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Research article

Epicardial and Perihepatic Fat as Cardiometabolic Risk Predictors in Girls with Turner Syndrome: A Cardiac Magnetic Resonance Study

Short running title: Epicardial and Perihepatic Fat in Turner Syndrome

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What is already known on this topic?
Turner syndrome (TS) patients are at high risk of cardiometabolic disorders.

What this study adds?
TS girls displayed adverse cardiometabolic profile during late childhood and adolescence.
CMR-derived epicardial fat-thickness (EFT) and perihepatic fat-thickness (PHFT) are emerging cardiometabolic risk predictors in TS patients.
Excess EFT rather than total body adiposity may contribute to altered metabolic profile among lean-Turner patients.

Abstract
Background: Turner syndrome (TS) patients are at high risk of cardiometabolic disorders. Cardiometabolic risk factors are more related to visceral rather than total body adiposity. Adipocytokines have been explored as a potential link between obesity and obesity-related cardiometabolic dysfunctions. We explored the validity of epicardial fat-thickness (EFT) and perihepatic fat-thickness (PHFT) as cardiometabolic-risk predictors in TS-girls in relation to standard obesity-indices and metabolic syndrome (MetS) components.
Methods: Forty-six TS girls and twenty-five controls (10-16 years) were subdivided into two age-groups (10-13 and 13-16). They were assessed for BMI-Z-scores, waist-circumference (WC), total-fat mass (FM) and trunk-FM by bioimpedance-technique, EFT and PHFT by cardiovascular magnetic resonance, lipid-profile, Homeostasis model assessment of insulin resistance (HOMA-IR), and serum chemerin. MetS was defined according to International Diabetes Federation criteria.
Results: Overweight/obesity and MetS were detected in 45.7% and 37% of TS-girls respectively. BMI-Z-score, WC, total-FM, trunk-FM, EFT and PHFT values were significantly higher in TS-age groups compared to age-matched control groups, being more pronounced in older group at which TS-girls eventually exposed to estrogen. Dyslipidemia, higher HOMA-IR, chemerin, EFT and PHFT values were observed in lean-Turner compared to BMI-Z-matched controls. EFT and PHFT were significantly correlated with chemerin and several components of MetS. EFT at a cutoff-value of 6.20 mm (AUC=0.814) can predict MetS in TS-girls.
Conclusion: TS-girls displayed adverse cardiometabolic profile during late childhood and adolescence. EFT and PHFT are emerging cardiometabolic risk predictors in TS-patients. Excess EFT rather than total body adiposity may contribute to altered metabolic profile among lean-Turner patients.
Keywords: Epicardial fat, Perihepatic fat, metabolic syndrome, Turner Syndrome

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Introduction

Childhood obesity represents a major public-health crisis that has continued to climb throughout recent decades at an alarming rate (1). Metabolic Syndrome (MetS) is a clustering of co-incident cardiometabolic risk factors which predict adverse cardiovascular outcomes in adulthood (2).
Turner syndrome (TS) is one of the most common chromosomal disorders in females caused by complete or partial deficit of the second X-chromosome (3). Current epidemiological evidence indicates that children and adolescents with TS are susceptible to a wide spectrum of cardiometabolic risk factors including; higher obesity-indices, impaired glucose metabolism and atherogenic lipid profile compared to age-matched controls (4-7).
According to the adipose tissue expandability hypothesis, excess visceral fat (within/or surrounding viscera) together with relatively less subcutaneous adipose tissue elicit a state of chronic low-grade inflammation that increases the risk for cardiovascular diseases (8). Currently, visceral fat deposition been identified as emerging marker of cardiovascular risk (9). Epicardial adipose tissue, visceral fat reservoir of heart, is enclosed between pericardium and myocardium layers and secrets several adipocytokines (10).

Cardiovascular magnetic resonance imaging (CMR) is considered the standard reference for epicardial adipose tissue quantification (11). Epicardial fat thickness (EFT) shows good correlation with visceral abdominal fat, components of MetS, and severity of cardiovascular diseases (12,13).
Chemerin, an adipocytokine that modulates glucose and lipid metabolism in adipocytes (14), and displayed strong associations with MetS components (15), and with EF in adults with coronary artery disease (16), thus, may form an integral link between obesity and obesity-related cardiometabolic dysfunctions (17).
Therefore, we aimed to explore for the first time the validity of EFT and perihepatic fat thickness (PHFT) as cardiometabolic risk predictors in girls with TS in relation to standard obesity-indices and components of MetS.

Methods

A case-control study included forty-six girls with TS (age range: 10-16 years) and twenty-five age-matched healthy girls. Girls with TS were recruited sequentially between September 2018 and November 2019 during their routine visits at Pediatric Endocrinology Clinic at Mansoura University Children`s Hospital. The study was approved by the Ethics Committee of Mansoura Faculty of Medicine-Institutional Research Board (IRB) (Code No. R.20.04.800). Informed consent was obtained from the parents of all participants included in the study.
Girls with TS follow a uniform protocol for growth hormone therapy (GHT); thirty-six girls are currently receiving GHT (0.05 mg/kg/day), and ten girls had stopped GHT at a mean age of 14.4±0.8 year as height velocity <3 cm/year. Estrogen replacement therapy (ERT), oral ethinylestradiol at initial dose of 2 mg/day, was initiated for girls who had exhibited no clinical signs of spontaneous puberty by 14 years. Seven girls had spontaneous puberty at a mean age of 13.9±0.4 years. Turner girls having chronic comorbidities, thyroid dysfunction, congenital/acquired heart diseases or currently receiving medications, other than GHT and/or ERT, were excluded from the study.

