assessed according to Tanner staging [16]. A radiograph of the left hand was used to determine BA using the Greulich-Pyle method [17] and read in a blinded fashion by a single pediatric endocrinologist BA was defined as advanced when greater than the subject's chronological age by 1 year or more [18].

Laboratory methods

Blood samples were withdrawn after an overnight fast for at least 12 hours at 8.00-10.00 a.m. before the first dose of steroids for assessment of the serum levels of total cholesterol (TC), triglycerides (TG), high density lipopro-tein cholesterol (HDL-c), low density lipoprotein cholesterol (LDL-c), glucose and insulin .Serum triglycerides (TG)

and total cholesterol (TC) were assessed by quantitative enzymatic colorimetric technique (Bio Merieux-Diagnostic Chemicals Ltd., Charlottetown, CA, USA). Serum high-density lipoproteins (HDL) were measured by the phosphotungstate precipitation method (Bio Merieux kit, Marcyl'Etoile, Craponne, France). LDL cholesterol was calculated by Friedewald's formula: (Total cholesterol) –(HDL cholesterol)– 1/5 (Triglycerides) [19]. Insulin resistance (IR) was calculated using the homeo-stasis model assessment (HOMA-IR) equation formula as follow: HOMA-IR = Fasting insulin (uU/mL) multiplied by fasting glucose (mmol/L) divided by 22.5. A cut-off level for diagnosing insulin resistance was 2.7 [20]. ACTH, plasma 17-OHP, serum cortisol, androstenedione and testosterone were also measured with commercially available RIA kits (Siemens Healthcare Diagnostics, Inc., LA, CA, USA). Plasma hsCRP The plasma level of hsCRP was measured using the HighSensitivity C-Reactive Protein (hsCRP) Enzyme Immunoassay Test (ELISA) kit for quantitative determination of the C-reactive protein concentration in human serum (catalog no. E29-056; Immunospec Corp., Canoga Park, CA, USA)

Echocardiographic examination

We performed all echocardiographic examinations according to the recommendations of the American Society of Echocardiography [21]. we employed a Philips Envisor Ultrasound System with a S4-2 Broadband Sector (Philips Medical Systems, Inc., Netherlands). Measurements were performed using the machine's incorporated analysis package A M-mode echocardiography was obtained at the left Sternal border. LV dimension, LV fractional shortening (FS) and LV ejection fraction (EF) were measured. LVM index (LVMI) was measured using LVMI calculator. LV diastolic function was evaluated by mitral inflow velocities obtained in the apical four-chamber view. Mitral filling was assessed with the peak velocity of the trans-mitral early filling wave (E) and the peak velocity of atrial late filling (A) and the ratio of both (E/A) was calculated. The interval from the early peak velocity to the zero intercept of the extrapolated deceleration slope (early filling mitral DcT) was measured. The interval between the end of the LV outflow velocity and the onset of mitral inflow (isovolumic relaxation time [IVRT]) obtained by pulsed-wave Doppler with the cursor placed in the LV out-flow near the anterior leaflet of the mitral valve, was measured from the end of the LV ejection to the onset of the mitral inflow.

Epicardial fat thickness measurement:

A two-dimensional (2D) echocardiogram using a standard procedure was performed, with the patient in the left lateral decubitus position. EAT thickness was measured by an experienced pediatric echocardiologist who was blinded to the subjects' clinical and demographic data using the procedure validated by Iacobellis et al.[9]. EAT was identified as the echolucent space between the external wall of the myocardium and the visceral layer of the pericardium. This thickness was measured perpendicularly on the free wall of the right ventricle at the end of systole in three cardiac cycles, using a parasternal long and in the parasternal short axis .The average value of 3 cardiac cycles from each echocardiographic view was used for the statistical analysis.

Carotid intima media thickness measurement:

All participants underwent an ultrasound scan to measure CA-IMT. The studies were performed in the morning between 7:30 and 9:30 a.m. after the children had fasted overnight. All ultrasound scans were performed by an experienced vascular operator who was unaware of children's clinical details. Examination of carotid artery intima-media thickness (CA-IMT) was manually performed using a color duplex flow imaging system (Acuson 128 XP; Acuson Corporation, Mountain View, CA, USA. The examinations were performed while the patients were in a supine position, with their necks slightly extended and their heads turned 45^{c} away from the examination side. From both sides of the head, three images were obtained from the distal common carotid artery, 1 -2 cm proximal to the carotid bulb at end diastole; these images then were stored for offline analyses. All studies were done according to a predetermined, standardized scanning protocol for the right and left carotid arteries [22]. All measurements were performed in all participants by the same pediatric cardiologist who was blind to the clinical and treatment status of the study participants. Reliability of echocardiographic measurements of CA-CMT and EFT were assessed by the intra-observer correlation coefficient in all subjects.