Clinical evaluation:

Anthropometric measurements including weight, height, and waist circumference (WC) were obtained by a trained nurse according to standardized techniques. Body mass index (BMI) was calculated as weight divided by squared height (kg/m^2). Height and BMI Z-scores were calculated using reference data for Egyptian children and adolescents (18). In girls with TS, BMI Z-score was corrected for patient's height age to adjust for the effect of short stature (19). Girls with TS were classified based on WHO BMI Z-score cut-offs into "lean-group" (BMI Z-score ≤+1SD); "overweight/obese-group" (BMI Z-score >+1SD) (20). Systolic and diastolic blood pressure (SBP/DBP) was measured by standard technique (21).
Participants were classified according to Tanner breast scale into pre-pubertal (stage I), early-puberty (stages II-III) and late-puberty (stages IV-V). Girls with TS were subdivided into two groups; pre-pubertal (10-13 years) and pubertal (13-16 years) groups, the latter group includes both early-and late-puberty stages. The controls were subdivided into two groups; early-puberty (10-13 years) and late-puberty (13-16 years) groups.

Definition of metabolic syndrome:

In Turner group, MetS was diagnosed according to the 2007 International Diabetes Federation (IDF) pediatric definition for MetS (2), with the exception of blood pressure for which “elevated blood pressure” was defined according to the 2017 Clinical Practice Guideline for Screening and Management of High Blood Pressure in Children and Adolescents (21). MetS was diagnosed by central adiposity (WC ≥90th percentile for age and gender) and the presence of at least 2 of remaining 4 criteria; fasting blood glucose ≥100 mg/dL (5.6 mmol/L); triglycerides levels ≥150 mg/dL (1.7 mmol/L); HDL-C level ≤40 mg/dL (1.03 mmol/L); and SBP and/or DBP ≥90th percentile for age, gender and height percentile.

Body fat composition evaluation:

Total-fat mass (FM; kg) and trunk-FM (kg), a marker of central (abdominal) adiposity, were measured by bioimpedance technique using Tanita BC-418MA body composition analyzer (Tanita Corp., Tokyo, Japan).

Biochemical evaluation:

Blood samples were collected in the morning, after a 12-hour overnight fast. Sera were stored at −20°C. Total cholesterol and triglycerides were measured by colorometric kit (Spinreact, Girona, Spain), and HDL-C was measured by colorometric kit (Human Diagnostics, Wiesbaden, Germany). Serum chemerin (ng/mL) was measured using ELISA-Sandwitch technique kits (SUN RED, Shanghai-China) (cat. no. 201-12-1436). Homeostasis model assessment of insulin resistance (HOMA-IR) was calculated as FBG (mg/dl) × Fasting insulin (mIU/L)/405.

Cardiac magnetic resonance imaging (CMR):

The measurements of EFT and PHFT were performed by single expert technician who was blinded to the study groups using 1.5 T Tesla MRI machine (Ingenia, Philips, Netherland). Electrocardiogram gated cine steady state free precession (SSFP) images were acquired in short axis-view (SA) (slice thickness 5 mm, slice gap -1 mm, TR=3.2 ms, TE=1.6 ms, matrix 175/352, FOV=350 mm^2, slices 25), while modified Dixon (mDixon) sequence was obtained in short axis plane (slice thickness 5 mm, slice gap -2.5 mm, TR=5.9 ms, TE=0.0 ms, matrix 151/320, FOV =400 mm^2, slices 92). Image analysis was performed by single radiologist (N.B.) who was also blinded to the study groups. Images were transferred to workstation (extended MR Workspace 2.6.3.5, Philips medical systems, Netherland). Maximum EFT was measured opposite right ventricular free wall in the following sequences; m-Dixon (SA-view) and cine SSFP (SA-view) at end systole and end diastole. Maximum PHFT was measured in cine SSFP (SA-view) (**Supplementary fig. 1**).

Statistical analysis

Data were analyzed using IBM SPSS Statistics 23.0 (SPSS Inc., Chicago, IL, USA). Categorical data presented as number and percent, and Chi-Square test or Fisher Exact test were used as appropriate for comparison of two groups. Continuous data were tested for normality using one-sample Kolmogorov-Smirnov test, then data presented as mean ±SD for parametric data and median (min-max) for non-parametric data. Two groups were compared using Student t-test for parametric data and Mann-Whitney test for non-parametric data. Pearson and Spearman correlation analysis were used to correlate parametric and non-parametric data respectively. Receiver operator characteristic (ROC) curves were constructed to analyze the discriminative power of EFT, and PHFT to predict MetS among girls with TS. Areas under curves (AUCs) and 95% CI, optimal cutoff-values with relevant specificity, sensitivity, and accuracy were determined. Statistical significance was set at $P < 0.05$.

Results

The study included two age-matched groups, Turner-group (n=46; mean age 13.14±3.15 years) and control-group (n=45; mean age 12.17±3.02). Based on Karyotype results, two groups were identified; 45,X group (n=24) and non-45,X group (n=22) that including isochromosome 46,X,i[Xq10] (n=12), deletion 46,X,del(Xp-) (n=4), and different forms of mosaicism (n=6). No significant differences were detected in cardiometabolic variables between 45,X and non-45,X groups (P>0.05) **(Supplementary table 1).**

Girls with TS in pre-pubertal (10-13 years) group displayed significantly higher total-FM, trunk-FM, serum cholesterol, HOMA-IR and EFT values compared to age-matched control groups. Girls with TS in pubertal (13-16 years) group displayed similar previous significances in addition to significantly higher BMI Z-score, WC, serum triglycerides, and PHFT values compared to age-matched control groups (Table 1).

Based on BMI status, the prevalence of overweight/obesity in girls with TS was 45.7% (21/46), distributed as 8/26 (30.8%) within 10-13 years-group and 13/20 (65%) within 13-16 years-group. Overweight/obese Turner-group were significantly older and had significantly higher BMI Z-score, WC, total-FM, trunk-FM, HOMA-IR, serum cholesterol and triglycerides, EFT and PHFT values compared to lean-Turner and control groups. Interestingly, lean-Turner had significantly higher HOMA-IR, serum cholesterol, EFT values compared to controls, although BMI Z-score, total-FM, and trunk-FM were significantly lower in lean-Turner compared to age-matched controls (Table 2).