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Statistical analysis:

Calculations were done with the statistical package of SPSS for windows, version 16.0 (SPSS Inc, Chicago, IL). Data were expressed as means + standard deviation, Comparisons of quantitative variables between the study groups were done using paired Student t test. Correlations between EFT and demographic, clinical, and laboratory variables were assessed using Pearson test. Multiple logistic regression analysis was used to determine the factors that were significantly associated with high EFT . The odds ratios (ORs), 95% confidence intervals CIs), and significances were calculated. For all tests, values of $P < .05$ were considered statistically significant.

Results:

Demographic and anthropometric data of the studied groups were shown in (Table 1). It shows that bone age in the patient group was advanced by an average of 2 years compared with chronological age. Compared with healthy controls, children with CAH exhibited increased visceral adiposity, as suggested by higher values of BMI SDS, waist circumference, hip circumference, and waist to height ratio. Moreover, CAH children had higher SBP and DBP [although all children had blood pressures within the normal ranges, i.e. normotensive].

Laboratory data of the studied groups were shown in (Table 2) , levels of TC, TG, LDL-c, fasting blood glucose, fasting insulin, and HOMA-IR hs-CRP , 17-OHP, androstenedione and testosterone were significantly higher while levels of HDL-c were significantly lower compared to control subjects.

The results of echocardiographic. EFT and CA-IMT examinations are shown in (Table 3).We reported no significant differences in EF and FS values between patients and control subjects. However, compared to control subjects, patients had higher LVMI value, indicating myocardial hypertrophy, and lower E/A ratio, higher IVRT values, and prolonged mitral DcT, indicating impaired diastolic function and increased CA-IMT and higher EFT. Intra-observer agreement on CA-CMT and EFT measurements were excellent: intra-observer correlation coefficient was 0.94 and 0.95, respectively, suggesting an excellent reproducibility of these measures. Compared to patients who were well controlled (n=16), patients who were uncontrolled (n=20) were older, had advanced bone ages, and had higher levels of 17-OHP, testosterone, and hs-CRP, and higher values of LVMI, mitral DcT, EFT and CA-IMT (Table 4).

(Table 5): EAT thickness showed a statistically significant positive correlation with body mass index , waist circumference ,SBP,DBP HOMA-IR ,hs-CRP , 17-OHP , testosterone ,LVMI, mitral DcT and CA-IMT .

In multivariate analysis in children with CAH, we reported that EFT was significantly correlated with waist circumference (OR = 1.9; 95% CI = 1.45-2.4, p = 0.05) , 17-OHP; nmol/l (OR =1.6; 95% CI = 1.33-2.89, p = 0.05) , HOMA-IR (OR = 1.3; 95% CI 1.04- 1.34, p = 0.05)testosterone , ng/dl(OR =1.7; 95% CI = 1.55-2.13, p = 0.01) ,mitral DcT (OR = 2.2; 95% CI 1.15-2.05, p = 0.01),CA-IMT (OR = 1.7; 95% CI 1.16-1.57, p = 0.01), and LVMI(OR = 1.1; 95% CI 1.08-1.13, p = 0.01) (Table 6).

Discussion

The results of the present study demonstrate that (1) children with classic CAH may have subclinical LV hypertrophy , diastolic dysfunction and subclinical atherosclerosis ; (2) EFT was higher in patients with CAH than in the healthy control; (2) EFT correlated to carotid intima media thickness, left ventricular mass and mitral deceleration time ; (3) EFT may be used a possible marker of endothelial dysfunction and myocardial dysfunction in children with CAH. The classical cardiovascular risk factors in children with CAH i.e. obesity ,hypertension , dyslipidemia , steroid treatment and others were previously discussed in many publication [4,5,6] and it is beyond the scope of this study.

To our knowledge, this study is the first to demonstrate that EFT was significantly increased in children with classic CAH compared with control children (P <0.001). In addition, we showed that EFT was correlated positively with CA-CMT. The multiple linear regression analysis showed that the CIMT was the variable that most influenced EAT . EFT was reported to be increased in children with positive family history of type 2 DM and considered as risk factor for early atherosclerosis. [23]A meta-analysis showed that EFT may be an effective marker for the prediction of coronary heart disease [24]. EAT is thought to play a pivotal role in the pathogenesis of coronary artery disease(CAD) as it releases a wide range of biologically active molecules that modulate vascular smooth-muscle contraction[25]. Their paracrine effects might be attributable to their location being close to the adventitia and extravascular bed [26]. Gastaldelli et al.[27] reported the existence of a link between EAT and hypertension, atherosclerosis, and coronary heart disease. Several studies have emphasized the link between EFT and the severity of CAD [28]. EFT has an important role in the inflammatory process within the atherosclerotic plaque [29]. Furthermore, it has been shown that EFT products induce increased cell surface expression of adhesion molecules, enhance adhesion monocytes to coronary artery endothelial cells, and facilitate migration of adherent monocytes [30]. . Echocardiographic EFT measurements provide some advantages when used to assess the cardiometabolic risk, being an objective, quantified, non-invasive, low cost, routine applicability, avoidance of exposure to radiation, and potential for monitoring therapeutic effects. It may be also used as a simple marker for identification of CAH patients with higher CV risk who may need further cardiac evaluation [31].

In the present study, children with CAH had echocardiographic changes indicating presence of LV hypertrophy as evidenced by increased LVMI. Moreover ,our study showed significant positive correlations between EFT levels and LVMI that remained significant with EFT after regression analysis which suggest the detrimental effect of EFT excess on the myocardium of patients with CAH .This is in agreement with Corradi et al.[32] who reported that EFT have an important role in left ventricular hypertrophy . Some mechanisms may be evoked to explain this correlation. We could assume that the increased visceral fat directly effects LV output and stroke volume to perfuse the increased body mass. Additionally, the biochemical properties of visceral adipose tissue, such as insulin resistance, high free fatty acids levels, and -adrenergic activity, could contribute to LV hypertrophy [33].

In the present study, children with CAH had echocardiographic changes indicating presence of diastolic dysfunction (as evidenced by reduced E/A ratio and prolonged IVRT and mitral DcT). Regression analysis revealed that EFT in CAH patients correlates with mitral DcT .This is in agreement with the study of Van der Meer which showed that myocardial fat has progressive and harmful effects on LV diastolic function [34]. Diastolic dysfunction has been considered as one of the first echocardiographic abnormalities to appear in patients with atherosclerotic cardiovascular disease with a high rate of release of free fatty acids (FFA) [35], which encounter no physical barrier or fascia before reaching the cardiomyocytes; [36] therefore, the myocardium receives a double dose of FFA from both the EFT and the systemic circulation. EFT is a source of several bioactive molecules that might directly influence the myocardium [37]. In metabolic and cardiovascular disease states, these fat tissues expand, becoming hypoxic and dysfunctional and recruiting phagocytic cells which would lead to reducing the production of protective cytokines and impaired cardiac function eventually[38,39].

BMI and waist circumference are the widely accepted measurements of generalized adiposity; however they are poor indicators for visceral obesity. It is well known that visceral adipose tissue accumulation is associated with subclinical atherosclerosis and increased cardiovascular mortality and morbidity .In this study , we found a very good correlation between EFT and waist circumference by multiple regression analysis in children with CAH , on the other hand ,we did not reported significant correlation between BMI-SDS and EFT ; these data indicate that waist circumference is a better anthropometric cardiovascular risk predictor and support the evidence that EFT is related to visceral fat, rather than total adiposity [40]. Mavri et al. [41] suggested that CA-IMT regression may also be achieved by weight reduction programs. Altin et al. [42] suggested that laparoscopic sleeve gastrectomy induced weigh loss result in regression of CA-IMT and EFT. Marked adipose mass reduction is associated with dramatic changes in circulating adipokine levels, with leptin reduction and adiponectin increase, thereby leading to reduced leptin/adiponectin ratio. Of note, such ratio was found to be directly correlated with CA- IMT in male subjects [43].