Based on IDF criteria,[2] the prevalence of MetS in Turner-group was 37% (17/46). Girls with TS were further classified as "MetS-group" (n=17) and "non-MetS group" (n=29). All girls in MetS-group were obese, while in non-MetS group, twelve girls (41.4%) were overweight/obese. BMI Z-score, WC, total-FM, trunk-FM, HOMA-IR, cholesterol, triglycerides, EFT and PHFT were significantly higher in MetS-group compared to non-MetS and control groups. Although non-MetS and control groups were matched for BMI Z-score and WC, non-MetS group displayed significantly higher total-FM, trunk-FM, HOMA-IR, cholesterol and EFT compared to control girls (Table 3).

Serum chemerin levels were significantly higher in Turner age-groups compared to age-matched control-groups; in overweight/obese-Turner compared to lean-Turner (P=0.014) and control (P<0.001) groups; in MetS-group compared to non-MetS (P=0.044) and control (P<0.001) groups; and in non-MetS and lean-Turner groups compared to controls. Serum chemerin was positively correlated with age, BMI Z-score, WC, total-FM, trunk-FM, HOMA-IR, triglycerides, EFT and PHFT in Turner group (Table 4). However, these correlations were not evident in control group (**Supplementary table 2**).

EFT and PHFT were positively correlated, EFT at different sequences were positively correlated with age, BMI Z-score, WC, HOMA-IR, triglycerides, total-FM, and trunk-FM, while PHFT showed similar correlations but not correlated with triglycerides (Table 4).

Regarding prediction of MetS among TS girls, EFT at mDixon SA-view with a cutoff-value of 6.20 mm has the highest discriminative power among CMR-derived parameters (AUC=0.814) (84.6% sensitivity; 73.5% specificity), while PHFT with a cutoff-value of 5.15 mm has the lowest discriminative power (AUC=0.685). AUC of EFT at mDixon SA-view was comparable to those of standard cardiometabolic risk factors; BMI Z-score (AUC=998), WC (AUC=955), HOMA-IR (AUC=0.899), and triglycerides (AUC=885). Finally, serum chemerin of more than 250 ng/ml can predict MetS in girls with TS with AUC=0.834, 76.9% sensitivity and 77.6% specificity (Table 5 and Figure 1a and 1b).

Interestingly, **supplementary fig. 1** represents CRM imaging of a lean Turner girl aged 15 years and 2 months (height age-adjusted BMI Z-score=0.9), although being lean, the results of EFT at different sequences and PHFT exceed the established cut-off values derived from ROC analysis (Table 5).

Discussion

In the current study, we explored for the first time the clinical relevance CMR-derived EFT and PHFT as cardiometabolic risk predictors in girls with TS in relation to standard obesity-indices and components of MetS.

Data on the prevalence of MetS in pediatric and adult Turner cohorts are limited. In the current study, the prevalence of overweight/obesity and MetS were 45.7% (21/46), and 37% (17/46) respectively. In adult Turner, Calcaterra et al. (22) reported a prevalence of MetS to be 4.7% (4/85), where Álvarez-Nava et al.(23) reported a prevalence of overweight/obesity and MetS to be 40% (35/88) and 49% (43/88), respectively. In a pediatric Turner cohort (n=19), O'Gorman et al. (5) found that 7/19 girls met one criterion for MetS, 8/19 met two criteria, and none fulfilled the diagnosis of MetS. The discrepancy in the prevalence of MetS mostly related to ethnic differences in rates of obesity and MetS components and different criteria used to define MetS.

We observed that although Turner girls in non-MetS group were age, BMI Z-score and WC-matched with control girls, they had significantly higher total-FM, trunk-FM, HOMA-IR, total cholesterol and EFT values. These findings can be explained by 41.4% of girls in non-MetS group were overweight/obese.

However, although BMI Z-score, total-FM, and trunk-FM values were significantly higher in control group compared to lean-Turner, interestingly, lean-Turner had significantly higher HOMA-IR, total cholesterol, EFT values compared to control girls, together with the significant associations between EFT and PHFT and triglycerides and HOMA-IR. These observations support for the independence of visceral adipose tissue deposition from traditional measurements of body adiposity, also emphasis on that visceral fat rather than subcutaneous adipose tissue is more metabolically active and has a key role in the development of different cardiometabolic risk factors (8).

Similar associations between EFT and cardiometabolic risk factors and with increased carotid intima-media thickness in obese children and adolescents were reported (24-26). In previous studies conducted among Turner cohorts, O'Gorman et al. (5) have demonstrated a significant increase in MRI-derived subcutaneous adipose tissue with no significant differences in MRI-derived intra-myocellular lipid measured by MRI or in bioimpedance-derived body-FM between young TS girls and age-and BMI Z-score-matched controls, while Ostberg et al. (27) demonstrated increased intrahepatocellular lipids using MRI in adult Turner cohorts.

In results of ROC analysis for the validity of EFT and PHFT in prediction of MetS in girls with TS revealed that EFT at mDixon SA-view with cutoff-value of 6.20 mm had the highest discriminative power (AUC=0.814), while PHFT has the

lowest discriminative power (AUC=0.685) among CMR-derived parameters. The results of EFT were nearly comparable to those of standard cardiometabolic risk factors and also to that of serum chemerin (AUC=0.834).

Currently, there is no consensus for EFT cutoff-values and most previous studies yielded different EFT cutoff-values, mostly related to several determinants such as age, ethnicity and degree of obesity. Abaci et al. (28) reported EFT cutoff-value of 4.1-mm for prediction of insulin resistance (90% sensitivity and 61% specificity), while Okyay et al. (29) reported EFT cutoff-value of 4.35-mm for prediction of MetS (61.7% sensitivity; 79.2% specificity) in obese children. Virtually, no data available concerned with PHFT measurements or reported a specific cutoff-value for PHFT in literature.

Interestingly, **supplementary fig. 1** represents CRM imaging of a lean Turner girl aged 15 years and 2 months (height age-adjusted BMI Z-score=0.9), although being lean, the results of EFT at different sequences and PHFT exceed the established cut-off values derived from ROC analysis in the current study.

Currently, two opposite phenotypes has been reported; "metabolically healthy obese" and "metabolically obese but normal-weight" (30). The underlying mechanism of such apparent dissociation is not fully understood; however, advances in non-invasive imaging techniques are making significant inroads allow understanding the fundamental contribution of visceral adiposity and fat distribution in such phenotypes, which potentially mediate their metabolic effects through adipocytokines production. Therefore, excess EFT rather than total-FM may explain the altered metabolic profile among lean-Turner group with potential role of chemerin as an adipocytokine.

Current results revealed higher serum chemerin levels in overweight/obese-Turner compared to lean-Turner and control groups and also in MetS-group compared to non-MetS and control groups. It is worth noting that serum chemerin was significantly higher in non-MetS than in control group despite being matched for age, BMI Z-scores, and WC, this could be due to that 41.4% of girls in non-MetS group were overweight/obese, however, serum chemerin was significantly higher in lean-Turner than control girls although, control girls had higher total-FM, and trunk-FM. The significantly increased EFT and PHFT in lean-Turner compared to control group together with significant correlation between chemerin and EFT and PHFT may point to the higher contribution of visceral fat (EF and PHF) rather than subcutaneous fat as a source of circulating chemerin.

In addition, positive correlations between chemerin and BMI Z-score, WC, FM, HOMA-IR, triglycerides, EFT, and PHFT were evident in Turner group but not in control group, supporting the possible role of chemerin in mediating metabolic derangement in Turner patients and indicate that serum chemerin increases only with pathological increase body mass and excess body adiposity. Likewise, serum chemerin in previous studies displayed strong associations with components of MetS (15), and with EF-volume (16), thus, chemerin may form a pivotal link between obesity and obesity-related cardiometabolic dysfunction (17).

In a recent study, chemerin was significantly higher in girls with TS compared with age-and BMI-matched controls but was not correlated with age, BMI Z-score, or any of glucolipid metabolism parameters (31).

In the current TS cohort, the risk for overweight/obesity and for the adverse cardiometabolic profile during late childhood and adolescence, whereas the metabolic derangements (high cholesterol and HOMA-IR), unfavorable body composition (increased total-FM and trunk-FM) and increased visceral adiposity (EFT) start to be evident in girls with TS in pre-pubertal (10-13 years) group, while girls with TS in pubertal (13-16 years) group who were eventually exposed to estrogen displayed similar previous significances in addition to significantly higher BMI Z-score, WC, serum triglycerides, and PHFT values compared to age-matched control groups. Likewise, in a recent longitudinal study, metabolic comorbidities were found to start in childhood, increasing the risk for CVD across the Turner patient's lifespan (7). These findings reinforce the importance of annual screening for cardiometabolic risk factors and early counseling regarding healthy nutrition and active lifestyle in young TS girls (3).

Limitation and strength:

The small sample size of the current TS cohort precluded the reliable evaluation of cardiometabolic profile, EFT and PHFT of different karyotypes groups and the cross-sectional design precluded the evaluation of the beneficial/or adverse effects of GHT and ERT in the context of cardiometabolic profile, EFT and PHFT. Nevertheless, our study is the first to identify the validity of EFT and PHFT as cardiometabolic risk predictors in TS patients, thus provides relevant data for future researches.

Conclusion:

Girls with TS display adverse cardiometabolic profile during late childhood and adolescence. CMR-derived EFT and PHFT are emerging tools for assessment of cardiometabolic risk and for prediction of MetS in high-risk population as TS patients. There is evident need to establish specific cutoff-values for EFT and PHFT that will improve the concomitant use of EFT and PHFT as screening tool and follow-up markers for cardiometabolic risks in high-risk population.

Authors Declarations

- **Author's contribution:** Nanees Salem, Wafaa Laimon, and Nihal Batouty conceived the main study idea and design. Nanees Salem and Wafaa Laimon involved in clinical data collection and data analysis; Nihal Batouty performed CMR imaging analysis; Shimaa Hendawy performed Biochemical analysis; Nanees Salem wrote the first draft of manuscript; all authors involved in data interpretation, manuscript revision and editing and literature search and approved the submitted version.
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List of Abbreviations:

BMI	Body mass index
CMR	Cardiovascular magnetic resonance
DBP	Diastolic blood pressure
EFT	Epicardial fat-thickness

ERT	Estrogen replacement therapy
FBG	Fasting blood glucose
FM	Fat mass
GHT	Growth hormone therapy
HDL-C	High-density lipoprotein cholesterol
HOMA-IR	Homeostasis model assessment of insulin resistance
IDF	International Diabetes Federation
MetS	Metabolic syndrome
PHFT	Perihepatic fat-thickness
SBP	Systolic blood pressure
TS	Turner syndrome
WC	Waist circumference