Testosterone levels were significantly higher in our subjects with CAH compared to controls particularly in children who were poorly controlled on medical treatment.In addition, testosterone levels correlated positively and significantly with EFT. Colgecen et al. [44] reported that subjects in advanced stages of androgenetic alopecia had higher echocardiographically measured EFT than controls. Moreover, Cakir et al. [45] reported strong positive correlation between testosterone levels and EFT in patients with polycystic ovarian disease .This suggest that androgen excess may be responsible for the increased EFT in patients with CAH. The treating physicians should be aware that amelioration of androgen excess in patients with CAH should also be considered as a target for cardiovascular prevention and is not only a way to improve hyperandrogenic symptoms**.**

In the present study, children with CAH had higher HOMA-IR than controls . EFT were correlated positively and significantly with HOMA-IR by multiple regression analysis . This in agreement with Manco et al.[46] who reported that EAT is a significant marker of increased insulin resistance . These observations suggest that epicardial fat is a tissue with high insulin resistance. [47]. EFT is associated with high lipolytic activity that is probably due to the reduced antilipolytic effect of insulin in this tissue and an increased expression of B-adrenergic receptors, especially B-3 receptors, whose stimulation activates lipolysis and increases the release of free fatty acids able to promote blood pressure increase through different pathways, including adrenergic stimulation, increased oxidative stress, endothelial dysfunction, and vascular cell growth[48] **.**

Study limitations:

We recognize that this study has some limitations:

1- The sample size was relatively small (although significant) and included a wide age range of patients due to the difficulties in enrolling and studying CAH children.

2- Cross-sectional design. The results cannot be generalized to the general population.

3- CA-IMT measurements were performed manually.

3- We could not confirm EFT using the standard MRI methods. However. Echocardiographic calculation of epicardial fat also showed good reliability when compared with magnetic resonance epicardial fat measurements [49].

4-Epicardial adipose tissue has a three dimensional distribution, 2-dimensional echocardiography may not completely assess the total amount of epicardial adiposity.

Conclusions:

EFT may be elevated in children with classic CAH particularly those with poor control and is correlated to carotid intima media thickness, left ventricular mass and mitral deceleration time. Measurement of EFT by Echocardiography in CAH children may help to identify those at high risk of developing left ventricular dysfunction and subclinical atherosclerosis. Future prospective much larger multicenter studies are required to confirm our results.

Authors' contributions

Kotb Abbass Metwalley participated in the design of the protocol of the study, coordination of the research, performance of the clinical part, analyses of the data, and writing the draft of the paper. Hekma Saad Farghaly participated in the design of the protocol of the study, performed the echocardiography, and participated in the analyses of the data and writing the draft of the paper. Abdelrahman Abdelhamid performed the laboratory investigation and participated in the analyses of the results. All the authors read and approved the manuscript. All the authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work

Compliance with ethical standard

Conflict of interest

All authors declared no potential conflicts of interest with respect to the research, authorship and/or publication of this article. All authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work.

Ethical approval :

The study protocol was approved by the local Ethics Committee of Assiut Children University Hospital, Assiut , Egypt and also by Ethics Committee of Faculty of Medicine, Assiut , Egypt.

Funding source: No funding was secured for this study.

Financial disclosure :The authors have no financial relationships relevant to this article to disclose

Informed consent :Written informed consents were obtained from the parents of all participants.

References:

1- Labarta JI, Bello E, Ruiz-Echarri M, Rueda C, Martul P, Mayayo E, FerrándezLongás A (2004) Childhood-onset congenital adrenal hyperplasia: long-term outcome and optimization of therapy. J Pediatr Endocrinol Metab 17(3):411–422

2 -White PC, Speiser PW (2000) Congenital adrenal hyperplasia due to 21-hydroxylase deficiency. Endocr Rev 21:245–291

3-Speiser PW, White PC (2003) Congenital adrenal hyperplasia. N Engl J Med 349:776–788

4- Amr NH, Ahmed AY, Ibrahim YA (2014) Carotid intima media thickness and other cardiovascular risk factors in children with congenital adrenal hyperplasia. J Endocrinol Investig 37(10):1001–1008

5-Marra AM, Improda N, Capalbo D, Salzano A, Arcopinto M, De Paulis A, Alessio M, Lenzi A, Isidori AM, Cittadini A, Salerno M (2015) Cardiovascular abnormalities and impaired exercise performance in adolescents with congenital adrenal hyperplasia. J Clin Endocrinol Metab 100(2):644–652

6-Mooij CF, Kroese JM, Claahsen-van der Grinten HL, Tack CJ, Hermus AR.Unfavourable trends in cardiovascular and metabolic risk in paediatric and adult patients with congenital adrenal hyperplasia? Clin Endocrinol(Oxf). 2010;73:137–146

7-Wasniewska M, Balsamo A, Valenzise M, Manganaro A, Faggioli G, Bombaci S, Conti V, Ferri M, Aversa T, Cicognani A, De Luca F (2013) Increased large artery intima media thickness in adolescents with either classical or non-classical congenital adrenal hyperplasia. J Endocrinol Investig 36(1):12–15

8-Fusaro, M. F. G. S., Zanini, J. L. S. S., & Silva, I. N. (2016). Increased carotid intima-media thickness in Brazilian adolescents with type 1 diabetes mellitus. Diabetology & metabolic syndrome, 8(1), 74.