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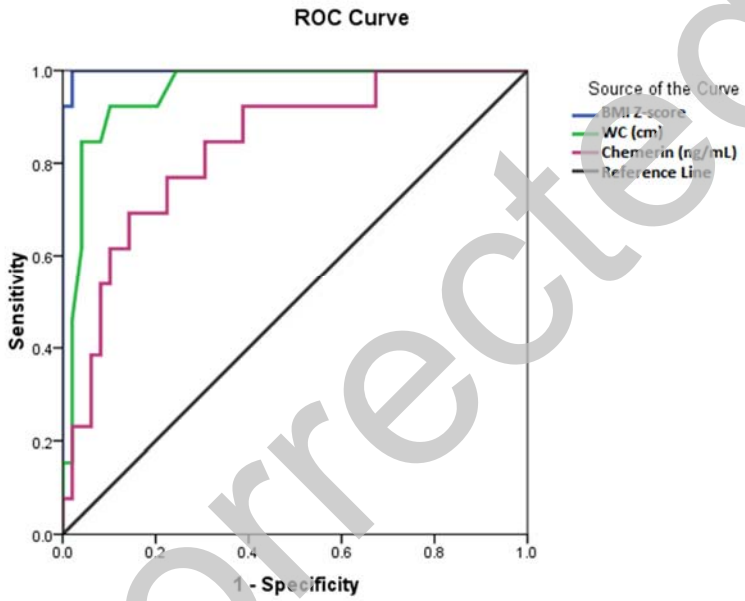
Figure 1.

(a) Receiver operating characteristic (ROC) curve for prediction of metabolic syndrome from BMI Z-score, WC and serum chemerin in girls with Turner syndrome



(b) Receiver operating characteristic (ROC) curve for prediction of metabolic syndrome from epicardial fat thickness sequences and perihepatic fat thickness in girls with Turner syndrome.

Abbreviations: BMI, body mass index; EFT, epicardial fat thickness; HOMA-IR, Homeostasis model assessment of insulin resistance; PHFT, perihepatic fat thickness; SA, short axis view; WC, waist circumference.

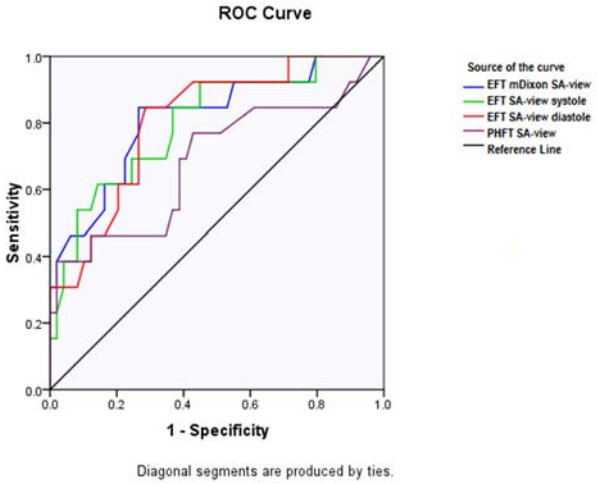


Table 1: Clinical, body composition, biochemical and CMR parameters among Turner syndrome and control age-groups

	Turner group (n=46)		Control group (n=25)		Test of significance	
	10-13 years (n=26)	13-16 years (n=20)	10-13 years (n=10)	13-16 years (n=15)	P1	P2
Clinical parameters						
BMI Z-score	0.43 (-1.1, 4)	1.5 (-1.5, 3.1)	0.9 (-0.6, 1.0)	0.7 (0.0, 1.0)	0.549	0.031*
WC (cm)	66.69±8.82	76.75±7.79	66.54±5.51	72.71±2.69	0.960	0.042*
Body composition parameters						
Total body FM (kg)	7.80 (5.5-17.4)	13.45 (7.2-28.9)	5.10 (3.43-8.6)	10.0 (5.4-13.8)	0.022*	0.019*
Trunk FM (Kg)	3.20 (2.5-7.9)	7.15 (3.4-15.9)	2.20 (0.7-3.8)	4.20 (0.6-6.5)	0.010*	0.025*
Biochemical parameters						
HOMA-IR	2.11 (0.96-5.43)	4.27 (0.92-8.26)	1.66 (0.79-2.01)	1.48 (1.06-2.23)	0.047*	0.001*
Cholesterol (mg/dl)	145.6±26.7	176.8±35.5	121.5±9.5	136.3±11.1	0.011*	<0.001*
Triglycerides (mg/dl)	95.5±27.5	111.8±40.5	80.4±10.7	78.1±13.8	0.088	0.041*
HDL (mg/dl)	54.61±13.73	55.66±13.07	56.83±11.68	49.57±5.25	0.139	0.242
Chemerin (ng/mL)	249.4 (108.5-388.5)	353.5 (109.3-630.3)	128.5 (35.5-136)	131.0 (119-175)	0.033*	0.008*
CMR parameters						
EFT-SA mDixon (mm)	5.26±1.65	7.64±1.80	4.68±0.51	5.18±1.17	0.294	0.002*
EFT-SA systole (mm)	6.35±1.64	9.19±2.04	4.55±0.51	5.17±0.63	0.002*	<0.001*
EFT-SA diastole (mm)	3.79±1.14	4.89±1.61	2.38±0.31	2.94±0.47	0.001*	0.004*
PHFT (mm)	5.62±1.86	6.31±2.37	4.57±0.38	4.99±1.06	0.134	0.023*

Data presented either as mean SD or median (minimum-maximum)
BMI, body mass index; CMR, cardiac magnetic resonance; EFT, epicardial fat thickness; FM, fat mass; HOMA-IR, Homeostasis model assessment of insulin resistance; HDL, high density lipoprotein; SA, short axis view; PHFT, perihepatic fat thickness; WC, waist circumference.
*Significant difference (P<0.05)
P1: Turner group vs. control group (10-13 years); P2: Turner group vs. control group (13-16 years).

Table 2: Clinical, body composition, biochemical and CMR parameters in girls with Turner syndrome according to BMI status compared to control group

	Turner group (n=46)		Control group (n=25)	Test of significance		
	Overweight/Obese (n=21)	Lean (n=25)		P1	P2	P3
Clinical parameters						
Age (years)	14.46±2.26	12.04±3.41	12.17±3.02	0.007*	0.879	0.006*
Height Z-score	-3.65 (-4.8--2.1)	-2.89 (-5.0--1.3)	0.90 (-1.5-1.7)	<0.001*	<0.001*	0.165
BMI Z-score	2.15 (1.1-4.0)	-0.15 (-1.7-1.0)	0.70 (0.0-1.0)	<0.001*	<0.001*	<0.001*
WC (cm)	78.81±6.66	63.72±7.96	66.02±7.28	<0.001*	0.292	<0.001*
Body composition parameters						
Total body FM (kg)	12.00 (4.6-31.9)	4.50 (2.4-14.0)	7.60 (2.6-10.8)	<0.001*	<0.001*	<0.001*
Trunk FM (kg)	4.70 (2.1-15.9)	1.60 (0.6-5.5)	3.20 (1.2-6.5)	0.001*	<0.001*	<0.001*
Biochemical parameters						
HOMA-IR	4.15 (1.82-8.26)	2.29 (0.92-4.71)	1.48 (0.35-2.23)	<0.001*	0.001*	0.004*
Total cholesterol (mg/dl)	185.33±39.33	155.16±15.49	117.04±10.35	<0.001*	0.010*	0.007*
Triglycerides (mg/dl)	116.52±35.79	74.72±9.24	80.04±10.97	<0.001*	0.161	0.007*
HDL (mg/dl)	52.90±14.07	51.84±13.73	51.80±10.11	0.759	0.991	0.797
Chemerin (ng/mL)	344.90 (109.3-630.3)	221.70 (45.0-290.1)	119.00 (20.4-175.0)	<0.001*	<0.001*	0.014*
CMR parameters						
EFT-SA mDixon (mm)	7.24±2.04	5.69±2.12	4.64±0.97	<0.001*	0.031*	0.016*
EFT-SA systole (mm)	8.71±2.73	6.54±2.22	4.58±0.77	<0.001*	<0.001*	0.005*
EFT-SA diastole (mm)	4.88±1.76	3.27±1.05	2.87±0.65	<0.001*	0.053	0.013*
PHFT (mm)	6.97±2.66	5.11±1.14	4.99±1.06	0.001*	0.702	0.003*

Data presented either as mean SD or median (minimum-maximum)
BMI, body mass index; CMR, cardiac magnetic resonance; EFT, epicardial fat thickness; FM, fat mass; HOMA-IR, Homeostasis model assessment of insulin resistance; HDL, high density lipoprotein; SA, short axis view; PHFT, perihepatic fat thickness; WC, waist circumference.
*Significant difference (P<0.05)
P1: overweight/obese TS vs. control; P2: Lean TS vs. control; P3: overweight/obese TS vs. Lean TS.

Table 3: Clinical, body composition, biochemical and CMRI parameters among Turner syndrome subgroups (with and without metabolic syndrome) and control group

	Turner Syndrome (n=46)		Control (n=25)	Test of significance		
	MetS group (n=17)	Non-MetS group (n=29)		P1	P2	P3
Clinical parameters						
Age (years)	15.26±1.77	13.86±2.07	13.45±2.04	0.012	0.515	0.046
BMI Z-score	2.53 (1.9,4.0)	0.54 (-1.5,2.1)	0.68 (0.2, 0.9)	<0.001*	0.607	<0.001*
WC (cm)	82.00 ± 4.30	68.46±7.85	66.82±5.51	<0.001*	0.867	<0.001*
Body composition parameters						
Total body FM (kg)	13.70 (11.2-31.9)	5.35 (3.4-14.0)	4.80 (2.6-5.1)	<0.001*	0.017*	<0.001*
Trunk FM (kg)	5.70 (3.4-15.9)	2.50 (0.6-7.2)	1.75 (1.2-2.2)	<0.001*	0.013*	<0.001*
Biochemical parameters						
HOMA-IR	5.81 (2.35-8.26)	3.13 (0.92-4.31)	1.48 (0.35-2.23)	<0.001*	<0.001*	0.003*
Total cholesterol (mg/dl)	183.53±34.87	154.50±18.97	118.68±9.89	<0.001*	<0.001*	0.029*
Triglycerides (mg/dl)	131.92±29.09	92.16±33.39	79.58±11.64	<0.001*	0.096	0.001*
HDL (mg/dl)	50.07±13.86	53.25±14.22	54.16±10.28	0.346	0.809	0.518
Chemerin (ng/mL)	378.80 (115.7-630.3)	245.95 (108.5-335.7)	104.50 (20.4-166.9)	<0.001*	<0.001*	0.044*
CMR parameters						
EFT-SA mDixon (mm)	7.87±2.07	6.22±1.86	4.90±0.82	<0.001*	0.004	0.019*
EFT-SA systole (mm)	9.06±2.75	6.72±1.98	4.78±0.63	<0.001*	<0.001*	0.042*
EFT-SA diastole (mm)	5.16±1.98	4.14±1.13	2.59±0.46	0.001*	<0.001*	0.053
PHFT (mm)	7.31±3.03	5.67±1.53	5.37±0.89	0.042*	0.452	0.034*

Data presented either as mean SD or median (minimum-maximum)

BMI, body mass index; CMR, cardiac magnetic resonance; EFT, epicardial fat thickness; FM, fat mass; HOMA-IR, Homeostasis model assessment of insulin resistance; HDL, high density lipoprotein; MetS, Metabolic syndrome; SA, short axis view; PHFT, perihepatic fat thickness; WC, waist circumference.

*Significant difference (P<0.05).

P1: MetS vs. control; P2: non-MetS vs. control; P3: MetS vs. non-MetS.