9- Iacobellis G, Assael F, Ribaudo MC, Zappaterreno A, Alessi G, Di Mario U, Leonetti F. Epicardial fat from echocardiography: a new method for visceral adipose tissue prediction. Obes Res 2003;11:304–310.

10-Bachar GN, Dicker D, Kornowski R, et al 2012 : Epicardial adipose tissue as a predictor of coronary artery disease in asymptomatic subjects. Am J Cardiol;110:534–538

11-Merke DP, Bornstein SR 2005 .Congenital adrenal hyperplasia.Lancet.;365:2125–2136

12-Labarta JI, Bello E, Ruiz-EcharriM, Rueda C, Martul P, Mayayo E, FerrándezLongás 2004 A. Childhood-onset congenital adrenal hyperplasia:long-term outcome and optimization of therapy. J Pediatr Endocrinol Metab J Pediatr Endocrinol Metab.;17 Suppl 3:411-422.

13-Joint LWPES/ESPE CAH Working Group.2002 Consensus statement on 21-hydroxylase deficiency from the Lawson Wilkins Pediatric Endocrine Society and the European Society for Paediatric Endocrinology. J Clin Endocrinol Metab.;87(9):4048-4053.

14- Finkielstain GP, Kim MS, Sinaii N, Nishitani M, Van Ryzin C, Hill SC, Reynolds JC, Hanna RM, Merke DP.2012 Clinical characteristics of a cohort of 244 patients with congenital adrenal hyperplasia. J Clin Endocrinol Metab.;97:4429–4438.

15-Diabetes Endocrine Metabolism Pediatric Unit, Cairo University Children's Hospital. Egyptian growth curves 2002. [http://dempuegypt.blogspot.com].

16-Tanner JM, Whitehouse RH.1975 A note on the bone age at which patients with true isolated growth hormone deficiency enter puberty. J Clin Endocrinol Metab.;41(4):788–790. [PMID:170299]

17-Greulich WW, Pyle SI. Stanford, CA: Stanford University Press; 1959. Radiographic Atlas of Skeletal Development of the Hand and Wrist
18-Bretones, P., Riche, B., Pichot, E., et al. (2016). Growth curves for congenital adrenal hyperplasia from a national retrospective cohort. Journal of Pediatric Endocrinology and Metabolism, 29(12), 1379-1388
19- Chen, Y., Zhang, X., Pan, B., Jin, X., Yao, H., Chen, B. Chen, H. (2010). A modified formula for calculating low-density lipoprotein cholesterol values. Lipids in health and disease, 9(1), 52.
20-Gutch M, Kumar S, Razi SM, Gupta KK, Gupta A.(2015) Assessment of insulin sensitivity/resistance. Indian Journal of Endocrinology and Metabolism.19(1):160-164.
21-Park MK (2008) Pediatric cardiology for practitioners. Noninvasive techniques (Chap. 6), 5th edn., An imprint of Elsevier, Part2: Special tools in evaluation of cardiac patients Elsevier, Mosby
22-Ja¨rvisalo MJ, Jartti L, Na ¨nto¨-Salonen K, Irjala K, Ro ¨nnemaa T, Hartiala JJ, Celermajer DS, Raitakari OT (2001) Increased aortic intima-media thickness: a marker of preclinical atherosclerosis in high-risk children. Circulation 104:2943–2947
23-Mahfouz, R. A., Alzaiat, A., & Yousry, A. (2015). Relationship of epicardial fat thickness with endothelial and cardiac functions in children with family history of type 2 diabetes mellitus. Echocardiography, 32(1), 28-33.
24-Baker AR, Silva NF, Quinn DW et al., (2006): Human epicardial adipose tissue expresses a pathogenic profile of adipocytokines in patients with cardiovascular disease. Cardiovasc. Diabetol. 5: 1
25. Bachar GN, Dicker D, Kornowski R, Atar E(2012)Epicardial adipose tissue as a predictor of coronary artery disease in asymptomatic subjects. Am J Cardiol.; 110 (4):534
26-Sengul C, Cevik C, Ozveren O, et al.(2012) Epicardial fat thickness is associated with non-dipper blood pressure pattern in patients with essential hypertension. Clin Exp Hypertens.;34:165–170.
27-Gastaldelli A, Basta G. Ectopic fat and cardiovascular disease: what is the link?2010 Nutr Metab Cardiovasc Dis.;20:481–490.
28. Nakanishi R, Rajani R, Cheng VY, Gransar H, Nakazato R, Shmilovich H, et al.2011 Increase in epicardial fat volume is associated with greater coronary artery calcification progression in subjects at intermediate risk by coronary calcium score: a serial study using non-contrast cardiac CT. Atherosclerosis.; 218 (2):363 -8.
29. Şengül C, Özveren O.2013 Epicardial adipose tissue: a review of physiology, pathophysiology, and clinical applications. Anadolu Kardiyol Derg.;13(3):261-5.
30-Cengel A. 2012Epicardial adipose tissue, metabolic syndrome, inflammation, and cardiovascular risk. Turk Kardiyol Dern Ars.;40(8):696-8
31-Iacobellis, Gianluca, and Howard J. Willens. 2009"Echocardiographic epicardial fat: a review of research and clinical applications." Journal of the American Society of Echocardiography 22.12 1311-1319.
32-Corradi D, Maestri R, Callegari S, et al., (2004): The ventricular epicardial fat is related to the myocardial mass in normal, ischemic and hypertrophic hearts. Cardiovasc Pathol; 13: 313-6.
33- Iacobellis G, Ribaudo MC, Zappaterreno A, et al., (2004): Relation between epicardial adipose tissue and left ventricular mass. Am J Cardiol; 94: 1084-7.
34-Van der Meer RW, Rijzewijk LJ, Diamant M, et al., (2008): The ageing male heart: myocardial triglyceride content as independent predictor of diastolic function. Eur Heart J 29:1516-1522.
35-Harada, M., & Tabako, S. (2016). Carotid atherosclerosis is associated with left ventricular diastolic function. Journal of echocardiography, 14(3), 120-129.
36-Iacobellis G, Corradi D and Sharma AM (2005): Epicardial adipose tissue: anatomic,nbiomolecular and clinical relationships with the heart. Nat. Clin. Pract. Cardiovasc. Med. 2:536–543.
37- Greenstein AS, Khavandi K, Withers SB, et al., (2009): Local inflammation and hypoxia abolish the protective anticontractile properties of perivascular fat in obese patients. Circulation 119: 1661–1670.
38-Henrichot E, Juge-Aubry CE, Pernin A, et al., (2005): Production of chemokines by perivascular adipose tissue: a role in the pathogenesis of atherosclerosis? Arterioscler Thromb Vasc Biol 25:2594–2599.
39- Xu Y, Cheng X, Hong K, et al., (2012): How to interpret epicardial adipose tissue as a cause of coronary artery disease: a meta-analysis. Coronary Artery Dis 23: 227-233.
40- Janssen I, Heymsfield SB, Allison DB, Kotler DP, Ross R 2002 Body mass index and waist circumference independently contribute to the prediction of non abdominal, abdominal subcutaneous, and visceral fat. Am J Clin Nutr. ;75(4):683-8.
41-Mavri, A., Stegnar, M., Sentočnik, J. T., & Videčnik, V. (2001). Impact of weight reduction on early carotid atherosclerosis in obese premenopausal women. Obesity research, 9(9), 511-516.
42-Altin, C., Erol, V., Aydin, E., Yilmaz, M., Tekindal, M. A., Sade, L. E., ... & Muderrisoglu, H. (2018). Impact of weight loss on epicardial fat and carotid intima media thickness after laparoscopic sleeve gastrectomy: A prospective study. *Nutrition, Metabolism and Cardiovascular Diseases*, *28*(5), 501-509.