Table 4: Correlation analyses of serum chemerin, epicardiac fat thickness and perihepatic fat thickness with clinical, biochemical, and body composition parameters in Turner syndrome group

		Serum Chemerin	PHFT	EFT-SA mDixon	EFT-SA systole	EFT-SA diastole
Age	r	0.508	0.406	0.621	0.622	0.369
	P	<0.001*	0.005*	<0.001*	<0.001*	0.012*
BMI Z-score	r	0.368	0.417	0.343	0.373	0.251
	p	0.012*	0.004*	0.020*	0.011*	0.093
WC	r	0.425	0.535	0.442	0.505	0.347
	p	0.003*	<0.001*	0.002*	<0.001*	0.018*
Total body FM	r	0.483	0.378	0.450	0.477	0.399
	p	0.001*	0.010*	0.002*	0.001*	0.006*
Trunk FM	r	0.431	0.326	0.363	0.393	0.340
	p	0.003*	0.027*	0.013*	0.007*	0.021*
HOMA-IR	r	0.652	0.358	0.430	0.372	0.306
	p	<0.001*	0.014*	0.003*	0.011*	0.039*
Triglycerides	r	0.500	0.157	0.344	0.268	0.228
	p	0.011*	0.296	0.019*	0.072	0.127
Chemerin	r	-	0.448	0.535	0.443	0.394
	p	-	0.002*	<0.001*	0.002*	0.007*
PHFT	r	-	-	0.494	0.491	0.416
	p	-	-	<0.001*	0.001*	0.004*

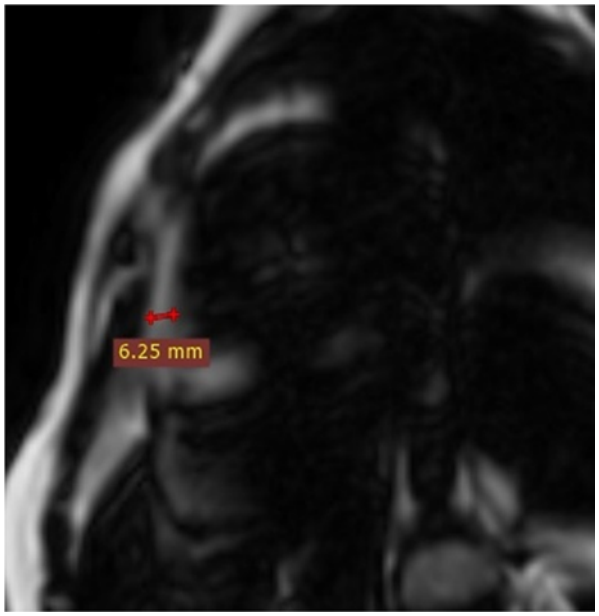
r, regression coefficient
*significant correlation
BMI, body mass index; EFT, epicardial fat thickness; FM, fat mass; HOMA-IR, Homeostasis model assessment of insulin resistance; SA, short axis view; PHFT, perihepatic fat thickness; WC, waist circumference.

Table 5: ROC curves for the traditional cardiometabolic risk factors, serum chemerin, epicardial fat thickness and perihepatic fat thickness in the diagnosis of metabolic syndrome in girls with Turner syndrome.

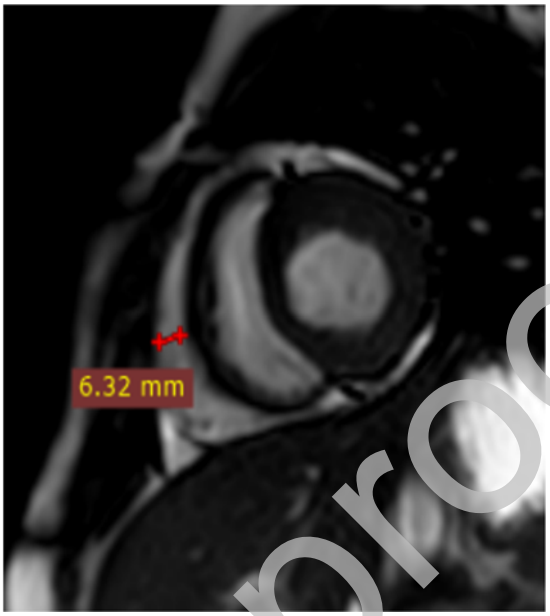
	AUC	95% CI	Cut-off	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Accuracy (%)
BMI Z-score	0.998	0.99-1.0	1.97	92.3	98	92.3	95.8	94.6
WC (cm)	0.955	0.905-1.0	76.50	92.0	90.8	75	95.2	86.4
HOMA-IR	0.899	0.819-0.979	3.32	91.5	77.6	66.7	92.8	81.1
Triglycerides (mg/dl)	0.885	0.799-0.972	94.0	92.1	69.4	63.1	92.8	78.4
Chemerin (ng/ml)	0.834	0.715-0.952	250.1	76.9	77.6	62.5	85.7	75.6
EFT-SA mDixon (mm)	0.814	0.677-0.951	6.20	84.6	73.5	64.7	90	78.4
EFT-SA systole (mm)	0.800	0.662-0.938	6.15	83.8	63.3	55	88.2	70.3
EFT-SA diastole (mm)	0.807	0.681-0.933	3.55	80.9	71.4	61.1	89.4	75.7
PHFT (mm)	0.685	0.502-0.868	5.15	72.7	57.1	50	82.3	64.8

AUC: area under the curve, CI: confidence interval, PPV: positive predictive value, NPV: Negative predictive value; BMI, body mass index; EFT, epicardial fat thickness; HOMA-IR, Homeostasis model assessment of insulin resistance; SA, short axis view; PHFT, perihepatic fat thickness; WC, waist circumference.