43-Norata, G. D., Raselli, S., Grigore, L., Garlaschelli, K., Dozio, E., Magni, P., & Catapano, A. L. (2007). Leptin: adiponectin ratio is an independent predictor of intima media thickness of the common carotid artery. *Stroke*, *38*(10), 2844-2846.
44-Colgecen, E., Ede, H., Erkoc, M. F., Akyuz, Y., & Erbay, A. R. (2016). The Relation of Androgenetic Alopecia Severity with Epicardial Fat Thickness. *Annals of dermatology*, *28*(2), 205-209.
45-Cakir, E., Doğan, M., Topaloglu, O., Ozbek, M., Cakal, E., Vural, M. G., ... & Delibasi, T. (2013). Subclinical atherosclerosis and hyperandrogenemia are independent risk factors for increased epicardial fat thickness in patients with PCOS and idiopathic hirsutism. Atherosclerosis, 226(1), 291-295.
46-Manco M, Morandi A, Marigliano M, Rigotti F, Manfredi R, Maffeis C. 2013 Epicardial fat, abdominal adiposity and insulin resistance in obese pre-pubertal and early pubertal children. Atherosclerosis. ;226:490---5
47 Marchington JM, Pond CM.1990 Site specific properties of pericardial and epicardial adipose tissue: the effects of insulin and high-fat feeding on lipogenesis and the incorporation of fatty acids in vivo. Int J Obesity. ;14:1013---22.
48-Fortier, M., Wang, S. P., Mauriège, P., Semache, M., Mfuma, L., Li, H., ... & Mitchell, G. A. (2004). Hormone-sensitive lipase-independent adipocyte lipolysis during β-adrenergic stimulation, fasting, and dietary fat loading. *American Journal of Physiology-Endocrinology And Metabolism*, *287*(2), E282-E288.
49-Iacobellis, G., Assael, F., Ribaudo, M. C., Zappaterreno, A., Alessi, G., Di Mario, U., & Leonetti, F. (2003). Epicardial fat from echocardiography: a new method for visceral adipose tissue prediction. *Obesity research*, *11*(2), 304-310.

Table (1) Demographic , anthropometric and clinical data of the studied groups

	CAH cases (n = 36)	Controls (n = 36)	P-value
Age, years	13.7±2.4	13.6±2.5	NS
Male/Female	11/25	7/27	-
SDS-BMI	1.02±0.92	-0.24±1.5	0.01
Waist circumference, cm	83±13	72±14	0.01
Hip circumference, cm	86±9	78±12	0.05
Waist to height ratio	0.55±0.08	0.47±0.07	0.001
SBP (mmHg)	119.76 ± 8.11	106.35 ± 7.47	0.001
DBP (mmHg)	74.70 ± 5.23	65.43 ± 4.91	0.001
Bone age, years	15.3±2.3	12.2 ± 1.2	0.001

Data are expressed as mean ± SD
SDS-BMI: standard deviation scores of body mass Index; SBP: systolic blood pressure; DBP: diastolic blood pressure;

Table (2) Laboratory data of the studied groups

	CAH cases (n = 36)	Controls (n = 36)	P-value
Total cholesterol (mg/dl)	173.65 ± 43.34	142.22 ± 18.14	0.01
Triglycerides (mg/dl)	138.22 ± 34.23	104.23 ± 12.21	0.01
LDL-c (mg/dl)	113.55 ± 65.21	73.66 ± 13.32	0.001
HDL-c (mg/dl)	44.12 ± 7.8	53.9 ± 7.8	0.01
Fasting blood glucose (mg/dl)	92.4 ± 15.8	81.6 ± 12.9	0.01
Fasting insulin (lU/mL)	15.2 ± 6.2	7.6 ± 2.8	0.001
HOMA-IR	3.21 ± 1.2	1.8 ± 0.8	0.001
hs-CRP(mg/L)	329±20.5	154.9±16.8	0.001
ACTH, pg/mL	102.5±12.7	26.3±2.2	0.001
17-OHP, nmol/l	184.2±54.9	1.75±0.95	0.001
Testosterone, ng/dl	544.3±195.7	181.6±62.5	0.001
Androstenedione, ng/d	182±15.2	89.3±17.2	0.001

Data are expressed as mean ± SD
LDL-c: low density lipoprotein cholesterol; HDL-c: high density lipoprotein cholesterol; HOMA-IR: the homeostasis model assessment of insulin resistance; 17-OHP, 17-hydroxyprogesterone, hs-CRP high-sensitivity C-reactive protein

Table (3) The echocardiographic , CA-IMT and EFT examination of the studied groups

	CAH children (n = 36)	Controls (n = 36)	P-value
LVEDD (mm)	43.7 ± 2.2	37.9 ± 3.8	0.001
LVESD (mm)	23.5± 2.1	22.2 ± 2.8	NS
EF(%)	67.5 ± 6.6	66.3 ± 4.1	NS
FS (%)	41.2 ±3.1	49.9 ± 4.2	NS
IVSWT (mm)	9.36 ±1.20	4.33 ± 1.32	0.001
LVPWT (mm)	7.54 ± 0.43	4.17 ± 0.45	0.001
LVMI (gm/m^2)	59.71 ± 7.24	42.29 ± 5.75	0.001
E/A ratio	1.28 ± 0.12	1.64 ± 0.22	0.01
IVRT (ms)	76.2 ± 6.3	46.5 ± 5.1	0.001

Mitral DcT (ms)	189.5 ± 16.2	129.4 ± 15.3	0.001
CA-IMT; mm	0.52 ± 0.20	0.43 ± 0.02	0.001
EFT (mm)	6.95 ±0.81	4.01 ±0.52	0.001

Data are expressed as mean ± SD
LVEDD: left ventricular end-diastolic diameter; LVESD: left ventricular end-systolic diameter; EF: ejection fraction; FS: fractional shortening; IVSWT; interventricular septal wall thickness; LVPWT: left ventricular posterior wall thickness; LVMI: left ventricular mass index; E/A: ratio of mitral E- to mitral A-wave peak velocity; IVRT: isovolumic relaxation time; DcT: deceleration time; EFT:epicardial fat thickness ; NS: non-significant

Table (4): The demographic, laboratory, echocardiographic characteristics of patients according to the degree of control on medical treatment

	Uncontrolled patients (n = 20)	Controlled patients (n = 16)	P value
Age; years	16.2 ± 0.8	13.1 ± 2.5	0.01
Bone age; years	17.1 ± 1.2	14.3 ± 2.2	0.01
HOMA-IR	4.42 ± 1.9	2.7 ± 1.9	0.001
hs-CRP(mg/L)	452±33.1	216.4±14.6	0.001
17-OHP; nmol/l	188.3 ± 32.5	8.21 ± 1.3	0.001
Testosterone; ng/dl	498.8 ± 191.2	34.5 ± 12.7	0.001
LVMI; gm/m^2	44.4 ± 6.5	32.4 ± 7.2	0.01
EFT (mm)	8.95 ±1.21	6.66 ±1.76	0.001
DcT; ms	187.0 ± 23.0	120.0 ± 25.0	0.01
CA-IMT; mm	0.54 ± 0.30	0.43 ± 0.02	0.05

Data are expressed as mean ± SD (standard deviation)

17-OHP, 17-hydroxyprogesterone, hs-CRP high-sensitivity C-reactive protein, LVMI: left ventricular mass index, DcT: deceleration time, CA-IMT carotid artery intima-media thickness

Table 5 The correlation between EFT and anthropometric ,laboratory and echocardiographic data in children with CAH

Parameter	(r and P values)
Age ;years	+0.639**
SDS-BMI	0.155
Waist circumference (cm)	+ 0.569**
Waist to height ratio	+0.657*
SBP(mmHg)	+0.432**
DBP(mmHg)	+0.361**
HOMA–IR	+0.562**
hs-CRP(mg/L)	+0.389**
Total cholesterol(mg/dl)	0.062
Triglycerides(mg/dl)	0.155
HDL-c(mg/dl)	- 0.658*
17-OHP; nmol/l	+0.743**
Testosterone, ng/dl	+ 0.659**
LVMI (gm/m^2)	+0.301*
Mitral DcT(ms)	+0.39**
CA-IMT (mm)	+0.415**

SDS-BMI: standard deviation scores of body Mass Index; LVMI: left ventricular mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure; HOMA-IR: the homeostasis model assessment of insulin resistance; HDL-c: high density lipoprotein cholesterol; EFT: Epicardial fat thickness; mitral DcT:mitral deceleration time; CA-IMT: carotid intima media thickness
*Significance: <0.05; **Significance: 0.01

Table (6) Multivariate correlation coefficients between EFT and various confounding variables in children with CAH

Confounding variables	**Odds ratio**	95% confidence interval (CI)
Waist circumference	1.9*	1.45-2.4
17-OHP; nmol/l	1.6*	1.33-2.89
Testosterone , ng/dl	1.7**	1.55-2.13
HOMA –IR	1.3*	1.04- 1.34
LVMI(gm/m^2)	1.1**	1.08-1.13
Mitral DcT(ms)	1.4**	1.15-2.05
CA-IMT(mm)	2.7**	1.16-1.57

EFT: Epicardial fat thickness ; HOMA-IR: the homeostasis model assessment of insulin resistance; LVMI: left ventricular mass index; mitral DcT: mitral deceleration time; CA-IMT: carotid intima media thickness
*Significance: <0.05; **Significance: 0.01