(A)



(B)



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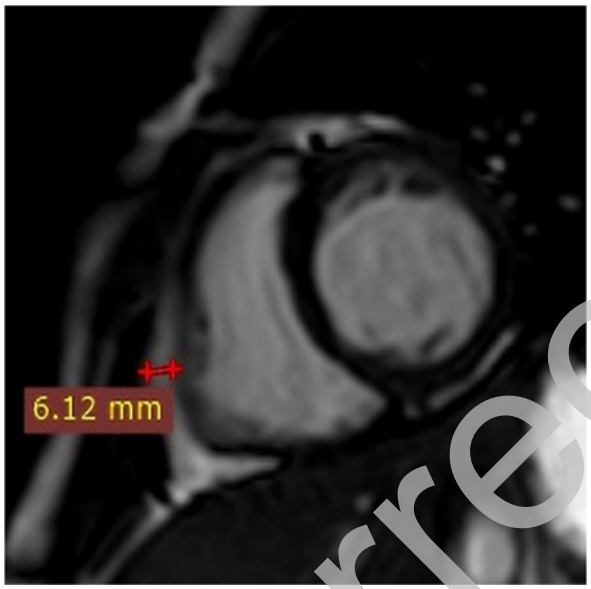

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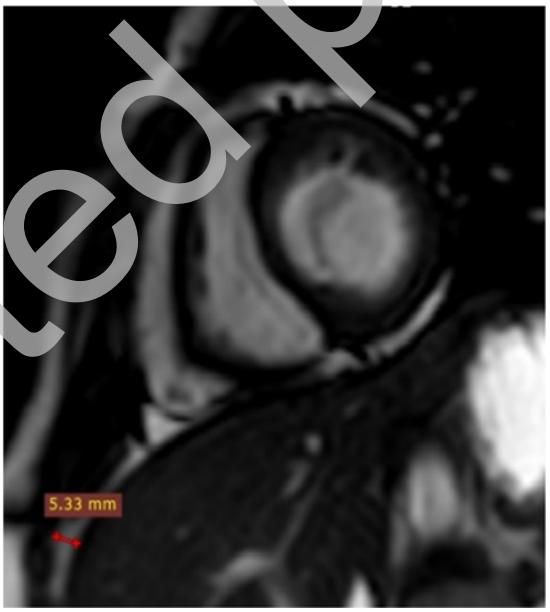

Supplementary figure 1:
Measurements of EFT (mm) at different sequences and PHFT (mm) using Cardiovascular MRI (CMR) imaging
Comment: CRM imaging of a lean Turner girl aged 15 years and 2 months, BMI Z-score corrected for height age=0.9, Interestingly, although being lean, the results of EFT at different sequences and PHFT exceed the established cut-off values derived from ROC analysis presented in table 5.

- (A): EFT-mDixon SA veiw (6.25 mm) (cutoff value=6.20mm)
- (B): EFT-Cine SSFP SA veiw end systole (6.32 mm) (cutoff value=6.15 mm)
- (C): EFT-Cine SSFP SA veiw end diastole (6.12 mm) (cutoff value=3.55 mm)
- (D): PHFT SA veiw (5.33 mm) (cutoff value=5.15 mm)

EFT, epicardial fat thickness; PHFT, perihepatic fat thickness; SA, short axis view

Supplementary Table 1:
Clinical, body composition, biochemical and CMR parameters among girls with Turner syndrome based on Karyotype results

	Turner group (n=46)		P-value
	45,X group (n=24)	Non-45,X group (n=22)	
Clinical parameters			
Height Z-score	-2.83 (-4.8--1.3)	-3.68 (-5.0--1.8)	0.101
BMI Z-score	0.48 (-1.5-3.2)	1.04 (-1.7-4.0)	0.356
WC (cm)	69.38±10.64	71.95±10.51	0.413
Body composition parameters			
Body FM (kg)	5.65 (2.7-18.5)	7.80 (2.4-31.9)	0.509
FM-index (kg/m^2)	3.44 (1.87-8.80)	4.41 (1.98-13.81)	0.860
TKF mass	.7-7.9)02.15 (	.6-15.9)03.40 (	0.454
Biochemical parameters			
HOMA-IR	2.76 (0.96-11.26)	4.42 (0.92-10.11)	0.113
Cholesterol (mg/dl)	164.12±37.97	174.18±39.24	0.382
Triglycerides (mg/dl)	101.83±39.29	100.95±32.62	0.935
HDL (mg/dl)	53.70±15.55	50.81±11.63	0.483
Chemerin (ng/mL)	232.15 (45.0-630.3)	323.30 (109.3-488.5)	0.099
CMR parameters			
EFT-SA mDixon (mm)	5.80±2.18	7.05±2.10	0.056
EFT-SA systole (mm)	7.17±2.92	7.93±2.37	0.340
EFT-SA diastole (mm)	4.10±1.81	4.59±1.53	0.330
PHFT (mm)	5.63±1.96	6.31±2.37	0.294

Data presented either as mean SD or median (minimum-maximum)
BMI, body mass index; CMR, cardiac magnetic resonance; EFT, epicardial fat thickness; FBG, fasting blood glucose; FM, fat mass; HOMA-IR, Homeostasis model assessment of insulin resistance; HDL, high density lipoprotein; SA, short axis view; PHFT, perihepatic fat thickness; WC, waist circumference.

Supplementary Table 2:
Correlation analyses of serum chemerin with clinical, biochemical, and body composition parameters in control group

	Chemerin (ng/ml)	
	r	*P*
Age (years)	0.288	0.162
BMI-Z score	0.190	0.362
WC (cm)	0.254	0.221
Total FM (kg)	0.280	0.176
Trunk-FM (kg)	0.332	0.105
HOMA-IR	0.280	0.174
Triglycerides (mg/dL)	0.294	0.154
EFT-SA mDixon (mm)	0.351	0.085
EFT-SA systole (mm)	0.433	0.056
EFT-SA diastole (mm)	0.016	0.938
PHFT (mm)	0.126	0.547

BMI, body mass index; EFT, epicardial fat thickness; FM, fat mass; HOMA-IR, Homeostasis model assessment of insulin resistance; SA, short axis view; PHFT, perihepatic fat thickness; WC, waist circumference.